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Biomass and Lipid Content of Heterotrophic *Spirogyra* sp by Using Cassava Starch Hydrolysate

Mohamad Agus Salim
Biology Department, Faculty of Science & Technology,
State Islamic University of Sunan Gunung Djati Bandung
Jl. A. H. Nasution No.105 Bandung, Indonesia

Abstract:- Research has been conducted on *Spirogyra* sp heterotrophic cultivation by providing cassava starch hydrolysate (CSH) as a carbon source under dark condition. The purpose of this research was to obtain the concentration of CSH which could increase biomass concentration and lipid content of *Spirogyra* sp under heterotrophic cultivation. The treatment of CSH (15 g/L) could produce the highest biomass concentration and lipid content of *Spirogyra* sp. After 6 weeks cultivation, the maximal biomass concentration and lipid content of 12.03 g/L and 5.23 % respectively was obtained in the culture added 15 g/L cassava starch hydrolysate under dark condition, which higher than under light condition. This study revealed that algae *Spirogyra* sp exhibit enhanced biomass concentration and lipid content under heterotrophic cultivation, in the presence of cassava starch hydrolysate.

Keywords:- cassava starch hydrolysate, heterotrophic, lipid content, *Sprirogyra* sp.

I. INTRODUCTION

In recent years, with the increase in anthropogenic green house gas emission and depleting fossil fuel reserves, mainly due to large scale use of this fuel for transport, electricity and thermal energy generation. It has become increasingly important to research on improving biofuel production has been accelerating for both ecological and economical reasons, primarily for its use as an alternative to petroleum based fuels [1]. There are several reports documenting the potential of algal biomass to generate biofuels [2]**.** Algae are currently considered the most promising biomass for biofuel production, based on their high lipid contents [3].

Algae may assume many types of metabolisms, such as autotrophic and heterotrophic**.** The autotrophic algae use photosynthesis to harness sunlight and fix the atmospheric CO_2 which is then assimilated in the form of lipids. There are many algal species which are heterotrophic and they are able to take up small organic molecules in the environment and turn them into the building blocks of their own which are mainly lipid which can then be processed to generate biofuels [3]. However, the use of algae can be a suitable alternative because algae are the most efficient biological producer of lipid on the planet and versatile biomass source and may soon be one of the earth's most important renewable fuel crops [4].

Spirogyra sp is one of the commonest of green algae. It is found in bright green free floating masses in the still water fresh water ponds, pools, lakes and ditches and also in flowing streams. The plant body is thallus which consists of a long green cylindrical thread about 1/10 mm across and several centimeters long. It is silky, hair like unbranched and often called a filament. The outer is a pectose layer covered with mucilage sheath [3]. Biodiesel can be produced from this species algae, although lipid content lower than microalgae [5]. In a previous study apparently capable of cultured heterotrophic algae on organic substrates without light. It can therefore occur because the presence of organic compounds as a carbon source will produce high levels of lipids in heterotrophic conditions [6]**.**

Algae are the fastest growing photosynthesizing organisms and can complete an entire growing cycle every few days. Some algae species have high lipid content (up to 60%) by weight and can produce up to 15,000 gallons of lipid per acre per year under optimum conditions. Algae contain lipids as membrane components, storage products metabolites and sources of energy. The lipids contents of algae vary in accordance with culture conditions [4].

The price of algal biofuel ultimately depends on the substrate cost, lipid yield, and the quality of the products formed by the downstream process [7]. The cost of carbon source represents 50% of the cost of medium in algal cultivation [8]**.** Recent studies have found that the biomass and lipid content of algae can be increased through changing cultivation conditions, such as CO_2 aeration fixation, temperature, salinity and nutrient concentration [9]. Particularly, the effects of carbon sources and concentrations on lipid accumulation of algae have been examined widely [10]. The effects of cassava starch hydrolysate as the sole organic carbon source could enhance the algal biomass content in heterotrophic culture medium. The utilization of complex

organic carbon substrate like cassava starch hydrolysate could stimulate the biosynthesis of lipids as the raw materials for biodiesel, while reduce the anabolism of photosynthetic pigments and proteins.

The ability of algae to survive in diverse and extreme conditions is reflected in the tremendous diversity and sometimes unusual pattern of cellular lipids obtained from these algae [11]. Moreover, some of these algae can also modify lipid metabolism efficiently in response to changes in environmental conditions [12]. The objective of this study was to investigate the potential of using cassava starch hydrolysate as the complex carbon substrate to produce algal biomass and biochemical components, such as lipids by *Spirogyra* sp.

II. MATERIALS AND METHODS

A. Algae Production

The study was conducted from September to November 2011**,** in Biology Laboratory of Science and Technology Faculty of State Islamic University, Bandung, Indonesia. Samples of *Spirogyra* sp were collected from freshwater pond of Gunung Masegit Kareumbi Cicalengka, Bandung. In the experiments, *Spirogyra* sp was cultivated in 500 ml Erlenmeyer flask with 300 ml working volume of basal bold medium (BBM), under 25 ±1 ^{o}C and 180 µmol $m^{-2}s^{-2}$ light intensity was measured by a light meter. For creating heterotrophic condition, every Erlenmeyer flask were covered by carbon paper, meanwhile for autotrophic condition as control without covering by carbon paper. The initial mass of *Spirogyra* sp was 20 gram fresh weight and the initial pH was 6.5. Cultures were aerated continuously with filtered (0.22 µm) mixtures via bubbling from the above of Erlenmeyer flask with an aeration rate of 200 $ml.min^{-1}$ (i.e., 0.25 vvm, volume gas per volume media per minute). Different concentrations of cassava starch hydrolysate were added to the BBM. The cultivation lasted for 6 weeks and then be harvested for measurement.

B. Biomass Concentration

Algal biomass concentrations were determined by measuring the dry cell weight. Cells were washed and rinsed twice with distilled water and dried at 70 $^{\circ}$C for 24 h to give the dry cell weight (g/L).

C. Lipid Extraction

Cells were harvested and washed with distilled water two times, and then dried by a freeze dryer. The dry biomass (100 mg) was homogenized in mortar and extracted with *n*-hexane (20 mL) for 30 minutes and centrifuged. The extraction process was repeated three times and supernatant was transferred to a pre-weighed glass vial and evaporated on rotary evaporator. The algae lipid was recovered after solvent evaporation and dried at 70 ^{o}C completely. The weight of glass vial containing oil was measured gravimetrically and the lipid content expressed as dry weight percentage (%).

D. Statistical Analysis

The mean value of all datas were compared by variances analysis (ANOVA) and then Duncan's Multiple Range Test (DMRT) for pair wise comparison was used at the 5% significance level [28].

III. RESULTS AND DISCUSSIONS

A. Biomass Concentration

Results presented in Figure 1 demonstrate the effects of cassava starch hydrolysate on biomass concentration of *Spirogyra* sp under heterotrophic cultivation (6 weeks). With the cultivation of *Spirogyra* sp under dark and light condition (as control), all of the cultures had an obvious growth except the control group. The samples supplied organic carbon sources (cassava starch hydrolysate) displayed the superiority in their growth compared with the autotrophic cultivation (light condition). After 6 weeks cultivation, the maximal biomass concentration of 12.03 g/L was obtained in the culture added 15 g/L cassava starch hydrolysate under dark condition, which higher than under light condition 6 times, however, it had no significant difference ($p<0.05$) with the culture medium supplied 10 g/L and 5 g/L cassava starch hydrolysate (7.79 g/L and 6.61 g/L) respectively. In photoautotrophic and heterotrophic growth there was two distinctive processes, photosynthesis and aerobic respiration. The former was influenced by light intensity and the latter was related to the organic substrate concentration (cassava starch hydrolysate). *Spirogyra* sp could grow with two condition under dark condition with providing carbon sources or light condition with illumination.

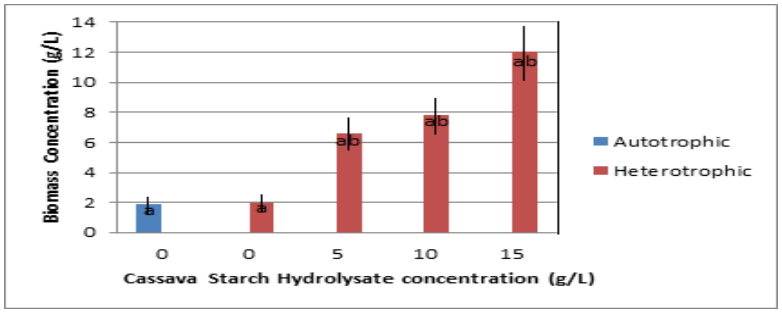

Figure 1. The effect of providing cassava starch hydrolysate concentration to biomass concentration of *Spirogyra* sp under heterotrophic cultivation for 6 weeks. (description: values of bars followed by different letters indicate difference at 5% level test).

From the above findings it could be concluded that the algae *Spirogyra* sp can efficiently utilize cassava starch hydrolysate as carbon source in culture media. According Miao & Wu [20] to overcome the limitation of light penetration, many algae that are able to grow heterotrophically by using organic carbon sources such as sugars or organic acids without light were studied widely.

The high biomass concentration of heterotrophic cultures demonstrates that the growth stimulating effects of cassava starch hydrolysate as a carbon source utilization in heterotrophic cultures were the strongest effect. In the case of the heterotrophic dark environment with the addition of cassava starch hydrolysate, the algae did not have access to a light source and was forced to convert the organic carbon that was initially provided into the energy it needed to survive.

Growing algae heterotrophically presents economic advantages. The algae can be grown significantly denser, allowing for greater yield, because light does not need to penetrate the algae. Also, the cost of cultivation will decline because the space and maintenance requirements are not as demanding [13]. By observing a heterotrophic growth condition, energy consumption and cost of algal cultivation will decrease, while feasibility of algal production will increase.

The heterotrophic growth cultivation had organic carbon directly imputed into the system as a source of energy for the algae, so it is clear that the algae were using the cassava starch hydrolysate as a source of energy. The autotrophic cultivation of growth is the least stressful to the algae. Algae are photosynthetic organisms, so the natural growth environment is autotrophic using light from the sun. Because the algae are in their naturally occurring, ordinary growth environment, the algae is not stressed in the autotrophic cultivation [14].

With cassava starch hydrolysate available to the algae *Spirogyra* sp, it had an abundance of nutrient source. This may have slightly stressed to *Spirogyra* sp because the algae are not acclimated to a medium with alternative source of carbon. The excess nutrient could account for the algal stress. Although algae *Spirogyra* sp are able to acclimate to the cassava starch hydrolysate-saturated water, the algae are not accustomed to forcing a different chemical process, and it is a large source of stress. The results prove that biomass concentration yields are, in fact, a true advantage of heterotrophic growth cultivation [15].

B. **Lipid Content**

This work demonstrates the feasibility of cassava starch hydrolysate as an alternative carbon substrate for algal cultivation and a cost reduction of carbon substrate feed in algal lipid production may be expected. In heterotrophic cultivation, the lipid contents were higher than (3-4 times) in the autotrophic cultivation (Fig.2). However, the heterotrophic cultivation supplied cassava starch hydrolysate experience an increase in lipid content that dependent on the increase in biomass concentration.

Figure 2 displays an analysis of lipid yield based on growth conditions. According to the data, the most optimal growth condition for lipid content was the heterotrophic cultivation. After 6 weeks of cultivation, higher cassava starch hydrolysate concentration led to an increase in lipid content, with the maximum lipid content of 5.23 % obtained by cultivation with cassava starch hydrolysate of 15 g/L. The algae *Spirogyra* sp can grow heterotrophic cultivation and accumulates much higher production of lipids and higher growth rate using cassava starch hydrolysate as carbon substrate.

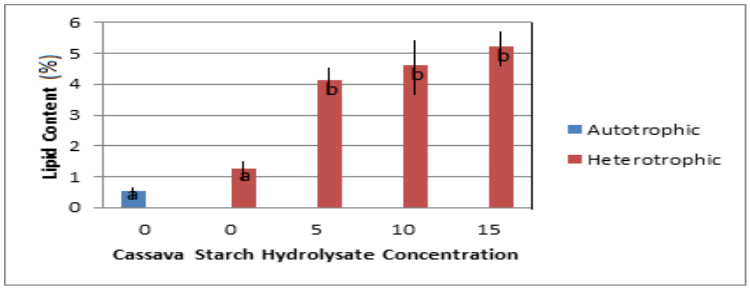

Figure 2. The effect of providing cassava starch hydrolysate concentration to lipid content of *Spirogyra* sp under heterotrophic cultivation for 6 weeks. (description: values of bars followed by different letters indicate difference at 5% level test).

In comparison to photoautotrophic cultivation, heterotrophic cultivation allows algae to accumulate much higher proportion of lipids within less time. When the photosynthetic process of algae is removed, algae gains energy from alternative organic processes that convert sugar into lipids [13]. Using cassava starch hydrolysate as an alternative source of energy was significantly less expensive than providing algae with light. Cassava starch hydrolysate was a complex carbon substrate that produces *Spirogyra* sp biomass and biochemical components of the algae such as lipids. If cassava starch hydrolysate was used as a source of carbon, cell growth rate and productivity could improve, which makes algae-derived biofuels more efficient [16]**.** The scale-up process is much easier and thus, it offers a potential pathway to produce lipids for diesel production in a large scale [17].

Production of lipids by algal species are very much importance in terms of their use in biodiesel, as lipids are the essential source of biofuel production. Many algal species contains a large amount of lipids, the production and content of lipids can be manipulated by introducing the algae with various carbon sources [18]. This supports the fact that algae produce profuse amounts of lipids when grown in stressful conditions [14]

Many species of algae can accumulate high amount of lipids under heterotrophic or environmental stress [19]. Under unfavorable environmental or stress conditions many algae alter their lipid biosynthetic pathways towards the formation and accumulation of lipids (20–50%), enabling the algae to endure these adverse conditions [20]. One who has grown algae under laboratory or outdoor condition is well aware of the fact that to obtain high lipid content, external stress or lipid induction techniques need to be applied [21].

These results are promising, because heterotrophic algae is more economical and feasible than photoautotrophic cultivation [22]. However, more economical carbon source should be employed substrate such as cassava starch hydrolysate. Lipid production of *Spirogyra* sp reached the maximum of 5.23 g/L at 15 g/L cassava starch hydrolysate were obtained. Indeed, carbon source concentration has been found to be the major impact factor for lipid accumulation by the algae [23]**.** This should be a reason for the growth increase in the heterotrophic culture.

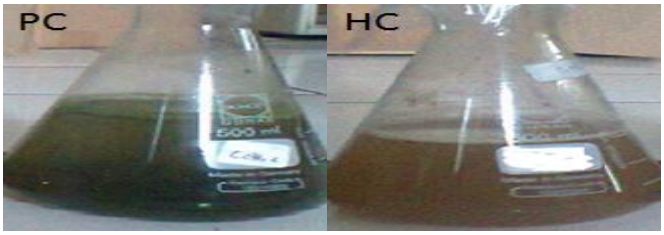

Figure 3. Photoautotropic Culture (PC) and Heterotropic Culture (HC) of *Spirogyra* sp.

According to previous reports, the utilization of an external organic carbon source may effect on the photoautotrophic growth processes in many aspects, such as photosynthesis and respiration [24]. However, with the supplement of cassava starch hydrolysate in heterotrophic cultures, the photosynthetic pigments contents decreased obviously and the cells were bleached are turned brown (Fig 3).

Without supporting CO_2 fixation under heterotrophic growth, which suggested that some aspects of CO_2 metabolism may be modulated by organic compounds. That may be the reason for the inhibition of photosynthetic pigments synthesis under heterotrophic growth. Yamane et al [27] indicated that a good production of chlorophyll and carotenoids were attained in the heterotrophic culture of *E. gracilis*, due to the highest fermenter productivity with respect to biomass as well as chlorophyll and carotenoids

The photoautotrophic mechanism in algae cells can convert atmospheric CO_2 into biomass, protein and lipid, as well as other biologically active substances; one of them is chlorophyll [25]. The main photosynthesis pigment chlorophyll are produced under photoautotrophic growth conditions. Algae have been reported to grow

on various light intensities exhibiting remarkable changes in their gross chemical composition, pigment content and photosynthetic activity [26].

IV. CONCLUSIONS

The ability of algae *Spirogyra* sp to adapt their metabolism to heterotrophic cultivation conditions provides opportunities to modify, control and thereby maximise the formation of targeted compounds. Heterotrophic cultivation of algae using organic carbon source offer several advantages over photoautotrophic cultivation including elimination of light, good control of cultivation process, high biomass and lipid content in cells. This work revealed that *Spirogyra* sp could grow on heterotrophic culture with providing CSH as carbon source under dark condition. Moreover this study has demonstrated the promise of biomass concentration and lipid content yields of *Spirogyra* sp heterotrophically grown on CSH. The results of this study suggests that *Spirogyra* sp had the potential to be an excellent biofuel producer due to the growth rate of the species could be stimulated by organic materials.

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