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# Growth Optimization of Microalgae *Chlorella vulgaris* to Increase Glucose Content for Bioethanol Feedstock.

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**ABSTRACT**

Research on biofuels production from microalgae has been done. But until now only a view amount of researches found on bioethanol production among many types of research which are a focus on biodiesel production. There are some species found with a relatively high carbohydrate content which is potential as a raw material in bioethanol production. The goal of this research is to optimize the microalga *Chlorella vulgaris* growth in order to increase the level of its carbohydrate content. Optimization includes the variation of light intensities and nitrogen sources. To obtain glucose levels, *Chlorella vulgaris* was hydrolysed by varying the initial concentration, temperature, sulphuric acid concentration and hydrolysis duration. The best *Chlorella vulgaris* growth required 2000 lux light intensity, ZA fertilizer as the nitrogen source. To hydrolyse *Chlorella vulgaris* used 2N $H_2SO_4$ at 120$^oC$ in 15 minutes producing 926.582 mg/L glucose.

**Keywords:** microalgae , *Chlorella vulgaris*, charbohydrate, glucose , bioethanol

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## INTRODUCTION

Microalgae also called phytoplankton are microscopic plants that about 3-30 µm have no roots, stems, and leaves. Microalgae have eukaryotic cells and has a different pigment, the green pigment (chlorophyll), brown (Fucoxanthin), turquoise (phycobilins), and red (phycoerythrin). Microalgae consist of vital ingredients that are beneficial, e.g., carbohydrates and fat so it can be used as a source of bioenergy. Research on biofuels from microalgae have been conducted, but mostly focused on biodiesel rather than bioethanol due to the high lipid content and rather a simple process. Many microalgae species are rich in lipid rather than the carbohydrate content, but there are also some species of microalgae which has a high carbohydrate content, such as *Chlorella*, *Dunaliella*, *Chlamydomonas,* and *Scenedesmus* are known for their particularly high the carbohydrate content of over 50% of the dry cell weight under specific culture conditions [1,2].

*Chlorella vulgaris* has great potential as a renewable energy source because of its availability easily obtained through culture, not compete with food crops, has a quick adaptation into the new environment and culture, fast growing, and fast in the harvesting. The *C.vulgaris* containing lignocellulosic materials such as cellulose, hemicellulose, and lignin in which the cellulose can be converted into glucose and used as an alternative substrate.

Cellulose is naturally bound by the hemicellulose and lignin are protected by the presence of lignin binder compound that causes lignocellulosic materials difficult to be hydrolyzed. The pretreatment aims to break down and reduce the amount of lignin and hemicellulose, damaging the crystal structure of cellulose and increase the porosity. The destruction of the cellulose crystal structure will facilitate the breakdown of cellulose into glucose. Furthermore, the simple sugars that will be fermented into ethanol by ethanol-producing microorganisms. However, the main challenge in the production of bioethanol from microalgae biomass is how to break down complex sugars from microalgae into simple sugars efficiently. Carbohydrates in green algae generally contain starch in the chloroplast and cellulose / polysaccharides in the cell wall. For that, it needs to be hydrolyzed to convert it into monomeric sugars before fermentation using microorganisms. There are two methods to hydrolyze Cellulose into glucose such as enzymatically by cellulolytic enzymes or chemically by sulfuric or other acids and by sodium hydroxide or other bases [3].

In this experiment, *C. vulgaris* was chosen because it implies a high carbohydrate reaches 50% of the dry weight and the relatively rapid growth compared with terrestrial plants. The previous growth of microalgae optimized use of nitrogen and light sources. In this research, optimization of hydrolysis using chemical methods with sulfuric acid due to hydrolyze cellulose used is usually sulfuric acid. To measure the levels of glucose in the sample used Nelson-Somogyi method as this method is cheaper, faster and accurately.

## MATERIAL AND METHODS

### Collect and cultivation conditions of microalgae

The *Chlorella vulgaris* microalgae were obtained from the collection of Central Brackish Water Development (BBPBAP) Jepara, Indonesia. Pure cells of the algae species were cultured in Bold Basal Medium (BBM). The medium composition is as follows: macronutrient ($NaNO_3$, $CaCl_2.2H_2O$, $MgSO4.7H_2O$, $K_2HPO_4$, $KH_2PO_4$, NaCl) and micronutrient (EDTA, trace element, $H_3BO_3$). To prevent contamination BBM was autoclaved and cooled to room temperature before use. The microalgae were cultured by bubbling air at room temperature and kept under constant illumination of 2000 lux with cool-white fluorescent tubes and with a 12-12 h light–dark cycle for 21 days. For optimization of microalgae growth medium modified by replacing the nitrogen source with urea (8.823 g / L) and ZA (19.411 g / L). Microalgae were harvested at exponential phase by centrifugation for 10 min at 2000 rpm at room temperature. Furthermore, the biomass was dried to obtain dry biomass.

### Determination of optimum hydrolysis condition

The hydrolysis optimum conditions were determined by varying concentrations of sulfuric acid, temperatures, and hydrolysis times. The effect of variation concentrations of sulfuric was obtained using nine concentration (5; 4; 3; 2; 1; 0,8; 0,6; 0,4; and 0,2 N). Each Concentration was added into nine Erlenmeyer containing 0.1 g of dry biomass, then incubated for 15 min at 121°C. After it is taken 1 mL, and added 1 mL

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Nelson-Somogyi reagent and placed into boiling water. After cooling to room temperature, the mixture is placed added 1 mL phosphomolybdate reagent, and 7 mL of distilled water and measured at the wavelength of 540 nm [4]. Influence of temperature on the glucose levels was determined by hydrolysis the mixture (concentrations of sulfuric acid optimum) at different temperatures (90, 100, 110, 120, 130, and 140°C). The influence of hydrolysis time on the glucose content of dry biomass by incubating the mixture (at concentrations of sulfuric acid and temperature optimum) for 5 to 20 minutes.

## RESULTS AND DISCUSSION

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### Effect of Light Source

The influence of different illumination on the growth of microalgae (Fig.1). The result showed that the growth of microalgae using cool white light faster than the growth using a solar source. It is observed from the absorbance (OD) on microalgae growth curve. High absorbance value indicates a number of cells in a growth medium and conversely, a low absorbance value indicates a bit number of cells in growth media [5]. In Fig. 1 the growth absorbance value of microalgae using cool white light is higher than using the sunlight source. This is due to the light intensity of solar is not constant while using the light intensity of cool white light is constant.

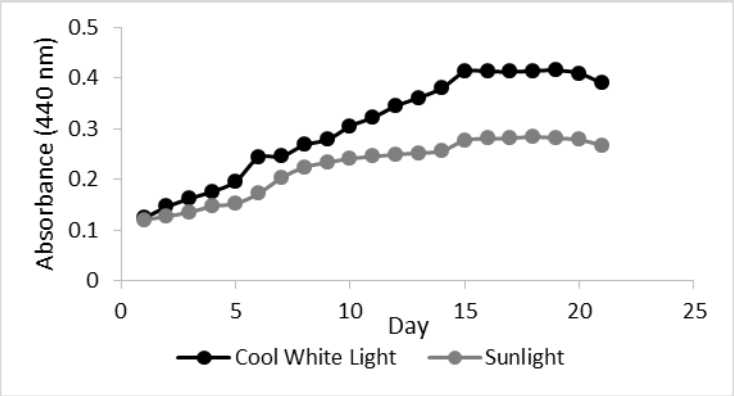


**Fig. 1 Growth curves of *Chlorella vulgaris* under different illumination.**

Moreover, the influence of the light source also affects the dried biomass and glucose contained in *Chlorella vulgaris*. The use of cool white light (2000 lux) produced the dry biomass is higher than using solar light (Table 1). This is due to the use of light with the intensity of 2000 lux as light sources on the growth of microalgae is more stable than using solar light. Conversely, glucose levels are higher using sunlight than using cool white light for their photosynthesis of green microalgae *Chlorella vulgaris*. As we know, that photosynthesis is part of the metabolism, excessive light can inhibit photosynthesis. Besides, a dark phase is also necessary for the regeneration of cofactors ($NAD^+$, $NADP^+$) required for phase I of photosynthesis [6,7].

**Table 1. Influence of light sources toward dry biomass (g/L) and carbohydrate content (mg/L) of *C. vulgaris* microalgae after 21 days of cultivation in BBM medium**

| Light sources | Dry Biomass (g/L) | Glucose Content (mg/L) |
|---|---|---|
| cool white | 0,222 | 876 |
| sunlight | 0,159 | 885 |

### Effect of nitrogen sources

Nitrogen is the most important nutrient for the microalgae growth. Nitrogen is needed in the synthesis of proteins, nucleic acids, cell growth and in other microalgae metabolism processes. However, different sources of nitrogen will affect the metabolic processes of their respective metabolites [8]. To prove the effects of nitrogen sources on microalgae growth, three different nitrogen sources (urea, ZA, and $NaNO_3$) were investigated in microalgae *C. vulgaris*.

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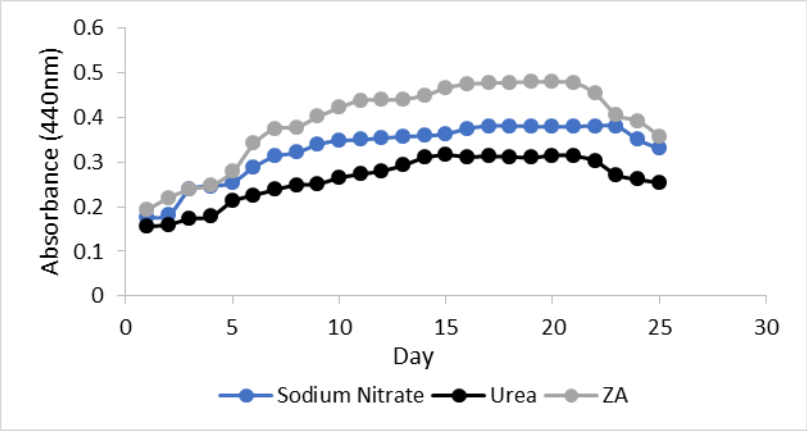


**Figure 2. Influence of nitrogen source on Growth curve of *C.vulgaris* microalgae**

As seen in fig. 2, the media contained ZA as nitrogen source has shown higher Optical Density than urea and $NaNO_3$. In addition to view microalgae growth through microalgae uptake at UV-Vis spectrophotometers around 440 nm, the effects of nitrogen sources on dry biomass and glucose levels in microalgae were also conducted (Table 2).

**Table 2. Influence of nitrogen source on dry biomass and carbohydrate microalgae of *C. vulgaris*.**

| Nitrogen Sources | Dry Biomass (g/L) | Glucose Content (mg/L) |
|---|---|---|
| $NaNO_3$ | 0,176 | 875 |
| Urea | 0,250 | 865 |
| ZA | 0,326 | 890 |

In Table 2 and Figure 2 it can be seen that the use of ZA as a nitrogen source has biomass and glucose levels as well as higher growth compared to other tested nitrogen sources. This is because the levels of N and S in ZA fertilizers are higher than that of Urea or $NaNO_3$ fertilizers that cause higher growth, biomass and glucose levels wherein the nitrogen content in microalgae serves as protein synthesis for growth and development of microalgae [9].

**Carbohydrates Hydrolysis**

For the hydrolysis of the carbohydrate-enriched biomass, $H_2SO_4$ were used with different concentrations (5; 4; 3; 2; 1 ; 0,8; 0,6; 0,4; and 0,2 N). The influence of sulfuric acid concentration on the glucose content of *C. vulgaris* is shown in Figure 3.

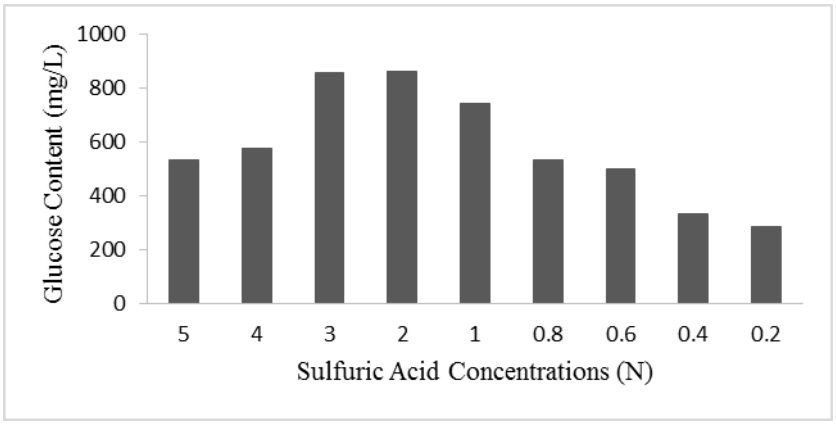


**Figure 3. Influence of sulfuric acid concentrations on the glucose content ( mg/L)**

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The result showed that the glucose content started increased up to 865 mg/L but dropped gradually of about concentration 2 N. In addition In Figure 3. It is apparent that the concentration of 3 N and 2 N sulfuric acid gives better results than others concentration of sulfuric acid. At over 3 N the sample is degraded into another product such as acids, formic acid, and hydroxymethylfulfural (HMF) [10]. However, In case of the environmental and the operating costs in hydrolysis process, the concentration of sulfuric acid at 2 N is more effective than the concentration of sulfuric acid at 3 N. For the optimization of hydrolysis with temperature variations carried 6 temperatures (90, 100, 10, 120, 130 and 140) °C.

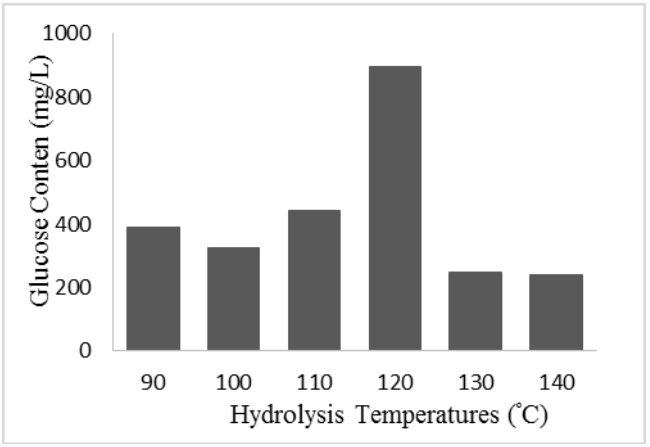


**Figure 4. Influence of hydrolysis temperatures on glucose content ( mg/L)**

Figure 4 shows the increasing hydrolysis temperature will also increasing the glucose level. This hydrolysis process is an endothermic reaction that requires heat for the reaction but over high temperature, the catalyst will evaporate which is affected by the slowing of the hydrolyzed reaction and will affect the concentration of glucose produced. The optimum temperature of hydrolysis for *C. vulgaris* is 120ºC.

Hydrolysis time using acid also has an effect on hydrolysis process. Glucose content in the dry biomass was determined at different hydrolysis time varied from 5-20 min (Figure 5). The optimum incubation time was 15 minutes. Subsequently, the glucose content was decreased significantly at longer hydrolysis time. The longer the hydrolysis time, caused the concentration of glucose concentration will also increase, as it will lengthen the acid chance to degrade the straight and long chain bonds of 1,4-β-glucose in cellulose. However, in Figure 5 occurs decreasing concentrations in the 20 minutes, this is probably because the $H^+$ ions on the acid have reached their optimum point releases the glycosidic chain linkage on cellulose [11].

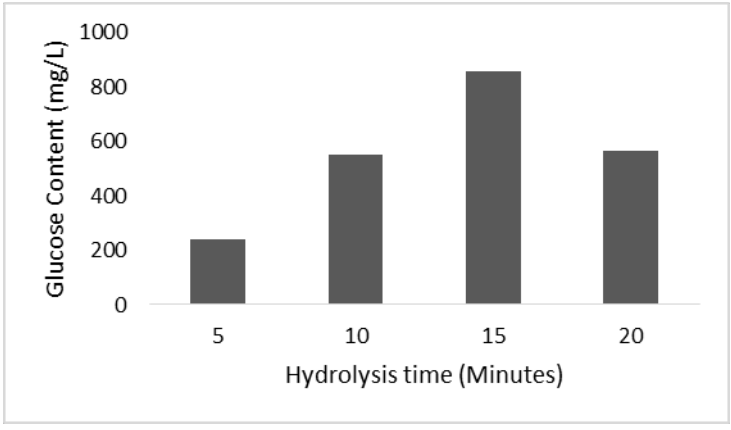


**Figure 5. Influence of hydrolysis time on the glucose content ( mg/L)**

After obtaining optimum growth using ZA as a nitrogen source and using 2000 lux light biomass dry light microalgae was hydrolyzed by using 2 N sulfuric acid for 15 minutes at 120ºC. Then the measurement of glucose level using Nelson-Somogyi method was obtained glucose level 926,582 mg / L at absorbance 0,375.

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## CONCLUSION

The growth of *Chlorella vulgaris* microalgae with ZA as nitrogen source gives better results compared to using nitrogen source urea or $NaNO_3$. The growth of *C. vulgaris* microalgae with fluorescent light source 2000 lux gives a better result than the solar light source. The optimum hydrolysis condition was found at sulfuric acid concentration 2N at 120°C for 15 minutes with glucose level 926,582 mg / L.

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