Different Concentration Organic Carbon Of Waste Cassava In Mixotrophic Cultivation On Growth Of Dunaliella Sp.

Prive Widya Antika, Happy Nursyam, Arning Wilujeng Ekawati

Abstract: Dunaliella sp. is one type of green microalgae that can be used as natural food because it is easy to digest. In mixotrophic culture, microalgae in their growth carry out the process of photosynthesis and use organic carbon nutrients to produce energy. Choice of organic carbon sources for growing Dunaliella sp. using waste cassava. The organic carbon content in waste cassava is still very complex, so that Dunaliella sp. for its growth, it can be done by breaking down carbohydrates into simple glucose using hydrolysis with the addition of a-amylase and glucoamylase enzymes. This research has 2 stages, stage 1 is the hydrolysis of waste cassava using a-amylase and glucoamylase enzymes. Phase 2 research on microalgae Dunaliella sp. mixotrophic cultured using organic carbon as a source of glucose from the hydrolysis of waste cassava. The results showed that the administration of the enzyme a-amylase 3.15 µl/g and glucoamylase 2.10 µl/g was able to break down carbohydrates into glucose in waste cassava by producing a glucose level of 34,285 mg/L. The administration of glucose organic carbon from different waste cassava under mixotrophic cultured significantly affected the growth of Dunaliella sp. The best glucose concentration was 0.30 g/l with the highest average density of 6.5 x 10^6 cells/ml, the specific growth rate was 1.04 $days^{-1}$ with a doubling time of 0.66 days.

Index Terms: Microalgae, System Culture, Glucose, Enzyme.

1 INTRODUCTION

Microalgae are photosynthetic organisms that have chlorophyll pigments so that they can utilize sunlight and carbon dioxide to produce glucose and oxygen [1]. The potential of phytoplankton in the aquatic environment is very large because almost all aquatic organisms utilize plankton as food [2]. Dunaliella sp. is a type of green microalgae that has the characteristics of oval, cylindrical, and elliptical cells [3]. Dunaliella sp. can be used as natural feed because it is easy to digest [4]. The nutritional content of Dunaliella sp. namely protein (57%), carbohydrates (32%), and fat (6%) [5].

Environmental factors can influence the growth of Dunaliella sp. including nutrients in culture media and water quality such as optimum salinity, pH, temperature, and light intensity [6]. Microalgae can change the nutritional content due to environmental influences so that they can be grouped into three forms, namely autotrophic, heterotrophic and mixotrophic. In mixotrophic cultured, microalgae in their growth carry out the process of photosynthesis and use organic carbon nutrients to produce energy [7]. Microalgae culture in mixotrophic conditions with the addition of organic carbon can increase biomass, so that it becomes an alternative to conventional photoautotrophic cultured [8]. In mixotrophic conditions, microalgae can grow denser. The required light intensity is lower than that of autotrophic culture [9]. The use of organic carbon sources in microalgae culture must meet the criteria, cheap, easy to sterilize, and able to increase microalgae growth [10]. It is recommended that organic carbon sources use inexpensive materials to reduce the cost of microalgae culture under mixotrophic conditions [11]. The choice of organic carbon source to grow Dunaliella sp. using waste cassava. Waste cassava is a by-product of processing cassava into tapioca [12]. Waste cassava contains 72.49–85.99% carbohydrates, 1.57% protein, 0.26% fat, and 20% crude fiber [13]. The organic carbon content in waste cassava is still very complex so that Dunaliella sp. For growth, carbohydrates can be broken down into glucose. Waste cassava which still contains fiber and starch can be hydrolyzed by the addition of enzymes. According to [14], waste cassava can be hydrolyzed by the a-amylase enzyme and the glucoamylase enzyme. Glucose is a complex carbon substrate that can produce biomass and biochemical components in microalgae [15]. Information on providing carbon sources from waste cassava to growth of microalgae Dunaliella sp. is still very limited. Therefore, research is needed on the application of organic carbon from waste cassava under mixotrophic conditions to the growth of Dunaliella sp.

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2 MATERIAL AND METHOD

2.1 Waste Cassava Hydrolysis Method

Grind the dried waste cassava, then 20 grams of waste cassava is put into an erlenmeyer and 175 ml of distilled water is added. Then the a-amylase enzyme was added (1.15; 3.15; 5.15) µl/g dry weight of the substrate at pH 5.5 and incubated at 100°C for 2 hours using a water bath. After preparation, the glucoamylase enzyme was added (1.10; 2.10; 3.10) µl/g dry weight of the hemp with a substrate pH of 4.5 and incubated in an incubator shaker at 60°C for 24 hours at 200 rpm. The hydrolyzate formed is filtered to separate solids and liquids and the liquid portion is analyzed for reducing sugars [14].

2.2 Microalgae Cultures and Medium

Dunaliella sp. was obtained from the Institute of Brackishwater Aquaculture, Situbondo, Indonesia. Walne medium was used to culture the cells. 1 mL of medium was supplemented with 1,000 mL of sterilized seawater. In terms of mixotrophic culture, different glucose of waste cassava concentrations (0, 0.15, 0.30, and 0.45 g/L) were added to the culture. For all treatments, algae cultures were aerated by the air pump with an airflow rate of 1 L/min and were incubated at the temperature of 28°C under constant illumination with a light

intensity of 3,000 lux. The inoculum was cultivated into 1 L bottles (working volume of 800 ml) at a salinity of 25 ppt and a pH of 7.5. Initial cell concentration was adjusted at 1 x 10^5 cells/mL. All treatments were carried out in triplicate.

2.3 Growth Analysis

Calculation of the density of Dunaliella sp. was carried out every day from the beginning of culture to the end of the experiment. Calculation of Dunaliella sp. using the method of calculating the concentration of cells using a 0.1 mm hemocytometer and using a microscope. According to [16], the density formula for Dunaliella sp. can be calculated using the following formula:

Cell density (sel/ml) = (n/counting chamber of hemocytometer) x 25 x 10^4 (1)

2.4 Specific Growth Rate Analysis

The calculation of the specific rate was carried out from the growth at the beginning of the culture to the peak of maximum concentration. The specific growth rate is calculated using the formula [17].

$\mu = (\ln Nt - \ln N0)/ t$ (2)

2.5 Doubling Time Analysis

Doubling time (td) or generation time (G) is the doubling time of Dunaliella sp. Cell doubling time (dt) is the average generation time of the concentration of cells dividing. Doubling Time (days) is calculated from the growth rate based on the formula [18].

$G = \text{Ln } 2/ \mu$ (3)

2.6 Statistic Analysis

Data analysis was calculated from each treatment, statistically tested using Microsoft Excel using analysis of variance (ANOVA) according to the design used, namely the Completely Randomized Factorial Design (RALF) for the hydrolysis of waste cassava using enzymes a-amylase and glucoamylase and the design Completely Randomized (CRD) for the treatment of Dunaliella sp. in a mixotrophic manner. At the 95% confidence level ($\alpha = 0.05$).

3 RESULT AND DISCUSSION

3.1 Hydrolysis of Waste Cassava Using a-amylase and Glucoamylase Enzymes

The results showed that the interaction of a-amylase and glucoamylase enzymes could increase glucose levels in waste cassava as shown in Table 1.

TABLE 1.
CONCENTRATION ENZYME A-AMILASE AND GLUCOAMYLASE FOR HYDROLYSIS WASTE CASSAVA TO GLUCOSE

Concentration a-amilase (µl/g)	Concentration glukoamilase (µl/g)	Glucose Content (mg/L)
A (1,15)	a (1,10)	13.756
	b (2,10)	25.073
	c (3,10)	19.089
B (3,15)	a (1,10)	29.968
	b (2,10)	34.285
	c (3,10)	28.228
C (5,15)	a (1,10)	23.892
	b (2,10)	27.859
	c (3,10)	23.785

The highest glucose concentration was produced when hydrolyzed using the enzyme a-amylase 3.15 µl/g and glucoamylase 2.10 µl/g with a glucose level of 34,285 mg/L while the lowest glucose concentration was produced in the a-amylase enzyme treatment 1.15 µl/g and glucoamylase 1.10 µl/g with the results of glucose levels 13,756 mg/L. The increase in glucose levels is directly proportional to the increase in enzyme concentration because the higher the enzyme concentration, the more substrate that binds to the active site of the enzyme so that the number of products produced will be more. The action of a-amylase enzyme is an endoenzyme that hydrolyzes a-1,4-glucoside bonds specifically along the starch chain (amylose and amylopectin) [19]. The action of the glucoamylase enzyme is exoamylose, which can hydrolyze starch chains into glucose molecules in the non-reducing part of the molecule, both a-1,4 and a-1,6 bonds can be hydrolyzed [20]. The addition of the enzyme concentration will increase the reaction rate. However, the increase in reaction rate will decrease for each increase in enzyme concentration. The increase in glucose levels will reach the limit point, after that point is exceeded there will be no change in higher glucose levels even though the enzyme concentration is added. This happens because the active site of the enzyme has been saturated by the substrate so that no more substrate can be attached to the active site [21]. This limit is referred to as the maximum speed, which is the speed at which the enzyme is saturated with the substrate. When the maximum speed is reached, all enzymes are present in the enzyme-substrate complex [22].

3.2 Growth of Microalgae Dunaliella sp.

The growth of microalgae Dunaliella sp. can be visually marked by a change in the color of the culture medium due to differences in cell concentrations. Data from Dunaliella sp. growth observations are shown in Figure 1. Growth of Dunaliella sp. increased according to the number of days during culture with an initial stocking concentration of 1 × 10^5 cells/ml. In the adaptation phase, there are enough nutrients available so that microalgae growth is fast and during this phase, metabolism is used to increase cell size [23]. During the study, Dunaliella sp. does not show an adaptation phase. This happened because the adaptation phase lasted less than 24 hours so it was not observed at the time of observation. Dunaliella sp. cell count increased after being stocked on media that had been given treatment. One of the factors that determine the length of the adaptation phase is the age of the culture used as the inoculant. The adaptation phase will be shorter or even invisible if the inoculated cells come from cultures that are in the exponential phase. The adaptation

phase is not visible on the treatment media, because the inoculated cells quickly adapt to new culture media and can grow and divide rapidly [24].

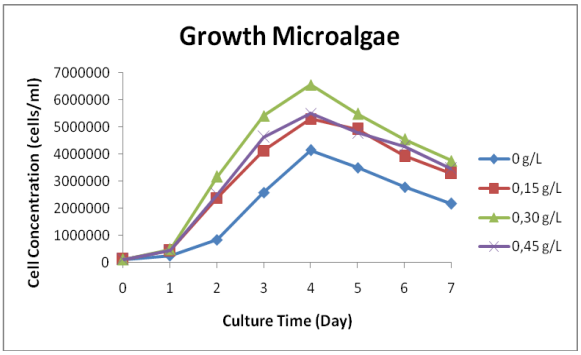

Fig. 1. *Cell growth of Dunaliella sp. under mixotrophic cultivation*

The exponential phase in this study occurred from day 1 to day 4 where every day the number of Dunaliella sp. increased. The fastest doubling time occurs during the exponential phase, which is the growth phase when cells divide rapidly. In this phase, cell growth and activity are at their maximum [25]. The exponential phase occurs when nutrients, light intensity, and media water quality conditions can still meet the needs of microalgae so that cells can reproduce. After experiencing an exponential phase, Dunaliella sp. will enter the stationary phase. The stationary phase is characterized by growth starting to decrease compared to the exponential phase. In this phase, the reproduction rate is the same as the death rate so that the density of microalgae tends to remain constant [4]. In this study, Dunaliella sp. did not appear to have a stationary phase because on the 4th day an exponential phase occurred, and on the 5th to 7th day, the density of Dunaliella sp. has decreased which indicates the culture has entered the death phase. The death phase is characterized by conditions of decreased water quality and reduced nutrient content which is unable to support cell growth, so that cell density decreases rapidly because the death rate of microalgae is higher than its growth rate [25]. Dunaliella sp. cell density. reached the highest peak on day 4 in each treatment. The highest average maximum cell density was found in the administration of glucose with a dose of 0.30 g/l (treatment C) of 6.55×10^6 cells/ml and the lowest was in the administration of glucose with a dose of 0 g/l (treatment A) of $4{,}14 \times 10^6$ cells/ml. The high cell density in mixotrophic culture indicates that cell growth is not completely dependent on photosynthetic reactions. Light energy is not an absolute limiting factor for microalgae growth, but an organic carbon source in the form of glucose can support microalgae growth [15].

3.2 Specific Growth Rate and Doubling Time of Dunaliella sp.

The growth of microalgae Dunaliella sp. which were cultured mixotrophic, the specific growth rate, and doubling time can be calculated in Table 2.

TABLE 2.
SPECIFIC GROWTH RATE AND DOUBLING TIME OF Dunaliella sp.

Glucose concentration of hydroliysis waste cassava (g/L)	Specific Growth Rate (day^{-1})	Doubling Time (day)
A (0,00)	0,931± 0,003	0,745 ± 0,002
B (0,15)	0,993 ± 0,004	0,698 ± 0,003
C (0,30)	1,046 ± 0,001	0,663 ± 0,001
D (0,45)	1,002 ± 0,004	0,692 ± 0,003

The growth of Dunaliella sp. which has a high value will increase the specific growth rate, in determining the best growth it is necessary to compare the specific growth rate and cell multiplication time. The specific growth rate describes the speed of microalgae cell growth per unit time which can be used as a benchmark to determine the carrying capacity of the medium or nutrients for the growth and division of microalgae cells [26]. The specific growth rate of Dunaliella sp. with the highest density of 1.046 per day with a glucose dose of 0.30 g/l (treatment C) with a doubling time of 0.663.days. The specific growth rate of Dunaliella sp. with the lowest density of 0.931 per day with a glucose dose of 0 g/l (treatment A) with a doubling time of 0.745 per day. Organic carbon sources such as glucose can increase the specific growth rate of microalgae [27]. In photoautotrophic culture the specific growth rate of Chlorella sorokiniana is 0.63 days and when cultured mixotrophic with an organic carbon source, namely glucose 4 g/l, the specific growth rate is 3.40 per day [28]. In this study, the specific growth rate of Dunaliella sp. The best results were obtained in the administration of the highest glucose dose of 0.30 g/L with a yield of 1.046 per day. These results indicate that the glucose dose given has an optimum point, if the glucose dose is reduced and increased then the density of Dunaliella sp. will experience a decline. Under optimum conditions, the rate of enzymatic reactions contained in the process of photosynthesis and cellular respiration will work optimally, so that an increased cell density is obtained. The rate of enzymatic reactions will increase with increasing enzyme concentration, but the reaction rate can reach a constant if the amount of substrate, namely glucose, continues to increase until it exceeds the limit of the enzyme's ability [29].

4 Conclusion

Glucose supplementation from the hydrolysis of waste cassava under mixotrophic culture significant effect on growth, specific growth rate, and doubling time Dunaliella sp. Mixotrophic cultivation of Dunaliella sp. causes higher growth than photoautotrophic cultured.

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