

Biosugar Production from *Kappaphycus alvarezii* by Hydrolysis Method using Fungi *Trichoderma harzianum*

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Abstract— *Kappaphycus alvarezii* is one of the natural resources containing high carbohydrate, and it has potential as an alternative raw material for sugar industry. This study aims to analyze the optimum conditions of the fungi *Trichoderma harzianum* in degradating polysaccharides from *K. alvarezii* into sugar. The seaweed was collected in Takalar Sea, South Sulawesi, Indonesia. The fungi was previously activated at 30°C for three days in 2% of seaweed substrate. The efficiency of the *T. harzianum* in producing biosugar was evaluated under various concentrations of inoculum (5%, 10%, 15% and 20%) and hydrolysis time (0 h, 24 h, 48 h, and 72 h). The result implied that *K. alvarezii* contain 55,58% of carbohydrate which was dominated by starch 35.83% and cellulose 12.21%. Furthermore, the highest content of sugar indicated by 12,27 g/L of reducing sugar was obtained at the combination of 20% of the fungi and 24h incubation. In conclusion, the fungi *T. harzianum* has ability to convert carbohydrate of *K.alvarezii* to sugar through hydrolysis process.

Keywords— seaweed; *K. alvarezii*; biosugar; *T. harzianum*; hydrolysis.

I. INTRODUCTION

Sugar is one of the basic human needs consumed by all levels of society both for household and industrial needs. Indonesian sugar needs in 2018 reached 6.6 million tons while the production of sugar based sugarcane only 2.17 million tons (Ministry of Industry, 2018). This lead to a significant increase in sugar import. Nowadays, based on data from Statistical Central Agency in 2018, Indonesia became the largest sugar importer in the world, reaching 4.6 million tons (Katadata.co.id, 2018).

Sugarcane as the main raw material of sugar industry continues declining due to limited availability of cultivation land (IDN TIMES, 2019). Various raw alternatives of the sugar industry have been reported for instances cassava, corn, and sago containing high level of carbohydrates (Rahmawati *et al.*, 2017; Mahyati *et al.*, 2017). However, the availability of raw materials is limited because of its main function as staple foods. Therefore, it is necessary to search for other alternatives as raw materials which are rich carbohydrate based natural resources in abundant availability e.g. seaweeds.

Indonesia is the largest producer of seaweeds with a total production of 9,9 million tons in 2019 (kcp.go.id, 2020). This country has contributed almost 40% of the total world seaweed production (FAO, 2018). In general, seaweeds are widely used in food, engineering, and medicinal industries (S. W. Kim *et al.*, 2015; Parenrengi & Sulaeman, 2007). South Sulawesi is one of the seaweed producing center, especially the red seaweeds *Kappaphycus alvarezii* and *Gracilaria sp* with a total production of 2,4 million tons in 2013 and increasing to 3,6 million tons in 2017 (Department of Marine and Fisheries South Sulawesi, 2018). The abundance of seaweed *K. alvarezii* (65% of the total production) becomes a potential alternative as raw material for sugar industry.

The constituent wall of seaweed cells consisted of agar, caragenan, cellulose, manan, and xylen can be converted into monosaccharides through various methods such as acid hydrolysis, enzymatic hydrolysis, hydrothermal, and fermentation (Meinita *et al.*, 2015; Parenrengi & Sulaeman, 2007). Several related studies have been reported, the hydrolysis of carbohydrates using

sulfuric acid besides yielding high sugar it also has side toxic compounds (Carvalho *et al.*, 2013). Decomposition of carbohydrates with hydrothermal methods showed less results due to high temperature causing damage of carbohydrate structure (D. H. Kim *et al.*, 2014). The enzymatic hydrolysis produced high oligosaccharide, environmental friendly, low side effects of toxic compounds but took relatively long time with high cost (Vanegas. 2015).

Acceleration of hydrolysis of seaweed carbohydrate using microbe which has enzyme activity is an effective alternative method. The potential microbe used in degradation of carbohydrates through the hydrolysis method is the fungi *Trichoderma harzianum* because its cellulosic properties (Lee *et al.*, 2017; Jamil *et al.*, 2009). The enzyme produced by *T. harzianum* is about 25% more efficient than *Trichoderma reesei* (Souza *et al.*, 2018). In the present study, we investigated biosugar production from the seaweed *K. alvarezii* using the fungi *Trichoderma harzianum*.

II. MATERIALS AND METHODS

2.1 Seaweed Materials

The sample *K. alvarezii* was collected from the Brackish Aquaculture Fisheries Hall, Takalar. It was washed with freshwater to remove attached mud and salts, then dried for two days. The dried seaweed was grinded using the hummer mill to obtain powder with size of 40 mesh.

2.2 Culture of *Trichoderma Harzianum*

The fungi *T. harzianum* was subcultured in a petri dish containing a PDA (Potato Dextrose Agar) medium and incubated at 30°C for 48 h. The fungi culture was stored in a refrigerator before its further use.

2.3 Activation process of *Trichoderma Harzianum*

The Fungi *T. harzianum* was activated in three stages. (1) A small amount of sub cultured fungi was picked up and inoculated into 5 mL of seaweed media then shake incubated at 30°C for 24 h. (2) 1 mL of the inoculum was diluted in 9 mL of seaweed media then incubated at the same condition of the previous stage. (3) 5 mL of the inoculum was activated in 45 mL of the seaweed media.

2.4 Hydrolysis Process

The hydrolysis process use seaweed that has been through the pretreatment process. The seaweed flour is dissolved in aquades and the heating process for 120 min with a warming temperature of $\pm 100^{\circ}\text{C}$ and then cooled to a temperature of $\pm 40^{\circ}\text{C}$. Then autoclave about ± 90 min. Selected parameters for optimization in experimental

hydrolysis using inoculum *T. harzianum* with varying concentrations (5%, 10%, 15% and 20%) and the duration hydrolysis (0 h, 24 h, 48 h and 72 h). After the reaction, the supernatant were separated from liquid by centrifugation at a speed of 9.000 RPM for 10 min. All preparation were performed at the Marine Microbiology Laboratory, Faculty of Marine Science and Fisheries, Hasanuddin University. The supernatant was collected to measured from the sugar level by the phenol sulfate acid.

2.5 Data Analysis

Data was analyzed by randomized design completed with factorial patterns. Total sugar was analyzed by two ways ANOVA to determine the influence of inoculum concentration and duration of hydrolysis on sugar production. If there was any influence, then proceed with the Tuckey test at a confidence level of 95% ($\alpha = 0.05$) to identify the optimum combination indicated by the highest amount of sugar.

III. RESULTS AND DISCUSSION

3.1 Analysis of carbohydrate content of seaweed *K. alvarezii*

Carbohydrate content of the seaweed *K. alvarezii* was evaluated and obtained that it was about 55,58% dominated by starch and cellulose which were 35,83% and 12,21%, respectively. Carbohydrates are also called polysaccharides which are combination of monosaccharides linked by the glycoside bonding, for instance polysaccharides are cellulose, glycogen, and starch.

Starch and cellulose are the main carbohydrates of *K. alvarezii*, other components are hemicellulose, pectin and lignin. Starch resulted in photosynthesis pathway of plants is deposited as energy for growing. It consists of amylose and amylopectin in different composition.

The seaweed *K. alvarezii* possesses starch higher than other seaweeds. This is supported by previous studies that *Caulerpa lentillifera* and *Ulva Ohnoi* contained starch of 29.82% and 21.4% (Tapotubun, 2018; Prabhu *et al.*, 2019).

On the other hand, cellulose is the main polysaccharides of seaweed. Cellulose is one of the most abundant biopolymers in nature and the major component of plant cell wall (Börjesson & Westman, 2015). It associates with other polysaccharides such as hemiselulose or lignin (Holtzaple *et al.*, 2003). Thallus of seaweed also has cell wall consisting of cellulose (Linder & Teeri, 1996). This study showed that *K. alvarezii* contained cellulose of 12.21%. It is supported by previous research on the red algae *Gracilaria* reported by Sari *et al.* (2013)

showing cellulose content of 20,17%. In addition, Milala *et al.* (2005) described cellulose content in plants reaching 40-50% of plant mass. High polysaccharides on the seaweed *K. alvarezii* can be converted into mono sugar by hydrolysis method.

3.2 Analysis of the effect of inoculum concentration on total sugar

Pretreated seaweed was hydrolyzed using activated inoculum *T. harzianum* with various concentrations of 5%, 10%, 15% and 20%, and hydrolysis time 0 h, 24 h, 48 h and 72 h. Samples were analyzed to determine the total sugar produced. Based on Two Way ANOVA and Tuckey test showed that the total sugar significant difference from each treatment ($p < 0,05$). Figure 1. shows total sugar produced by every single treatment combination.

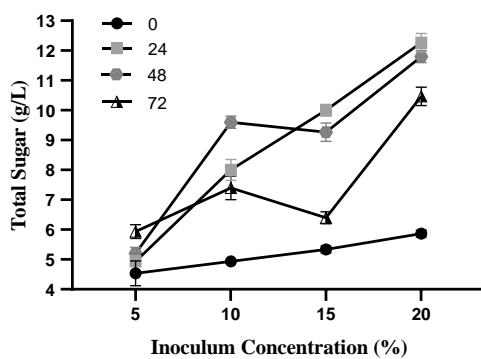


Fig.1: Total sugar from various inoculum concentration.

The result showed that generally, conversion of polysaccharide to total sugar increases due to increased inoculum concentration and hydrolysis time. The tendency is shown by the treatment of hydrolysis time 48 and 72 which increased to 10% inoculum concentration then decreased at a concentration of 15% and increased at the next concentration. Hydrolysis for 24 h showed an increase in sugar production with increasing inoculum concentration. The interesting from the results of this study is the control at the 0 h also shows the increase in total sugar with increasing inoculum concentration. Total sugar detected is sugar produced during the activation process of *T. harzianum*. Thus, the calculation of the total sugar produced must be corrected with the sugar content at the 0 h for all concentrations. The addition of 20% inoculum resulted in relatively equal total sugar after hydrolysis for 24 and 48 hours.

The hydrolysis process of carbohydrates into total sugars is carried out using the fungi *T.harzianum* with several parameters of concentration and duration of hydrolysis. Hydrolysis is a chemical reaction that splits a

molecule into two parts by adding water molecules with the aim of converting polysaccharides into monosakarida. Cellulose is the main element of seaweed cell walls that can be degraded into monosugar by the hydrolysis process. Under normal conditions, only a few reactions occur during the hydrolysis process, therefore it is necessary to have a catalyst that can accelerate reactions such as the addition of acids, alkali or enzymes. Enzymes can work 10^8 to 10^{11} times faster than the rate of reaction without a catalyst (Poedjadi, 2006).

T.harzianum produces cellulase enzymes that can degrade cellulosic substrates into sugar (Jamil *et al.*, 2009). The use of microbes in hydrolysis provides the advantage of a fast process and does not produce toxic compounds. Souza *et al.*, 2018 stated that β -glucosidase produced by *T. harzianum* strains was higher than *T. reesei* by using lactose as a carbon source.

The results indicated that the inoculum concentration and duration of hydrolysis have the significant effect on reducing sugars and there were significant interactions between the two treatments. The highest reducing sugar was 12,2g/L obtained from an inoculum concentration of 20% and a hydrolysis duration of 24 h. Moreover, the lowest reducing sugar is at 5% inoculum concentration treatment and 0 h hydrolysis time. Figure 1. shows the tendency to total sugar levels increases with increasing inoculum concentration. This research indicated that the activity of *T. harzianum* in hydrolyzing cellulose to sugar increases with increasing inoculum concentration. However, high cellulose content in seaweed is a source of carbon needed to obtain energy for the growth of *T. harzianum* in producing cellulase enzymes and degrading cellulose into monosugars. Ul-Haq *et al.*, 2005 stated *Trichoderma sp.* is a producer of cellulases and crude enzymes. *T. harzianum* is a potential fungus in producing cellulase enzymes that can hydrolyze chain β -glucosidase (cellobiase) that break down cellobiose to produce glucose (Wang *et al.*, 2020). The fungi also produces higher levels of cellulolytic activity and β -glucosidase activity compared to *T. reesei* (Souza *et al.*, 2018).

The carbohydrates contained in *K. Alvarezii* are applicative media for the *Trichoderma* growth. High cellulose and starch elements in the substance can be potential source of nutrients for fungal growth. The 20 percent concentration of inoculum seaweed into the media can produce high cellulase enzymes for the degradation of carbohydrates into monosaccharides. Brijwani *et al.* (2010) stated that *T. Harzianum* has ability to produce enzymes by the growth medium consisting of nitrogen and carbon. The results of the research of Yong Syuan *et al.* (2018) shows

rice straw as substrate of *T. Harzianum* potentially produce cellulase and xylanase. Higher substrate concentrations indicate the number of molecular substrates involved with enzyme activity. Moreover, low substrate concentrations mean fewer substrate molecules that can be attached to the enzyme causing reduced enzyme activity.

3.3 Analysis of the effect of hydrolysis time on total sugar

The hydrolysis time affects the total sugar produced. This is related to the ability of the fungi *T.harzianum* in degrading carbohydrates into monosugar. Based on the Tuckey test, it showed the significant difference between treatments ($P < 0.05$).

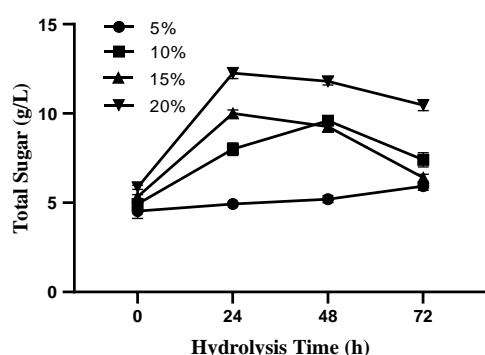


Fig.2: Total sugar from various hydrolysis time

The hydrolysis time affects the total sugar produced. This is related to the activity of *T. harzianum* which able to produce cellulase enzymes to degrade carbohydrates. The treatments with concentrations of 15 and 20% showing a significant increase in the incubation time of 24 h and decreasing in the following hours (Figure 2). This shows that the cellulase enzyme produced by *T. harzianum* is only able to work for a maximum of 24 h. In the treatment of 48 h and 72 h the enzyme activity decreases as indicated by a decrease in sugar levels.

To increase reducing sugar levels from 0 h to 24 h shows the activity of *T.harzianum* in producing cellulase enzymes to degrade carbohydrates from *K. alvarezii* seaweed. Treatment with an inoculum concentration of 20% is an excellent source of nutrition for *T. harzianum* to produce enzymes. At the beginning of the hydrolysis process, there are many sources of nutrients for *T. harzianum* available. After 24 h, growth nutrients for *T. harzianum* are reduced because they have been used in producing enzymes to degrade seaweed carbohydrates. This causes the concentration of the enzyme to become unbalanced, causing the enzyme performance to also decrease.

Treatment with an inoculum of 20% concentration after 24 h showed the amount of reducing sugar produced from the hydrolysis process decreased. This is caused by the accumulation of the final product of the hydrolysis process which inhibits the performance of the cellulase enzyme (Binod *et al.*, 2019). Glucose and ethanol are the end products that can inhibit cellulase performance (Chen & Jin, 2006). At constant seaweed substrate concentrations, the rate of enzymatic reaction increases with increasing enzyme concentration. This indicated that the more enzymes to a certain extent, the more substrates are converted because the higher the enzyme activity. In addition, enzymes are specific to the substrate, excessive concentrations will also affect the rate of the enzymatic reaction.

IV. CONCLUSIONS

This research has provided information about the potential of seaweed which used as raw material for producing sugar. Previous studies have shown in sugar production from seaweed uses several methods. However, this study is the first method using *T. harzianum* in degrading polysaccharides into monosugar. The finding of this research can demonstrated the ability of *T.harzianum* to convert carbohydrate from the *K. alvarezii* to biosugar production through the hydrolysis process.

ACKNOWLEDGMENTS

Special thanks to LPDP for providing funding for this research.

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