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Application of fungal pretreatment to produce monosaccharide from algae *Spirogyra peipingensis*

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Abstract: *Spirogyra peipingensis* is a species of green algae known to have a high carbohydrate content, making it a promising alternative raw material for biosugar production. This study aimed to determine the effects of inoculum size and pretreatment duration on biosugar yield. The research was conducted in three stages: pretreatment, hydrolysis, and fermentation. Pretreatment was carried out to release polysaccharides, hydrolysis was performed to convert cellulose and hemicellulose into simpler forms, and fermentation was conducted to transform disaccharides into monosaccharides. Monosaccharide measurement was performed using an ATC refractometer with a refractive index method to estimate the total sugar content. The results showed that the combination of a 7.5% inoculum size with a fermentation duration of 24 hours produced the most optimal monosaccharide yield, amounting to 0.28 g/g substrate. These findings emphasize that the regulation of inoculum and pretreatment parameters plays a crucial role in enhancing the efficiency of algal biomass conversion into biosugar. Overall, this study contributes to the development of bioconversion technologies based on local biological resources and highlights the potential of *Spirogyra peipingensis* as an environmentally friendly raw material candidate for future biosugar industries.

Keywords: biosugar, fermentation, carbohydrates, *Spirogyra peipingensis*, *Trichoderma harzianum*

Abstrak: *Spirogyra peipingensis* merupakan salah satu jenis alga hijau yang diketahui memiliki kandungan karbohidrat tinggi, sehingga berpotensi besar untuk dimanfaatkan sebagai bahan baku alternatif dalam produksi gula (biosugar). Penelitian ini bertujuan untuk mengetahui pengaruh ukuran inokulum dan lama *pretreatment* terhadap produksi biosugar. Penelitian ini dilakukan melalui tiga tahap, yaitu *pretreatment*, hidrolisis, dan fermentasi. *Pretreatment* dilakukan untuk melepaskan polisakarida, hidrolisis dilakukan untuk mengubah selulosa dan hemiselulosa menjadi bentuk yang lebih sederhana, dan fermentasi dilakukan untuk mengubah disakarida menjadi monosakarida. Pengukuran monosakarida dilakukan menggunakan alat refraktometer merk ATC dengan metode pengukuran indeks bias larutan untuk memperkirakan kadar gula total. Hasil penelitian menunjukkan bahwa kombinasi ukuran inokulum sebesar 7,5% dengan lama fermentasi 24 jam menghasilkan kadar monosakarida paling optimal, yaitu sebesar 0,28 g/g substrat. Temuan ini menegaskan bahwa pengaturan parameter inokulum dan *pretreatment* berperan krusial dalam meningkatkan efisiensi konversi biomassa alga menjadi biosugar. Secara keseluruhan, penelitian ini memberikan kontribusi terhadap pengembangan teknologi biokonversi berbasis sumber daya hayati lokal, serta membuka peluang pemanfaatan *Spirogyra peipingensis* sebagai kandidat bahan baku ramah lingkungan untuk industri gula biologis di masa depan.

Kata Kunci: biosugar, fermentasi, karbohidrat, *Spirogyra peipingensis*, *Trichoderma harzianum*

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INTRODUCTION

Sugar is one of the important basic needs (Sulfahri et al., 2019). Until currently, the main raw material for sugar production is sugar cane which every year has decreased production by 35.8% (Mizar et al., 2020). The decreasing of sugar production caused by land conversion and the transfer of crops from sugar cane into other plantation crops (Daulay et al., 2015). Therefore, efforts are needed to find alternative raw materials for sugar production that are potential for cultivation and rich in carbohydrates.

Alga is one of the raw materials that have a high potential to replace sugar cane for sugar production because of its high carbohydrate content (Sulfahri et al., 2017). One of the potential algae in Indonesia is the Spirogyra algae, because it has a high carbohydrate content (64%) and its rapid growth (Becker, 2006). The high carbohydrate content of Spirogyra algae has the potential to be developed into raw material for sugarproduction. The conversion process of carbohydrates from algae Spirogyra can perform using two stages, namely the pretreatment process and the hydrolysis process. The pretreatment process serves to break the cell wall of Spirogyra algae so that the carbohydrates can be converted into sugar through the hydrolysis process. Generally, various pretreatment methods that have been developed are ozonolysis, hydrothermal, and chemical. However, these methods are not environmentally friendly and require high costs. Therefore, efforts to develop inexpensive and environmentally friendly pretreatment methods are important. One potential pretreatment method is to utilize microbes that can produce enzymes to break down algal cell walls. One of the potential microbes in the hydrolysis of microbes is *Trichoderma harzianum*, because of its ability to produce cellulase enzymes (Abdullah et al., 2011; Harchi et al., 2018).

The biological pretreatment process is a method of breaking down biomass into sugars by utilizing enzymes. One microorganism with potential in this process is *T. harzianum*. This fungus can efficiently degrade algal cell walls through its enzymatic mechanisms, including the activities of cellulase, pectinase, and amylase. These enzymes break down the polysaccharides in the algal cell walls, releasing sugars that can subsequently be used in fermentation. The use of *T. harzianum* on algal and lignocellulosic biomass has been shown to significantly increase the amount of reducing sugars (Bader et al., 2020).

The disruption of algal cell walls can be achieved through the fermentation process. Fermentation is influenced by various factors, including fermentation nutrients, fermentation duration, pH, and inoculum size. Inoculum size is an important factor because it affects both production costs and sugar yield (Harchi et al., 2018). This study aims to determine the effects of inoculum size and fermentation duration followed by enzymatic hydrolysis on sugar production from the algae *S. peipingensis*. This research may provide a scientific basis for optimizing the bioconversion process of algae into biosugar, thereby supporting the development of renewable energy sources and environmentally friendly raw materials for the food and bioenergy industries.

RESEARCH METHODS

In this study, fungal pretreatment was applied to the alga *S. peipingensis*. The fungus *T. harzianum* used in this experiment was isolated from rice straw waste collected in East Java, Indonesia, while the alga *S. peipingensis* was obtained from South Sulawesi, Indonesia. The stages carried out in this study include:

1. Substrat and inoculum preparation

In this study, *S. peipingensis* algae were used as a substrate for the fungus *T. harzianum*. The algae were dried and ground using a hammer mill, then sieved through an 80-mesh sieve. The fraction that passed through the sieve was mixed with water to obtain a suspension containing 10% algae. The suspension was subsequently heated on a hotplate at 100°C for 2 hours and cooled to reach the optimum enzyme temperature of 45°C. Once the temperature reached 45°C, the algae suspension was transferred into a fermenter bottle and sterilized using an autoclave. The *T. harzianum* isolate was inoculated into the sterilized algae suspension at an inoculum concentration of 10%, followed by incubation in a rotary shaker at 30 °C with an agitation speed of 15 rpm for 8 hours. The resulting culture was then used as inoculum in the fermentation process. This study was conducted in two replications, and the parameter observed was the sugar content.

2. Biosugar production

The *T. harzianum* inoculum was inoculated into a 100 ml fermenter bottle containing fermented S. peipingensis alga substrate with varying duration (0 hours, 24 hours, 48 hours, 72 hours and 96 hours) and various inoculums (2.5%, 5.0%, 7.5%, 10.0%, and 12.5%) at room temperature (± 30ºC).

3. Measurement of sugar levels

In this study, sugars levels were measured using the Agilent 1100 HPLC system (Agilent Technologies,Santa Clara, CA, USA). The HPLC was fitted with a refractive index detector, equipped with a Bio-RadAminex HPX-87H column (300 × 7.8 mm). The column was maintained at 65°C with a flow of 0.6 mL/min of 5 mM H_2SO_4 mobile phase.

4. Statistical analysis

Data in the form of sugar content were statistically analyzed using Analysis of Variance (ANOVA) at a95% confidence interval ($\alpha = 0.05$) to determine the effect of inoculum size (2.5%, 5.0%, 7.5%, 10.0%, and 12.5%) and fermentation duration (0 hours, 24 hours, 48 hours, 72 hours and 96 hours) on sugar production. If there is influence then proceed with the Tukey test at a 95% confidence interval ($\alpha = 0.05$)to find pairs of the same and different data groups at each treatment. The software used for statistical analysis is Prism 8 For Mac.

RESULTS AND DISCUSSION

1. Effect of inoculum size on sugar production

In this study, *S. peipingensis* algae were pretreated using *T. harzianum* with varying inoculum concentrations (2.5%, 5.0%, 7.5%, 10.0%, and 12.5%). *Tricoderma* can degrade cell wall that contain cellulose using the cellulase enzyme that it produces (Pandey, *et al*., 2015). The results showed that sugar levels increasedwith increasing concentrations of inoculum used. At an inoculum concentration of 2.5% and 5.0% inoculum concentration yields an average sugar content of 0.24 g/g, while an inoculum concentration of 7.5%, 10.0% and an inoculum concentration of 12.5% produces an average sugar content of 0.28 g/g. The histogram of the optimum sugar level measurement results achieved in 24-hour duration, is presented in Figure 1.

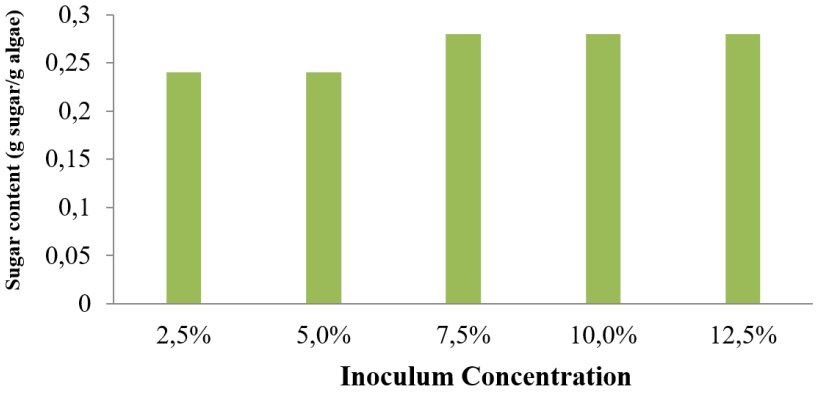

Figure 1. Sugar level at 24 H of fermentation duration

Based on the results of statistical analysis using ANOVA at a 95% confidence interval it can be seenthat the concentration of *T. harzianum* inoculums has a significant effect on the sugar content produced, the highest sugar content produced at a 7.5% inoculum concentration yields an average sugar content of 0.28 g/g. Based on the Tukey test that has been done at a 95% confidence interval, it is significantly different or significantly influences the sugar content produced. The concentration of the inoculum is an important factor because it will affect the cost of productionand the level of sugar produced, the inoculum has an important role in supporting the success of the fermentation process (Harchi et al., 2018). The maximum yield is obtained at an inoculum concentration of 6% and thendecreases, the addition of more yeast cells will cause the growth of yeast cells to multiply and will produce more optimal results (Ojewumi et al., 2018). When the yield produced has reached the maximum limit and the sizeof the inoculum is increasing, then the production of yields will begin to decrease because yeast cells stop growing due to competition in consuming nutrients that are starting to decrease (Ojewumi et al., 2018).

Tabel 1. Comparative study of inoculum size from several references

Microorganisms	Inoculum Size (%)	Raw Materials	Sugar Yield (g/L/h)	References
Saccharomyces cerevisiae	6	Sweet potato	6,39 g/L Ethanol	Ojewumi et al., 2018
Saccharomyces cerevisiae ZU-10	5	Corncob	0,57 g/L/h Ethanol	Azhar et al., 2017
Kluyveromyce smarxianus K213	5	Water hyacinth	0,31 g/L/h Ethanol	Yan et al., 2015
Saccharomyces cerevisiae GIM-2	6	Paper powder	0,59 g/L/h Ethanol	Peng & Chen, 2011
Saccharomyces cerevisiae CHFY0321	5	Cassava	2,41 g/L/h Ethanol	Moon et al., 2012
Saccharomyces cerevisiae	7	Sweet potato	4,76 g/L/h Ethanol	Zhang, et al., 2011
T. harzianum	7,5	*S. peipingensis*	0,58 g/L/h Gula	This study

1. Effect of fermentation duration on sugar production

In this study, the fermentation process was performed for 96 hours in anaerobic conditions with a time interval of 24 hours. The results showed that an increase in sugar levels with an increasing duration of fermentation. Based on the results of statistical analysis using ANOVA at 95% confidence interval, it can be seen that the incubation duration of *T. harzianum* has a significant effect on the sugar content produced, the

highest sugar content produced at the 24-hour incubation duration produces a sugar content of 0.28 g/g. Based on the Tukey test that has been done at a 95% confidence interval, it is significantly different or significantly influences the sugar content produced.

The lowest sugar level occurs at 0 hours, this happens because there is no fermentation process at that time. The fermentation process begins after the fermentation duration is 0 hours. Therefore, measurements made at 24-hour incubation duration have shown optimal sugar levels produced in the fermentation process. Then, in the 48-hour fermentation duration there was no increase but, there was a decrease in sugar levels, whereas in the 72-hour and 96-hour fermentation duration there was no increase or decrease in sugar levels. This is because the fermentation process does not occur again. That is, the polysaccharides contained in S. peipingensis algae have reached optimum conditions in their breakdowninto monosaccharides. Supported by previous research (Wu et al., 2014; Sulfahri et al., 2016; Sulfahri et al., 2020; Sulfahri et al., 2020), that glucose will be consumed by microbes in 24-hours incubation duration.

T. harzianum enzymatically degrades the cell wall of *S. peipingensis* via targeted hydrolysis of structural polysaccharides, including cellulose, β-1,3-glucan, and pectin. It produces a halophilic β-glucosidase that efficiently hydrolyzes laminarin, a common algal β-1,3-glucan (Sun et al., 2022). Additionally, *T. harzianum* expresses cellulases, pectinases, and proteases that act synergistically to dismantle the cell wall matrix, releasing monosaccharides such as glucose (Bader et al., 2020). Evidence from *Sargassum* biomass bioconversion further confirms the genus's capacity to degrade algal polysaccharides through laminarinase and cellulase activity (Agabo-García et al., 2025). Collectively, the coordinated action of these enzymes facilitates gradual cell wall disintegration, increasing soluble sugar availability for fermentation processes. The curve of the measurement of sugar content is presented in Table 2.

Tabel 2. Effect of fermentation duration on sugar production

Inoculum Size (%)	Sugar Yields (g/g)				
	0 h	24 h	48 h	72 h	96 h
2,5	0 g/g^{a}	0,24 g/g ^{b}	0,24 g/g ^{b}	0,24 g/g ^{b}	0, 24 g/g ^{b}
5,0	0 g/g^{a}	0,24 g/g ^{b}	0,23 g/g ^{b}	0,26 g/g^{c}	0,24 g/g ^{b}
7,5	0 g/g^{a}	0,28 g/g^{d}	0,24 g/g ^{b}	0,24 g/g ^{b}	0,28 g/g ^{d}
10,0	0 g/g ^{a}	0,28 g/g ^{d}	0,23 g/g ^{b}	0,28 g/g^{d}	0,28 g/g ^{d}
12,5	0 g/g^{a}	0,28 g/g ^{d}	0,26 g/g ^{c}	0,26 g/g ^{c}	0,26 g/g ^{c}

Note: The same letter in each column and row shows no significant difference according to the Tukeytest at 95% confidence intervals

Based on the results of statistical analysis using ANOVA at 95% confidence interval, the concentration of inoculum and the duration of incubation of *T. harzianum* have a significant effect on the level of sugar produced, the highest sugar content produced at 7.5% inoculum concentration with 24hours incubation duration produces sugar content as much as 0.28 g/g. Based on the Tukey test at a 95% confidence interval, the concentration of inoculum and the duration of incubation of *T. harzianum* was significantly different on the sugar produced.

CONCLUSION

The concentration of inoculum of incubation of T. harzianum has a significant effect on the level of sugar produced. The most optimal level of sugar produced was 0.28 g/g at a 7.5% inoculum concentration. The duration of incubation has a significant effect

on the level of sugar produced. The most optimal level of sugar produced is as much as 0.28 g/g on the incubation duration of 24 hours.

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