

***Production of Bioethanol from Rice Straw Assisted by Cellulosic Enzyme Oyster Mushroom Stem Using Simultaneous Saccharification and Fermentation (SSF)***

***Pembuatan Bioetanol dari Jerami Padi Berbantuan Ekstrak Enzim Selulase Batang Jamur Tiram Menggunakan Metode Simultaneous Saccharification and Fermentation (SSF)***

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***Abstract***

*Bioethanol is an alternative energy source to replace fossil fuels that can be produced from agricultural waste such as rice straw. The fermentation process can help hydrolyze lignocellulosic compounds in rice straw into simple sugars and convert them into bioethanol. This study aims to determine the effect of fermentation time and the amount of intracellular cellulase enzyme extract from oyster mushroom stem on the concentrations of bioethanol produced. The method used in the production of bioethanol is Simultaneous Saccharification and Fermentation (SSF). The bioethanol produced was then analyzed by gas chromatography and UV-Vis spectrophotometer. The result showed that the highest concentration of bioethanol was 14.52% obtained at 10 days of fermentation time and the amount of cellulase enzyme extract was 25 mL.*

**Keywords:** *bioethanol, rice straw, oyster mushroom, cellulose, SSF*

***Abstrak***

Bioetanol merupakan salah satu sumber energi alternatif pengganti bahan bakar fosil yang dapat dihasilkan dari limbah pertanian seperti jerami padi. Proses fermentasi dapat membantu menghidrolisis senyawaan lignoselulosa dalam jerami padi menjadi gula-gula sederhana dan mengubahnya menjadi bioetanol. Penelitian ini bertujuan untuk mengetahui pengaruh waktu fermentasi dan jumlah ekstrak enzim selulase intraseluler dari batang jamur tiram terhadap kadar bioetanol yang dihasilkan. Metode yang digunakan dalam pembuatan bioetanol ini adalah *Simultaneous Saccharification and Fermentation* (SSF). Bioetanol yang dihasilkan selanjutnya dianalisis dengan kromatografi gas dan spektrofotometer UV-Vis. Hasil penelitian menunjukkan bahwa kadar bioetanol yang tertinggi adalah 14,52% yang diperoleh pada waktu fermentasi 10 hari dan jumlah ekstrak enzim selulase sebanyak 25 mL.

**Kata kunci:** bioetanol, jerami padi, jamur tiram, selulase, SSF

## Introduction

The development of biofuel such as bioethanol from biomass as a source of renewable raw materials is an alternative to petroleum that has positive values from environmental and social aspects (Costa, 2012; Nenad, 2018). Bioethanol can be produced by organic waste (biomass) which contains a lot of lignocellulose such as rice straw (Binod, 2010; Belal, 2013; Takano, 2018). So far, the utilization of rice straw waste is still not optimal. Generally, rice straw is only used for animal feed and the rest is burned or left to rot which will produce pollutants that can damage the environment and contribute to greenhouse gases. Rice straw production can be abundant depending on variations in location and varieties of rice plants used. Rice straw is known to have high cellulose content, which reaches 34.2% dry weight, 24.5% hemicellulose and lignin content up to 23.4% (Yulianto, 2009).

In general, bioethanol synthesis derived from biomass consists of two main stages, namely hydrolysis, and fermentation. In the previous method, the process of hydrolysis and fermentation is done separately or often called Separated Hydrolysis and Fermentation (SHF) and the latest is the

Simultaneous Saccharification and Fermentation (SSF) process (Dahnum, 2015; Suttikul, 2016). One of the advantages of the SSF process is that hydrolysis and fermentation are carried out simultaneously in one container or reactor so that it can take place efficiently (Pinaki, 2016). The hydrolysis stage in the SSF process is usually carried out by conventional methods using sulfuric acid ( $H_2SO_4$ ) or hydrochloric acid (HCl), but this method is less environmentally friendly.

Development of bioprocess technology using enzymes in its hydrolysis process is believed to be a more environmentally friendly process (Pandey et al., 2000). The advantages of hydrolysis with enzymes include reducing the use of acid so as to reduce the negative effects on the environment. Then after the hydrolysis process is carried out fermentation using yeast such as *S. cerevisiae* to convert glucose to ethanol. Enzymes that are used as hydrolyzing agents such as cellulase enzymes are generally commercially available, thus requiring quite expensive costs to get it. One of the cellulase-producing biomass that has not been used optimally is the oyster mushroom stem. On the stem of the oyster mushrooms, there is mycelium which

functions as a cellulase enzyme producer. Mycelium as a whole has the power to absorb nutrients. Mycelium is directly related to the substrate and secretes enzymes that can break down complex organic components into simple components which can then be absorbed diffusion through the mycelium wall (Kalmis, 2008). In this study, cellulase enzymes from oyster mushroom stems will be used as hydrolysis agents in the production of bioethanol from rice straw using the SSF method.

The conversion of rice straw into bioethanol is a smart move, which provides three benefits at once, namely (i) economic value, (ii) being a solution for handling rice straw waste and (iii) producing environmentally friendly bioethanol products.

## **Experimental Methods**

### **Materials**

#### **Preparation of Cellulose Enzyme Extract of Oyster Mushroom Stem**

The preparation of cellulase enzyme extract of oyster mushroom stem is done by weighing 100 g of oyster mushroom stem and adding a pH of 5.5 acetate buffer of 100 mL. After that, blend until smooth. The solution mixture is allowed to stand for 1 day to get the

oyster mushroom stem cellulase enzyme extract and filtered using a vacuum filter.

#### **Production of bioethanol using SSF with a variation of the amount of cellulase enzyme extract**

A total of 10 g rice straw powder is put in a 250 mL glass bottle. Then added 100 mL aquadest and 4 g of yeast containing *S. cerevisiae* which then added oyster mushroom stem extract (cellulase enzyme) with various variations, namely 0, 10, 15, 20 and 25 mL then stirred until homogeneous, tightly closed and fermented during 9 days.

#### **Production of bioethanol using SSF with a variation of fermentation time**

A total of 10 g rice straw powder is put in a 250 mL glass bottle. Then added 100 mL aquadest, 4 g of traditional yeast tape containing *Saccharomyces cerevisiae* (*S. cerevisiae*) and 25 mL of oyster mushroom stem extract (cellulase enzyme) then stirred until homogeneous, tightly closed and fermented during various fermentation times at 2, 4, 6, 8 and 10 days.

#### **Determination of bioethanol**

Bioethanol was qualitatively analyzed using Gas Chromatography compared by the standard of ethanol. The concentration of bioethanol was determined using Spectrophotometer UV-Vis with the addition of Jones

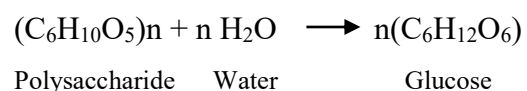
reagent and analyzed at a maximum wavelength of 580 nm.

## Discussion

Simultaneous Saccharification and Fermentation (SSF) is a method of hydrolysis and fermentation carried out simultaneously. The raw material containing cellulose undergoes a hydrolysis process first into glucose and then it is fermented directly into ethanol. This method is used because Simultaneous Saccharification and Fermentation (SSF) has several advantages, namely hydrolysis by cellulase enzymes and fermentation by microbes can be done simultaneously so that only using one reactor, in addition cellulose which is hydrolyzed into glucose will be directly fermented into ethanol. This hydrolysis and fermentation process will be very efficient and effective if carried out in a sustainable manner without going through a long period of time.

The first step in the SSF method is the hydrolysis process. Hydrolysis is the process of breaking down polysaccharides in lignocellulosic biomass, which is hemicellulose and cellulose into its constituent sugar monomers. Hydrolysis reaction is a reaction involving water or acid as a

reactant so that a compound can be split or decomposed. The hydrolysis reaction is a reaction that takes place slowly, therefore to accelerate the rate of catalyst often added. The usual catalyst used in the hydrolysis reaction is an acid and enzyme catalyst because the use of acids is classified as dangerous because of its corrosive nature, so in this study hydrolysis using enzymes was conducted. The reactions that occur in the hydrolysis process are as follows:

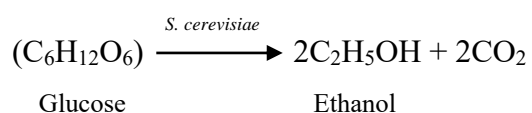


The enzyme used to hydrolyze cellulose to glucose is the cellulase enzyme. Effective conversion of cellulose to monosaccharides is only possible by the synergistic work of the following three cellulase subgroups:

1. Endo- $\beta$ -1,4-D-glucanase which breaks the internal bonds of glucosidic that is between the intact glucan chain.
2. Exo- $\beta$ -1,4-D-glucanase / exo- $\beta$ -1,4-D-selobiohydrolase which breaks down the cellulobiosa dimer from the glucan chain and releases it into solution.
3.  $\beta$ -glucosidase which completes the hydrolysis of cellulose into glucose by breaking down cellulobiose into glucose monomers (Pinaki, 2016).

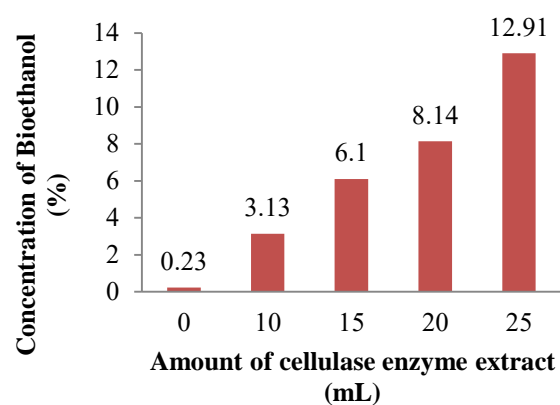
Then in the second stage, the hydrolyzed sample will experience

fermentation. Fermentation is the process of breaking down sugar into ethanol and carbon dioxide caused by enzymes produced by microbial cell masses. Changes that occur during the fermentation process are changes in glucose into ethanol by *S. cerevisiae* cells. Glucose fermentation is one type of anaerobic fermentation or without using oxygen in the process. Glucose fermentation in rice straw is carried out using 4 g NKL brand yeast tape as a source of *S. cerevisiae* which can live anaerobically in the fermentation media. *S. cerevisiae* is used because there are many types of yeast in yeast, but only one species is known to be able to convert sugar to ethanol which is very high, *S. cerevisiae*. The reactions that occur in the fermentation process are:

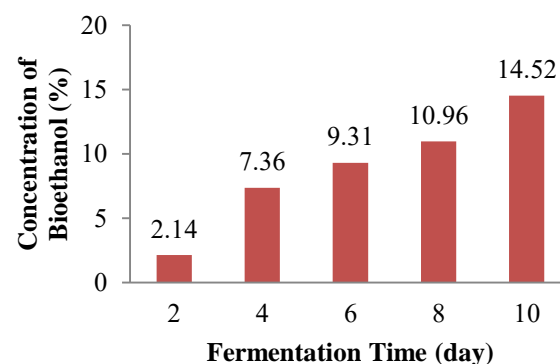


In this study, cellulase enzymes were obtained from oyster mushroom stems. Oyster mushrooms are known to have cellulase enzymes. The stem is part of oyster mushroom which is not used in its processing as food and is thrown away. In general, cellulase enzymes are obtained as extracellular enzymes isolated from its growth media. The

emergence of ethanol from the SSF process shows the activity of the lignocellulose enzyme in the oyster mushroom stem. The greater the amount of enzyme extract added the higher the bioethanol content obtained. The highest Data on the effect of the amount of oyster mushroom stem enzyme extract on the bioethanol content obtained from rice straw by the SSF method is presented in Figure 1.



**Figure 1.** Effect of volume of cellulase enzyme extract to concentration



**Figure 2.** Effect of fermentation time to concentration of bioethanol

Fermentation time also affects the concentration of bioethanol. The longer the fermentation time is applied, the

higher the concentration of bioethanol produced.

From these data, it appears that the exponential phase of growth of *S. cerevisiae* is still ongoing during the fermentation process using the SSF method. This shows that the enzymatic hydrolysis process is still ongoing until the fermentation time of 10 days.

### Conclusion

Bioethanol can be made from rice straw using SSF (Saccharification and Simultaneous Fermentation) method, the cellulose mixture from rice straw will be hydrolyzed with the help of cellulase enzymes into lead, then it will be transferred to ethanol by *S. cerevisiae* yeast. The more the volume of the cellulase enzyme, the faster the hydrolysis of cellulose into glucose. This is because the use of enzymes in the reaction reduces the activation energy so that the energy needed to reach the final product is smaller than the reaction without the enzyme. The highest ethanol content results in 25 mL cellulase enzyme volume that is equal to 12.91% in 10 days of fermentation time.

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