PROCEEDINGS
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PRODUCTION OF BIOETHANOL FROM *SARGASSUM CRASSIFOLIUM* USING SELLULASE ENZYMES FROM *TRICHODERMA RESEEI* AND *ASPERGILLUS NIGER*

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Abstract

Indonesia has the potential as the world's largest bioethanol producer. The efforts to produce bioethanol using non-crop raw material are recently developed. *Sargassum crassifolium* is a promising raw material for biofuel production since containing a high concentration of polysaccharides. The purpose of this study to determine *Sargassum crassifolium* bioethanol production by using enzymes from *Trichoderma reesai* and *Aspergillus niger*. Pre-treatment of *Sargassum crassifolium* at working pressure of 15 Psi, temperature of 121 ^{0} C and sulphuric acid concentration of 0.2 M increased the reducing sugars concentration up to 0,000558 mg/L. The application of cellulase enzymes from *Trichoderma reesai* and *Aspergillus niger* (3:1) helped increasing the reducing sugars concentration up to 1031,67 mg/L Fermentation using a naturally yeast, *Saccharomyces cereviceae* (JCM 3012) (0.15 g/mL), produced bioethanol up to 0,727 % during fermentation that assisted the degradation of *Sargassum crassifolium.*
Key words: Bioethanol, cellulase, *Sargassum crassifolium, Trichoderma sellulase reesai and Aspergillus niger*

Introduction

Indonesia is a country with high energy consumption in the world. Indonesia is quite high energy consumption nearly 95% met by fossil fuels. Of the total, nearly 50% of it is fuel oil (BBM). Fuel consumption is high enough to be a problem for Indonesia. As a non-renewable energy sources, fuel reserves are limited (ESDM, 2012). One of the alternative energy that can replace petroleum fuel is bioethanol.

According to Sari *et al*., (2014), many studies reveal that waste containing cellulose can be used as a source of sugar that is inexpensive and easy to replace starch material in the fermentation process. High cellulose content in the Sargassum sp. can be used as feedstock to produce bioethanol. Sargassum sp. many contain polysaccharide alginate used for industrial food and beverage, cosmetic and pharmaceutical.

Other polysaccharide is cellulose. Cellulose degradation process can be done chemically or biologically using cellulolytic organisms derived from bacteria or fungi, degradation of cellulose into simple sugars either cellobiose or glucose with the aid of a catalyst. Enzymatic hydrolysis using cellulase enzymes. Cellulase enzymes can be produced from microbial Selulotik of fungi and bacteria. Selulotik mushroom commonly used of the type Trichoderma and Aspergillus. The purpose of this study was to determine the production of bioethanol from Fermentation of Sargassum with an enzyme produced by *T.reesai* and *A.niger (*Kasmiran Ariani dan Tarmizi. 2012, Pujiarti *et.al*, 2012)\

Research methods

Cultivation *T.reesei* and *A. niger*

Growth of *T. reesei* and *A. niger* using a PDA (Potato Dextrose Agar). The first step: PDA boiled, after boiling sterilized using an autoclave for 15 minutes, and let cool then each mushroom cultivated on PDA using a wire loop, then incubated at room temperature for 7 days in a petri dish (Safaria *et al*., 2013).

Preparation of Sargassum sp.

Sargasssum sp. taken on the beach Krakal Gunung Kidul then the sample is washed with fresh water, and dried in the sun, but not exposed to direct sunlight. After drying, the samples blended, to form a powder.

Hydrolysis Sargassum sp.

Sargassum powder sp.10 g put into erlenmeyer, and soaking H_2SO_4 at a concentration of 0.2 M, 0.3 M, 0.4 m, 0.5M. Then the samples were stored 120 minutes and the initial pH was measured and adjusted to pH neutral (7) (Saputra, 2012).

Preparation of a solution of tween 80%

Tween solution 1mL 80% reconstituted with 1 liter of distilled water. Tween 80% solution serves as a non-ionic surfactant that can reduce the surface tension between the water and the spores T.reesei and A.niger. (Safaria *et al*., 2013).

Cellulase enzyme production

Cellulase enzyme production begins by mixing 10 grams of powder Sargassum sp., 25 mL nutrient solution, *T.reesei*, *A. niger* and 80% tween solution into erlenmayer 250 mL, then covered with cotton and covered with aluminum foil, incubated for 1 day at 37 °C and then centrifuged to get the enzyme sellulase.

Bioethanol production

Bioethanol production is done by mixing the powder of Sargassum, with 25 mL nutrient solution, T.reesei and A. niger and allowed to stand for 24 hours and then given a 15% *Saccharomyces cerevisiae* and the fermented 3 days.

Measuring levels of Bioethanol

Bioethanol content measurement is done using Gas Chromatography (GC-14B) Shimadzu FID system. (Febriani *et al*., 2014).

Result and Discussion

Table 1. Result of Sugar reduction

***Sargassum* sp. with H_2SO_4**	**Test Glukosa (µg/g)**	**Test Fruktosa (µg/g)**	**Test Sukrosa (µg/g)**
0,2 M	557,79	225,62	622,59
0,3 M	226,57	236,77	304,70
0,4 M	378,56	200,28	251,57
0,5 M	<4,2	200,38	<2,9

The content of glucose, sucrose, and fructose at the lowest concentrations of H_2SO_4 0.5M this case due to the increased concentration of the acid hydrolysis process results in glucose and other glucose compounds to be much degraded. Time used in hydrolysis of H_2SO_4 is 120 minutes because the longer the time of hydrolysis the higher the glucose levels obtained (Saputra *et al*., 2012)

Table 2. Level of Bioethanol

Trichoderma reesei **and** *Aspergillus niger*	**Replikasi 1 (%)**	**Replikasi 2 (%)**	**Rates Level of Bioetahol (%)**
0:1	0,1567	0,1554	0,1560
1:1	0,1254	0,1460	0,1357
1:0	0,1788	0,1590	0,1689
1:3	0,1340	0,1329	0,1334
3:1	0,6760	0,7789	0,7274
2:1	0,1097	0,1096	0,1096
1:2	0,1739	0,1816	0,1777

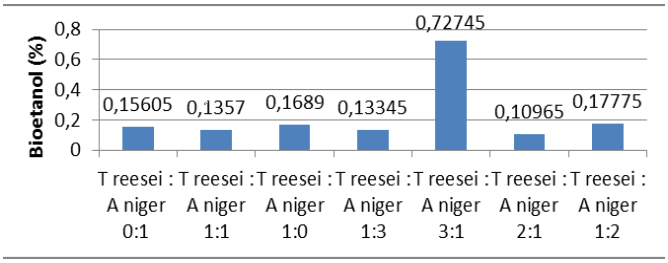

Figure 1. The Level of Bioethanol

The highest levels of ethanol at a concentration of T. reesei: A. niger 3: 1 with an average of 0.7274%, it is because the enzyme cellulase from T. reesei and A. niger higher can turn cellulose into glucose, thus increasing levels of bioethanol fermented with S.cerevisiae. (Khaira *et al*., 2015, Gunam *et.al*, 2010). Bioethaol lowest levels found in T. reesei: A. niger 2: 1 with the average value was 0.1096%, this is because the enzymes contained in T. reesei a bit so it is not able to change sellolosa into glucose, so the lower un bioethanol fermentation results , It fits Zely opinion (2014), T. reesei to produce endoglucanase and eksoglukanase 80% but lower β-glukosidasenya so the main product instead of glucose, but cellobiose hydrolysis. While required in bioethanol production is fermentation of glucose by S.cerevisiae convert glucose into ethanol.

4. Conclusion

Based on the results of this study concluded that the highest amount of bioethanol production in the treatment T.reesai: A.niger 3: 1 with a bioethanol content of 0.7274%

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