

***Trichoderma Reesei* single cell protein production from rice straw pulp in solid state fermentation**

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Abstract. The dependency on fish meal as a major protein source for animal feed can lead to price instability in line with the increasing in meat production and consumption in Indonesia. In order to deal with this problem, an effort to produce an alternative protein sources production is needed. This scenario is possible due to the abundant availability of agricultural residues such as rice straw which could be utilized as substrate for production of single cell proteins as an alternative protein source. This work investigated the potential utilization of rice straw pulp and urea mixture as substrate for the production of local *Trichoderma reesei* single cell protein in solid state fermentation system. Some parameters have been analyzed to evaluate the effect of ratio of rice straw pulp to urea on mixed single cell protein biomass (mixed SCP biomass) composition, such as total crude protein (analyzed by kjedhal method) and lignin content (TAPPI method). The results showed that crude protein content in mixed SCP biomass increases with the increasing in fermentation time, otherwise it decreases with the increasing in substrate carbon to nitrogen (C/N) ratio. Residual lignin content in mixed SCP biomass decreases from 7% to 0.63% during fermentation proceeded of 21 days. The highest crude protein content in mixed SCP biomass was obtained at substrate C/N ratio 20:1 of 25%.

1. Introduction

The consumption of meat in Indonesia continues to increase from year to year along with the increasing in population and people's income. This trend will affect the increasing demand on animal feed which in turn will disrupt the market stability of feed prices. The increase in feed prices is due to the higher price of raw materials of fish meal which has limited availability. One way to reduce the dependency on fish meal is to find out alternative sources such as single cell protein (SCP). Single cell proteins can be produced from various organic substrates by various microorganisms in fermentation process. One of the promising organic substrate to be utilized is agricultural residues such as rice straw. The utilization of rice straw has attracted much attention among researchers since it is available abundantly and as low-cost carbon source for SCP production [1-3].

In utilizing lignocellulosic biomass as substrate, it is required to select an appropriate microorganisms that have the ability to consume lignocellulosic biomass carbon source for its growth. Among of the microorganisms having these capabilities are fungal families such as *Trichoderma reesei*. According to Ugalde et al and Schuster, et al [3,4], *T. reesei* is a fungi which has good capability on digesting lignocellulosic material, as it can produce cellulolytic and hemicellulolytic enzymes. However, studies on the performance of *T. reesei* in utilizing rice straw pulp as substrate for its growth is still lacking. In this study, the potential of local *T. reesei* to produce



single cell protein (SCP) with rice straw pulp as substrate in solid state fermentation and the capability of *T. reesei* in degrading the lignin compounds contained within the substrate were examined.

2. Materials and methods

2.1. Rice straw liquid hot water pretreatment.

Rice straw which was used in this study was obtained from a local rice field in Aceh Besar District, Indonesia. Rice straw was collected and then put into a plastic bag and stored in a cool place. It was then crushed using a blender to increase surface area about 60 mesh. Pretreatment was conducted in an autoclave by using liquid hot water at 121 °C for 60 minutes and sequentially rinsed by distilled water and dried in oven at 50 °C for 2 days.

2.2. Microorganism

The fungus used was *Trichoderma reesei* which obtained from Microbiology Laboratory, School of Life Sciences and Technology, Bandung Institute of Technology, Indonesia. It was subcultured periodically on potato dextrose agar (PDA) plates.

2.3. Inoculum

Aerial spores of *T. reesei* were removed from 7 days old potato dextrose agar plates by scraping with a spatula and suspending in sterile water. This suspension of spores was used to inoculate 200 ml medium in a 500 ml erlenmeyer flask. The flask was incubated for 3 days in an orbital shaker at 150 rpm and 30 °C. The resulting biomass was used as an inoculum for the medium in solid state fermentation. Media for inoculum consists of 40 g/l of molasses and 7 g/l of ammonium sulfate.

2.4. Solid state fermentation (SSF)

Single cell protein production experiment were conducted using 500 ml erlenmeyer flask. 100 grams of sterilized rice straw pulp were put into the flask and added with urea solution according to C/N ratio variation, and then added 100 ml of the inoculum were sequentially added in. The cultivation was carried out for 21 days at temperature of 30 °C.

2.5. Measurement of crude protein content

The total amount of SCP in fermented product was estimated as crude protein contents by Kjeldahl method [5]. Subsequently 0.51 grams of samples (for incubation time of 4, 7, 14 and 21 days), was put into 100 ml of kjedahl flask. Added 2 grams of selenium mixture and 25 ml of concentrated H₂SO₄. Heated over an electric heater until boiling and the solution becomes clear greenish (about 2 hours). Allow to cool and then diluted and put into a 100 ml measuring flask, right up to the line mark. Pipetted 5 ml of solution and put in to the distiller, added 5 ml of 30% sodium hydroxide and several drops of phenolphthalein indicator. Distilled for approximately 10 minutes, distillate dropping into the flask contained 10 ml of 2% boric acid solution and indicator. Titrated with 0.01 N HCl solution.

Calculation:

$$\text{crudeproteincontent (\%)} = \frac{(V_1 - V_2) \times N \times 0.014 \times f_k \times f_p}{w} \times 100\% \quad (1)$$

where :

w : weight of sample (gram)

V₁ : volume of HCl used for sample (ml)

V₂ : volume of HCl used for blank test (ml)

N : normality of HCl (N)

f_k : conversion Factor (6.25)

f_p : dilution factor

2.6. Lignin content analysis

Analysis of lignin content using kappa number test [6]. This Standard procedure is adopted from TAPPI Useful Method 246-modified micro Kappa number of standard methods for Kappa number (T-236). After the moisture content of the pulp samples was determined, 0.5 gram (dry weight) pulp was taken. Samples were soaked with 10 ml of distilled water in a beaker glass for 10 minutes. Then, the mixture is transferred into the rotary blender with 40 ml of distilled water. After a minute, the mixture was transferred into the 250 ml beaker glass and rinsed with 20 ml distilled water before stirred by a magnetic stirrer. A mixture of 10 ml of 0.02 M KMnO_4 and 2.0 M H_2SO_4 solution that has been prepared before was inserted into the beaker. After 10 minutes, 2 ml of 1.0 M KI added to the beaker followed by titration with a solution of 0.1 M sodium thiosulfate solution and a few drops of 0.2% starch. Total volume of 0.1 M sodium thiosulfate solution used was recorded. Control test without the dissolved pulp sample was performed with the same procedure. Kappa number was calculated by the following equations:

$$p = \frac{(b-a)N_1}{0.1} \quad (2)$$

$$\log K = \log \frac{p}{w} + 0.00093 (p - 50) \quad (3)$$

where

- p : volume of potassium permanganate actually consumed by the test specimen, ml
- b : volume of sodium thiosulfate consumed in black test, ml
- a : volume of sodium thiosulfate consumed by pulp sample test, ml
- N_1 : normality of sodium thiosulfate
- w : weight of sample (pulp), gram
- K : Kappa number

The obtained kappa number is converted to lignin content using the following equation:

$$\% \text{ lignin} = 0.147 \times \text{Kappa Number} \quad (4)$$

3. Results and discussion

3.1 Degradation of lignin

The main obstacles on utilization of agricultural waste such as rice straw as animal feed are related to low nutritional content and digestibility level. Lignin chemically binds to carbohydrates and physically acts as a barrier to the process of reshuffling cell walls by microorganisms in the digestive process. According to Bhargav, et al., [7], lignin is a biopolymer with complex phenylpropanoid structure which can deteriorate digestion ability. In this experiment, liquid hot water pretreatment was used for eliminating lignin content in rice straw substrate.

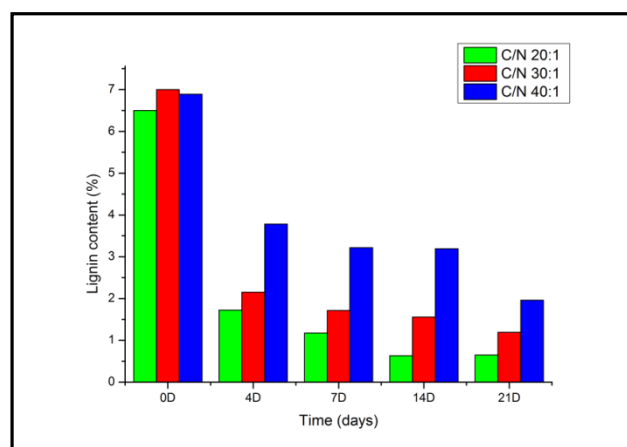
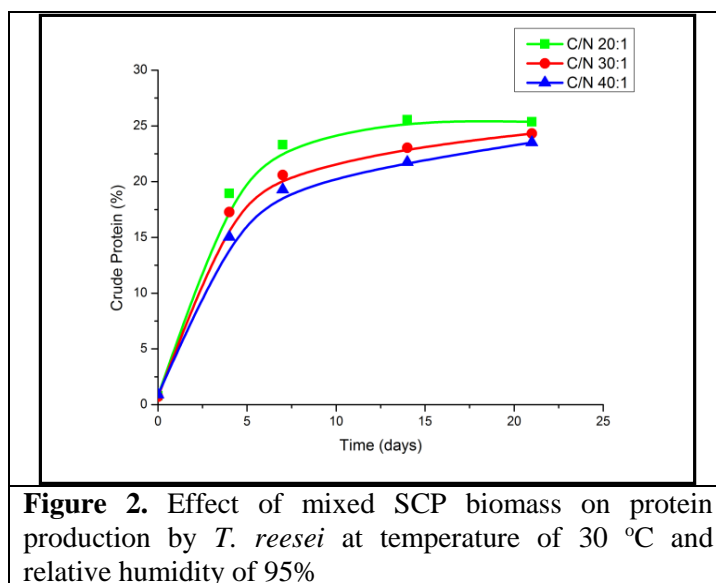


Figure 1. The lignin content of mixed SCP biomass.

But, unfortunately the pulp result from LHW pretreatment still has lignin content of 7% (dry basis). Therefore to fulfill the poultry feed standard [8] which is lignin content in the feed not allowed, the efforts should be made to eliminate the remaining lignin content in rice straw substrate using *T. reesei*. Schuster, et al., [4] reported that *Trichoderma reesei* is a fungus possessing ability to digesting lignocellulosic material very well, as of it can produce cellulolytic and hemicellulolytic enzymes, the same result reported by Helal [9]. In this study the ability of local *T. Reesei* in removal of the remaining lignin content in rice straw pulp was evaluated. In Figure 1 shows the lignin content in mixed SCP biomass against fermentation time. The minimum lignin content in mixed SCP biomass (0.63%) was observed at 14 days. From the Figure 1, it can be seen that the lignin content is influenced by the C/N ratio of the substrate. The lower C/N ratio (20: 1), the greater decrease of lignin level. At 14 days, the lignin content in mixed SCP biomass was 0.63%. This evidence is due to higher fungal cellular growth on the substrate with a lower C/N ratio. The increase infungal cells growth on substrate would affected the increasing of lignin disruption level.

3.2 Effect of initial C/N ratio on crude protein production

Crude protein is a single cell protein which extracted from mixed single cell biomass (mixed SCP biomass). Mixed SCP biomass is a fermentation product containing SCP and undigested pulp. The profile of crude protein production at different initial carbon to nitrogen ratio are shown in Figure 2. The increase of crude protein content in all mixed SCP biomass begin after 4 days incubation and reached the maximum after 14 days. It can be seen from the Figure 2, that the crude protein increase with decreasing initial C/N ratio. This trend is due to different amount of nitrogen supplied in substrate [10,11]. The substrate initial C/N ratio of 20:1 had more nitrogen content, so that the cell of *T. reesei* could utilize more carbon source and therefore would produce more single cell biomass than other C/N ratio combinations. The maximum crude protein in substrate with C/N ratio 20:1 was 25% (based on ratio of crude protein/mixed SCP biomass) after 14 days of fermentation.



4. Conclusion

This study shows that it is possible to grow a locally *Trichoderma reesei* on mixed urea-rice straw pulp substrate in solid state fermentation to produce single cell protein. *T. Reesei* proves to have ability to reduce lignin content in substrate from 7% (dry basis of biomass) to 0.63%. The maximum of total crude protein produced on substrate with C/N ratio of 20:1 was 25%.

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