Solid State Fermentation of Rice Straw Pulp with a Local *Trichoderma reesei* for Single Cell Protein Production: Effects of Temperature, Initial Medium pH and Fermentation Time

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Abstract. Indonesia is one among countries that produce abundant of rice straw every year generated as post-harvesting residue from paddy field all over country. This abundant amount residue is highly potential to be utilized as carbon source in producing single cell protein (SCP) in solid state fermentation system. SCP is a microbial protein that can be used as alternative protein for substitution of available conventional protein sources such as; fish meal, soy bean, and corn Stover for animal feed production. This research was conducted to evaluating the effects of initial medium pH of substrate (mixed rice straw pulp-urea), fermentation time and fermentation temperature on SCP production by *Trichoderma reesei* in solid state fermentation (SSF). The results showed that the highest SCP yield (19.71%) was produced at the initial medium pH of 5 and temperature of 30 °C at SSF conditions of C/N ratio 20:1 for 12 days of fermentation time.

1. Introduction

In Indonesia, recently, the problem on finding a cheaper and better quality of alternative protein sources has been faced by poultry farmers which are now being a serious concern in order to maintain a competitive production cost. An increase in population has a direct consequence on the increasing need for animal protein. Poultry is a potential source of animal protein that is in growing demand by Indonesian consumers. Unfortunately, the price of chicken meat is often fluctuated because it is strongly influenced by the unstable price of poultry feed. On the other hand, poultry feed price is largely determined by the cost of its raw materials, especially fish meal as the main ingredient for poultry feed production. The price of fish meal tends to increase due to the decline in fish population as a consequence of the decreasing quality of marine ecosystem and climate change effect. To overcome the dependence of feed processing mills on fish meal, single cell protein (SCP) can be used as an alternative option for the substitution of fish meal. Suman et al, 2015, stated that single cell protein from fungi or yeast is suitable for use as poultry feed because it has 50-55% protein, high protein-carbohydrates ratio, balance of amino acids and high B-complex vitamins [1].

The efforts on development an alternative applicable and friendly technology on poultry feed production would be encouraged the poultry feed integrated production of domestic animal feed at affordable prices and with good nutritional quality [1]. One of the promising protein potential is single
cell protein which can be produced from fermentation of organic materials containing cellulose such as rice straw. It can be used for animal feed as well as a good substitute to protein rich meat and fish. With the growing population, it would be necessary to find an alternative source to conventional protein feed and SCP is recently being a promising one[3-4]. Considering these challenges and opportunities, it is necessary to study the effects of process variables such as temperature, initial pH medium and fermentation time that involved in the production of SCP in SSF system of mixed rice straw pulp-urea using a local Trichoderma reesei.

2. Materials and Methods

2.1 Rice straw pulp preparation
Rice straw used in this experiment was obtained from a local farmer in Limpok paddy field, Aceh Besar District, Indonesia, on harvesting period of January 2018. One kilograms of rice straw collected and air-dried in an isolated room. It was cut into 2-3 cm and crushed in a mechanical crusher. After mesh sieving of 60 mesh were obtained, it was digested using hot water at temperature 121 °C for 60 minutes. Subsequently, the pre-treated rice straw pulp was rinsed with distilled water and oven dried at 60 °C for 48 hours prior to be utilized as substrate in SSF system.

2.2 Microorganism and inoculum
A local Trichoderma reesei which obtained from Microbiology Laboratory, School of Life Sciences and Technology, Bandung Institute of Technology, Indonesia was used as bioconversion agent in the present study was maintained on potato dextrose agar (PDA) slant at temperature 30 °C.

2.3 Solid state fermentation of rice straw pulp
Solid state fermentation (SSF) of rice straw pulp was carried out according to the modified method of our previous study [5]. Briefly, flasks containing rice straw pulp as substrate were incubated at controlled relative humidity (RH) of 95±1% for certain days and the temperature varied at 24, 27, 30, 33, and 36 °C. The effect of fermentation time was investigated at various times i.e: 3, 6, 9, 12, 15 days at constant initial C/N ratio of 20:1 and at varied pH 3, 4, 5 and 6, respectively. Each experiment was carried out in two sets.

2.4 SCP content analysis
SCP content in fermentation product was analyzed in term of crude protein by Kjeldahl method using the similar analysis procedure as reported in our previous work [5].

3. Results and Discussion

3.1. Effect of temperature on the SSF rice straw pulp
The effect of temperature on SCP production by Trichoderma reesei in the SSF using the mixed rice straw pulp-urea as substrate was evaluated. Figure 1 shows the SCP production at various temperatures. The maximum SCP production was observed for substrate which was incubated at 30 °C for 12 days fermentation time. The similar trends were also found by other researchers[6]–[9] which used Trichoderma reesei in producing cellulose enzyme from biomass. It might be presumed that the appropriate temperature for microbial growth on mixed rice straw pulp-urea substrate was at 30 °C and had a cooperated effect both on significant microbial cells growth and enzyme production in SSF system as indicated by maximum SCP yield.
3.2. Effect of initial medium pH on SSF rice straw pulp

In this study, the effect of the initial medium pH of the substrate was observed ranging at pH values from 3 to 6 which adjusted by using 1% sulfuric acid. Figure 2 indicated that the maximum crude protein yield was obtained at pH 5.

The effect of pH was studied by adjusting different ranges of initial medium pH of solid substrate ranging from 3 to 6 on SCP yield. The results showed that crude protein production increased from pH of 3 to 5 and reached maximum crude protein yield at pH 5. Further increasing in initial medium pH affected the decreasing of crude protein production. It was verified that the highest crude protein production obtained at initial medium pH of 5.
3.3. Effect of fermentation time on SSF rice straw pulp

The conversion of mixed rice straw pulp-urea to SCP by Trichoderma reesei is influenced by the fermentation time. SCP production was started from 0 to 15 days as shown in Figure 3 and reached the maximum protein production (19.71%) at 12 days fermentation time. Refer to Figure 3, the production of SCP started an exponential growth rate during the 6 to 12 days growth period and began constant at 12 days fermentation time and it tend to slightly decrease at extended fermentation time afterwards. This evidence was presumed that 12 days is as the most appropriate and productive period of cells growth and enzyme activities of Trichoderma reesei cellulose production in fermentation process in SSF system as previously reported by Maurya et al., 2012 [9] which lead to maximum crude protein production.

![Figure 3. Effect of fermentation time on SCP production](image)

4. Conclusion

The experimental results showed that the observed variables process; temperature, initial medium pH, and fermentation time were significantly affected the crude protein yield on SCP production of mixed rice straw pulp-urea pulp by using Trichoderma reesei on SSF system. The highest crude protein content (19.71%) was occurred at SSF conditions of C/N ratio 20:1, initial medium pH 5, and fermentation time and temperature 12 days and 30 °C, respectively.

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