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Single cell protein production by a local *Aspergillus niger* in solid state fermentation using rice straw pulp as carbon source: effects of fermentation variables

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Abstract. In this study, solid-state fermentation was carried out to produce single cell protein by local *Aspergillus niger*. Rice straw pulp produced from the pretreatment by liquid hot water at temperature 121 °C for 60 minutes of cooking time was used as carbon source. The effects of nitrogen sources, initial medium pH, C/N ratios of substrates and fermentation time on SCP production were evaluated in these experiments. The single cell protein was measured as crude protein content in the fermentation product by the Kjeldahl analysis method. The maximum crude protein of 18.9 % (w/w) contained in the fermentation product occurred at the fermentation condition with C/N ratio 30:1, the initial medium pH 4, temperature 30 °C, moisture content 75 % and relative humidity 95 %.

1. Introduction

Providing animal feed supply for a sustainable food production scenario will be the major concern all over the world, especially in developing countries. Indonesia is one of the biggest agricultural countries producing an abundant amount of rice straw as agricultural residue from paddy field every year. Based on an FAO report [1], the estimated rice straw production amounts about 740.95–1111.42 million tons per year globally while Indonesia itself contributed about 70.84–106.26 million metric tons of rice straw in 2014. This cellulosic biomass provides a low-cost resource with large possibility to support a large-scale production of various biomass-derived products [2];[3]. Rice straw is among major lignocellulosic agricultural residues with a great potential as substrate to produce single cell protein (SCP) with a useful nutrient for animal feed [4]. However, available data on treating rice straw applied as a suitable substrate for SCP production are relatively scarce, therefore, efforts to develop SCP from rice straw still need exploring more information. There are some important aspects affect the SCP production in solid state fermentation (SSF) include to select a pretreatment method with ability to produce a suitable substrate for a selected fungus and to determine the appropriate SSF process conditions for optimum microbial growth such as pH, the moisture content, temperature, C/N ratio, and fermentation time. One of the potential pretreatment methods for preparing a substrate from lignocellulosic materials is liquid hot water (LHW). This simple and low-cost pretreatment method can generate reactive cellulose fibers to make them easily accessible for microbial growth and produce degradation products with little inhibition



for a subsequent fermentation process [5]. Therefore, LHW pretreatment of rice straw as substrate for solid state fermentation using local *Aspergillus niger* is a promising cost-effective and environmental-friendly technology for developing countries. *Aspergillus niger* is one species with ability to convert cellulose materials into SCP [6]. Besides, it has an optimum growth temperature in the range of 35–37 °C, hence, it requires less energy for reaching optimal growth temperature in the fermentation process. Unfortunately, the utilization of rice straw pulp as a carbon source for SCP production by local *Aspergillus niger* is still less reported by researchers. This research evaluates the possibility to utilize LHW pretreatment rice straw pulp for SCP production by a local *Aspergillus niger* and the effects of initial pH, initial moisture content, the C/N ratio and fermentation time on crude protein yield.

2. Materials and Methods

2.1 Chemical composition and crude protein content analysis

The chemical composition of rice straw was determined using the method described by Goering and Van Soest [7]. Ash content was measured by thermogravimetric method. The crude protein content was determined by total nitrogen content which measured by Kjeldahl method [8] then multiplying with a traditional conversion factor of 6.25.

2.2 Microorganism and inoculum preparation

The local fungus used in this study was *Aspergillus niger* obtained from Microbiology Laboratory, School of Life Sciences and Technology, Bandung Institute of Technology, Indonesia. It was maintained on potato dextrose agar and sub-cultured periodically on the agar plate for 7 days. The spores produced on the agar were dislodged and suspended in sterile water under aseptic condition. The suspension of 107 spores/mL was inoculated into 200 mL medium in 500 mL Erlenmeyer flasks. The medium consisted of 40 g/L molasses and 7 g/L ammonium sulfate. The flask was incubated at a temperature of 30 °C and agitation speed of 150 rpm in an orbital incubator for 3 days. The incubation broth was used as culture inoculum for SSF.

2.3 Rice straw pretreatments

The rice straw used as substrate was obtained from a local farmer in the Aceh Besar district of Indonesia. It was collected in a plastic bag and placed in a room with an approximate temperature of 30 °C. Rice straw was cut into 2-3 cm, crushed in a blender, and passed through a screen sieve 60 mesh. Furthermore, rice straw was pretreated using LHW in autoclave at a temperature of 121 °C for 60 minutes. Rice straw pulp from autoclave was rinsed with distilled water and dried in an oven at a temperature of 60 °C for 2 days.

2.4 Solid state fermentation

The effects of nitrogen sources, C/N ratio, initial medium pH and fermentation time on the SCP production (determined as crude protein) in SSF were studied. The SSF was performed in each 500 mL Erlenmeyer flask containing 10 g of rice straw pulp at a certain C/N ratio and 75 % moisture content. Nitrogen source and distilled water were added into the flasks to adjust the C/N ratio and moisture content of the solid substrate (rice straw pulp). The flasks were sterilized at 121 °C for 15 minutes. The substrate in the flask was mixed properly using sterile spatula after adding culture inoculums of 10 mL into each flask. Subsequently, all flasks were incubated in the environment chamber at controlled temperature and relative humidity of 30±1 °C and 95±1 % for 12 days. Three types of fertilizer, namely urea, ammonium sulfate and NPK as nitrogen sources were used for evaluating the effect of the nitrogen source on the crude protein yield. The nitrogen source with the highest crude protein content in the fermentation product would be selected for C/N ratio assessment. The C/N ratio of substrate was evaluated at the various C/N ratios of 20:1, 30:1, 40:1, 50:1 and 60:1. The effect of the initial pH of solid substrate was investigated at pH of 3, 4, 5, and 6. The effect of incubation time was investigated

at various times, i.e 3, 6, 9, 12, 15 days at a constantly initial C/N ratio 30:1 and pH of 4. Each experiment was carried out two times.

2.5 Determination of initial medium pH

One gram of the sample was mixed with 10.0 mL of distilled water, shaken in an orbital shaker for 10 minutes, and filtered out. Subsequently pH of the filtrate was measured by pH meter (HORIBA pH 1100).

3. Results and Discussion

3.1 Liquid Hot Water Pretreatment of Rice Straw

The liquid hot water (LHW) pretreatment of rice straw was performed in autoclave at temperature condition of 121 °C for 60 minutes. The composition of untreated rice straw and rice straw pulp expressed in percentage was showed in Table 1. LHW pretreatment was able to reduce the lignin content from 11.18 % in rice straw to 7.07 % in rice straw pulp (pretreated rice straw), on the other hand cellulose and hemicellulose content respectively enhanced from 48.29 % and 16.89 % in rice straw to 54.33 % and 21.35 % in rice straw pulp. Rahnema et al., [9] used NaOH solution with low concentration of 0.5 % (w/v) and the ratio of rice straw to solution 1:10 (1 g rice straw /10 mL alkali solution) for delignification process, and revealed decrease of lignin content of rice straw from 9.22 % to 5.91 % after pretreatment with an alkaline solution which conducted in an autoclave at temperature 121 °C for 20 minutes. Referring to the report, LHW pretreatment has a comparable delignification effect to alkaline pretreatment but need longer operation time.

Table 1. Chemical composition of rice straw and rice straw pulp

Samples	Lignin (%)	Cellulose (%)	Hemicellulose (%)	Ash (%)
Rice straw	11.18 %	48.29 %	16.89 %	13.25 %
Rice straw pulp	7.07 %	54.33 %	21.35 %	14.33 %

3.2 Time courses of SCP production

The effect of fermentation time on SCP production was evaluated in solid substrate (mixed rice straw pulp-urea) with the C/N ratios of 20 and 30, and the fermentation condition of the initial medium pH 4, temperature 30 °C, moisture content 75 %, and relative humidity 95 %. The time course of SCP (determined as crude protein) production was shown in Fig. 1. SCP production increased with the time course from 0–12 days and reached the maximum protein production (18.9 %) in 12 days. SCP production began getting constant after 12 days of fermentation. Hamdy [10], explained the similar effect of fermentation time on SCP production by *Aspergillus niger* which was fermented in orange peel media. The total protein in fermentation product yielded by *Aspergillus niger* of 40.1 % was reached at 144 h (6 d) fermentation. The longer fermentation time was needed and less protein content in fermentation product resulted in rice straw pulp fermentation compared to fermentation in orange peel media. It was probably because of higher lignin content remained and lower soluble sugars contained in rice straw pulp.

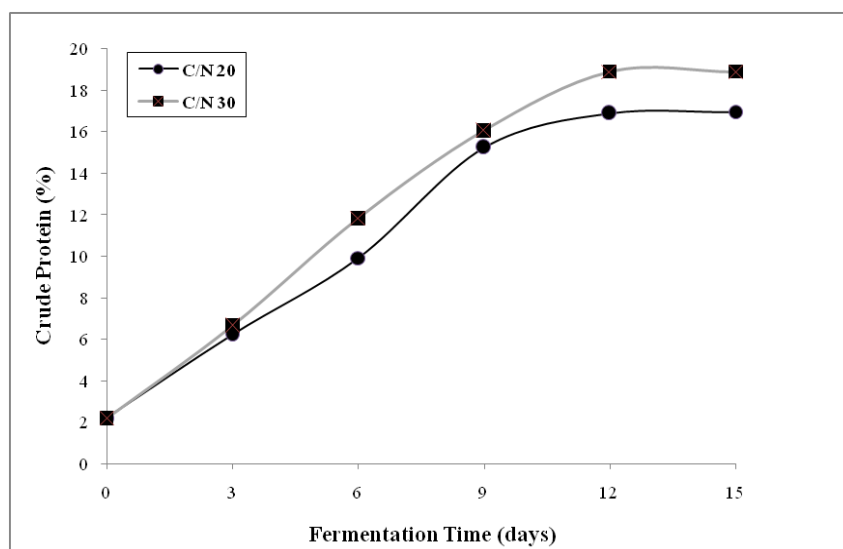


Figure 1. The time course of single cell protein production

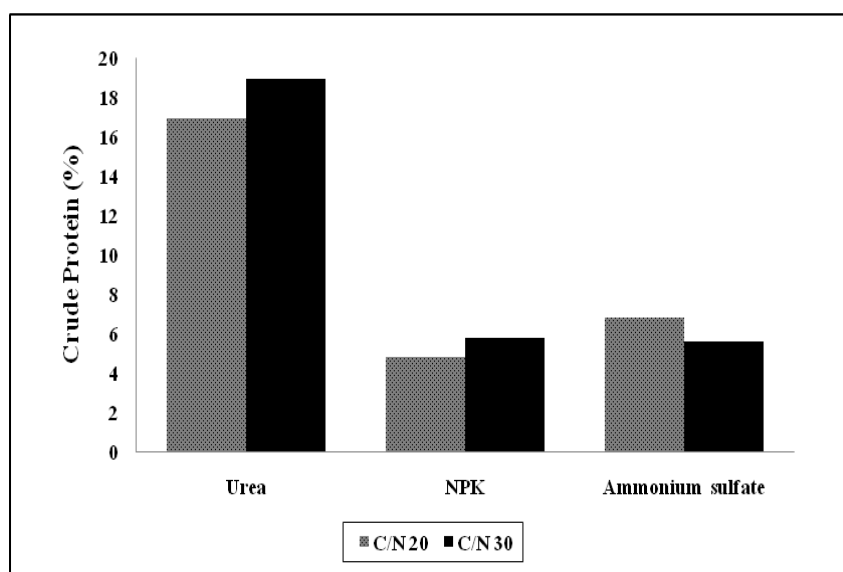


Figure 2. The effect of the various nitrogen sources on SCP production

3.3 Effect of the nitrogen source on SCP production

In order to select the optimum nitrogen source for maximum SCP production, various types of fertilizers which available in a local market (urea, ammonium sulfate and NPK) were used as the nitrogen source in the growth medium. Fig. 2 presented the effect of the nitrogen source on crude protein content in the fermentation product after 12 days fermentation time. The results revealed that the highest protein content (18.9 %, w/w) was produced on solid substrate with urea as nitrogen source. It is presumably that the possible reason urea might have a higher solubility in water compared to those other two nitrogen sources. It gave higher nitrogen concentration on the surface of substrate which was consumed by fungi as nutrient for their growth as the microbial activities take place in or near the surface of substrate. Hence, a higher crude protein yield reached (18.9 %) as compared to other nitrogen sources. The results revealed that urea, a low-cost fertilizer, supported maximum crude protein production.

Oshoma and Eguakun-Owie [11] who studied the effect of different anorganic nitrogen supplement on food waste for the production of *Aspergillus niger* SCP reported that ammonium nitrate and ammonium sulfate addition gave higher protein content.

3.4 Effect of the C/N ratio in the substrate

The effect of the carbon to nitrogen (C/N) ratio of the substrate on a crude protein with urea as a nitrogen source in fermentation product was evaluated. Carbon and nitrogen sources are important factor which will be consumed as nutrient for fungal growth and microbial metabolism during fermentation process. Optimum concentrations of these nutrients were found to increase the growth performance and fungal biomass. Hence, it is necessary to maintain the accurate composition of growth media for effective fermentation process to keep the C/N ratio within the desired range. In Fig. 3 showed that the crude protein content increased with the increasing of the C/N ratio from 10:1 to 30:1, but in contrary, further increasing in the C/N ratio from 30:1 to 50:1 gave a decreasing of crude protein content. The maximum crude protein content (18.9 %) was found at the C/N ratio of 30:1.

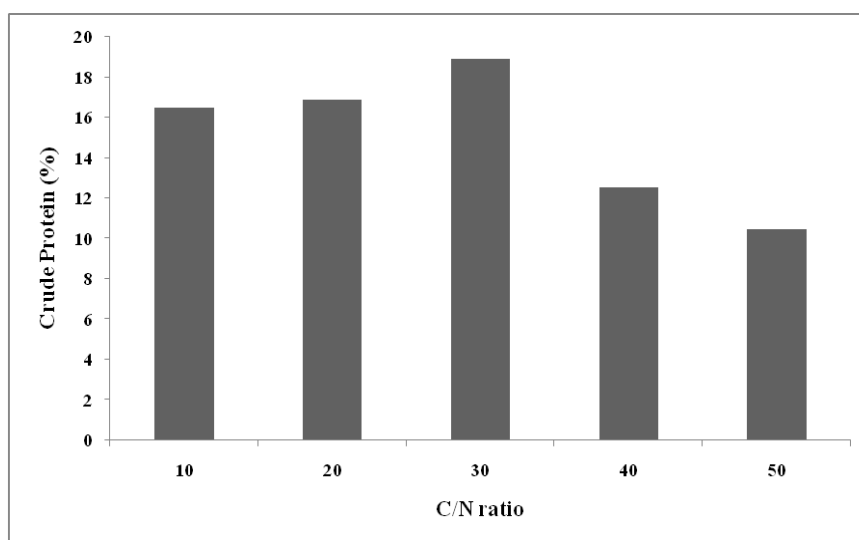


Figure 3. The effect of the C/N ratio on SCP production

A previous investigation also reported a significant enhancement in microbial biomass protein production using corn stover by sequential culture fermentation of *Candida utilis* and *Arachniotus sp.* was observed at 30:1 C/N ratio in 6 % (w/v) corn stover [12]. Thus, determination of appropriate amount of C/N ratio is the vital factor to harvest maximum microbial biomass protein.

3.5 Effect of initial medium pH

The effect of pH was studied by adjusting different ranges of an initial medium pH of the solid substrate in the range of 3–7 on crude protein yield. The results (Fig. 3) shown that crude protein production increased from pH of 3 to 4 and reached maximum crude protein yield at pH 4 (18.9 %, w/w). Further increasing in initial medium pH affected the decreasing of crude protein production. It was observed that the highest crude protein production obtained was at the initial medium pH of 4. This result is in agreement with Maftukhah and Abdullah [13] who used rice straw as substrate for cellulose enzyme production on solid state fermentation by *Aspergillus niger* which found that the maximum enzyme production occurred at pH 4. Sohail et al. [14] also reported that during an exponential phase of growth in solid state fermentation of lignocellulosic substrates, *Aspergillus niger* MS82 produced the highest production of cellulase at initial pH 4 and temperature 35 °C. These results in general also confirmed

that initial pH 4 is optimum for *Aspergillus niger* to produce the maximum crude protein or cellulase production in solid state fermentation using cellulosic substrate.

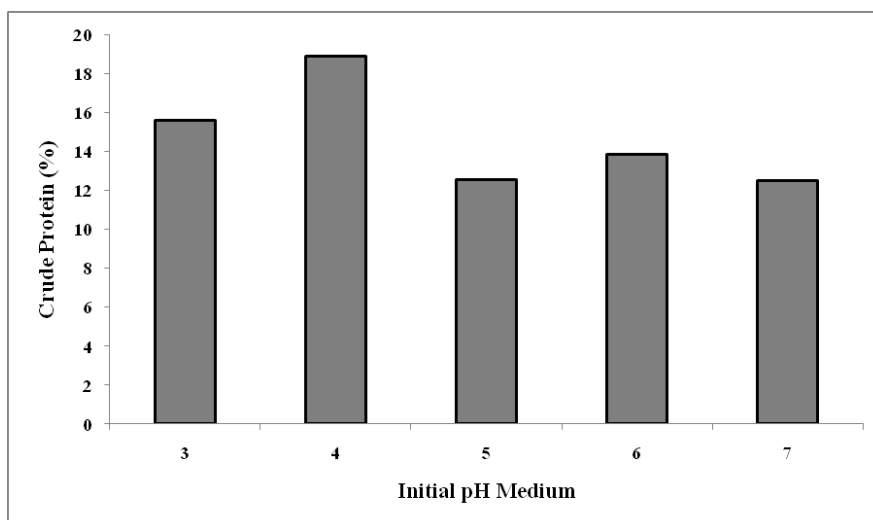


Figure 4. The effect of initial pH medium on SCP production for 12 days at 30 °C and 75 % moisture content

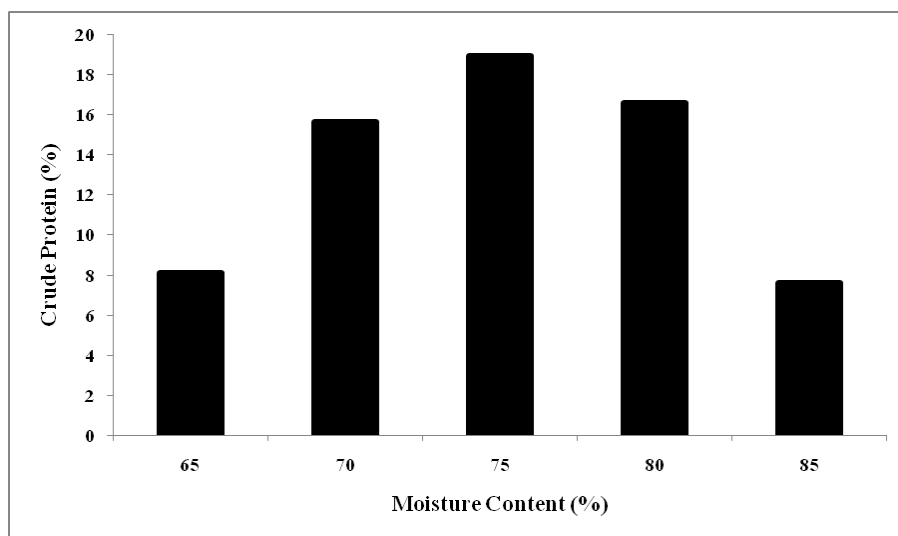


Figure 5. The effect of the initial moisture content on the SCP production for 12 days at 30 °C and relative humidity 95 %

3.6 Effect of moisture content

The initial moisture content of the substrate plays a vital role on microorganisms activities during the crude protein production by *A. niger* cultivated in SSF bioreactor. The moisture contents of substrates were evaluated ranging from 65 % to 85 %. Fig.5 shows the maximum crude protein yield (18.9 %) was obtained at the moisture content of 75 % and tends to decrease at higher moisture content level. Hamdy [10], studied the effect of moisture content in the range of 50 % to 70 %, and revealed that the initial moisture content of orange peels media of 55 % gave the highest protein yield in *A. niger* fermentation product. Water got involved in most vital metabolic activities, and the majority of

microbial viable required a moisture content of 70–80 % for reproduction [15]. But the optimum initial moisture content required by the fungus for maximum growth depends on the physicochemical characteristics of the substrate. High moisture content could developed water film which cover the porosity of solid surface and reduce oxygen transfer for the fungal growth. Conversely, low moisture content will limit the fungal growth [16]. This evaluation suggested that the optimal initial moisture content plays an important role for the maximum microbial biomass production.

4. Conclusion

This study showed that it was possible to develop a single cell protein production system by utilizing the solid substrate (mixed rice straw pulp-urea) in a SSF with a local *Aspergillus niger* as bioconversion agent. The present work revealed that *Aspergillus niger* had a good ability to consume the prepared substrate. The highest crude protein content (18.9 %) in the fermentation product occurred at a fermentation condition of C/N ratio 30:1, the initial medium pH 4, temperature 30 °C, moisture content 75 % and relative humidity 95 %. In addition, LHW pretreatment has ability to reduce lignin content in rice straw to the level in which the pretreated substrate (rice straw pulp) is appropriate for *A. niger* growth.

Acknowledgements

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