Production of single cell protein by a local *Trichoderma reesei* in solid state fermentation: effects of process variables

S D Said, M Zaki, E Novita and T M Asnawi

Chemical Engineering Department, Faculty of Engineering, Syiah Kuala University, Darussalam, Banda Aceh, 23111, Indonesia

E-mail: syahiddin@che.unsyiah.ac.id

Abstract. Single cell protein (SCP) has attracted a lot of attention as an alternative protein source to support the increasing demand and to reduce the dependence on fishmeal in the animal feed and poultry industries. The SCP can be produced using lignocellulosic materials which are abundantly generated continuously as agricultural residues by *Trichoderma reesei* in solid state fermentation system. In this work, we evaluated the effects of various carbon sources (rice straw, corncob, bagasse, and coffee husk) which were combined with various nitrogen sources (Urea, Ammonium Sulphate, NPK) on SCP production. The rice straw and coffee husk gave a higher SCP yield when combined with urea or NPK at C/N ratio of 20:1 and 30:1. The solid substrate which contained rice straw or coffee husk gave a higher SCP yield when combined with urea or NPK at C/N ratio of 20:1 and 30:1. The initial moisture content of the solid substrate gave a significant contribution to the SCP yield, the highest yield of SCP developed in the substrate with the initial water content of 75%. On the other hand, the ratio of inoculum size to the solid substrate of 1:1 (w/v) gave the maximum SCP yield.

1. Introduction

Lignocellulosic agricultural waste is the most widely available waste in the world. The International Grains Council reported that 737 million tons of wheat, 984 million tons of maize and 474 million tons of rice were harvested globally [1]. This abundant lignocellulosic biomass could be utilized as a substrate in the production of SCP. The agricultural waste needs a suitable pretreatment to meet the appropriate fermentable substrate for conducive cell growth to reach high yield SCP [2]. The utilization of the waste for SCP will simultaneously solve the disposal problems [3]. However, efforts to develop SCP production system from various carbon sources available in Indonesia such as rice straw, corncob, bagasse and coffee husk needs a lot of information regarding process variables in obtaining high substrate conversion to be SCP.

One of a promising and low-cost pretreatment for lignocellulosic materials is Liquid Hot Water (LHW) method. LHW is an environmentally friendly process due to it does not require any chemicals. This simple method can delignify lignin content in the materials with less inhibition effect to subsequent hydrolysis and fermentation process [4,5]. *Trichoderma reesei* species is one of the fungi that can utilize lignocellulosic materials as the source of carbon and energy for their growth [6,7]. Consider each strain of *T.*
Reesei has its unique capability in consuming different cellulosic materials in different condition for their growth so that it is necessary to study the effects of operating variables that involved in the production of SCP in the solid state fermentation system.

2. Materials and Methods

2.1. Microorganism and Inoculum

A local T. 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, and it was maintained on potato dextrose agar (PDA) slant. The spore suspension for inoculation was prepared by adding with 10 ml sterile water on aerial spore of T. reesei which was produced on 7 days old PDA plate. The spore was removed by scraping with a spatula, and the suspension (1x10⁷ spores/ml) was used to inoculate 200 ml medium contained 40 g/l of molasses and 7 g/l of ammonium sulfate in a 500 ml Erlenmeyer flask. The broth from 3 days incubated flask at 30 °C and 150 rpm was used as culture inoculum in solid state fermentation system.

2.2. Solid State Fermentation

Carbon sources of agricultural solid waste: rice straw, corncob and bagasse were obtained from Aceh Besar District, while coffee husk was obtained from Aceh Tengah District, Indonesia. All types of these carbon sources were collected and put into a plastic bag and keep in a cool place. The carbon source was crushed separately with a blender, then passed to screen sieve of 60 mesh. Liquid hot water pretreatment was conducted in an autoclave by using hot water at 121 °C for 60 minutes. After pretreatment, the carbon source was rinsed with distilled water and oven-dried at 60 °C for 2 days.

2.3. Inoculum Size and Initial Moisture Content

Effects of inoculum size (as the ratio of solid substrate to inoculum) on SCP was evaluated by varying the ratio at 1:0.5, 1:1, 1:1.5, 1:2, 1:2.5 (w/v; g/ml). While the effect of initial moisture content of solid substrate was examined by setting moisture content at 65, 70, 75, 80, 85% using a mixture of culture broth of inoculum and sterile distilled water. Moisture content was measured by using dry weight method by drying sample to a constant weight at 105 °C in an oven.

2.4. Carbon and Nitrogen Sources

The effects of various carbon sources: rice straw, corncob, bagasse, and coffee husk, were evaluated separately by adding each carbon source and urea as a solid substrate with C/N ratio of 20:1 into the flask. While the effect of various nitrogen sources on SCP yield i.e: urea, ammonium sulfate and NPK were studied by feeding rice straw pulp and each nitrogen source separately into the flask at various substrate C/N ratios.

2.5. Analysis of Crude Protein

SCP content in the fermentation product was determined in term of crude protein by Kjeldahl method [8]. The total nitrogen content obtained by the method then multiplied by conversion factor of 6.25 to convert the total measured nitrogen to a protein concentration.

3. Results and Discussion

3.1. Effect of Carbon Source on SCP Production

The effect of different carbon sources on SCP production was evaluated separately in solid substrate fermentation with urea fertilizer was used as nitrogen source. The fermentation process condition was adjusted at temperature 30 °C, pH 5, moisture content 75%, relative humidity (RH) 95%, and C/N ratio of 20:1. Figure 1 shows the SCP production (in terms of crude protein) by T. reesei in different solid substrates.
All type of solid substrates which have been tested being able to be well consumed by *T. reesei* and gave crude protein production with a comparable high percentage (more than 15%). The amount of crude protein produced by *T. reesei* in solid substrate containing rice straw or coffee husk gave a higher yield, 22% and 21%, respectively. While in the bagasse or corn cob solid substrate, the amount of crude protein produced individually were 16% and 17%.

![Figure 1. Effect of carbon sources on SCP production for 12 days fermentation at temperature 30 °C, pH 5, RH 95%, and moisture content 75%](image1)

3.2. *Effect of Nitrogen Source on SCP Production*

The effect of various fertilizers as nitrogen source on SCP production (in term of crude protein) by *T. reesei* in solid state fermentation by using rice straw pulp as the carbon source for 12 days fermentation was depicted in figure 2. It was observed that urea and NPK fertilizer provided a similar effect on SCP production which gave a significant increased of crude protein content from C/N ratio of 10:1 to 20:1, but then decreased at higher C/N ratio.

![Figure 2. Effect of various nitrogen source on SCP production at temperature 30 °C, pH 5, RH 95%, and moisture content 75%](image2)
Rice straw pulp medium with urea or NPK, each type of this nitrogen source at C/N ratio of 20:1, contributed maximum SCP production in the fermentation product of 22% and 21.6%, respectively. Conversely, the medium supplied with ammonium sulfate fertilizer yielded a different trend of SCP production, which decreased with the increasing of C/N ratio from 10:1 to 30:1. Maximum SCP production by *T. Reesei* in medium with ammonium sulfate as nitrogen source was 18%.

3.3. Effect of Moisture Content
In solid state fermentation, the moisture content is an important factor affecting the cell growth by swelling the substrate and supporting the utilization of substrate by microorganism [9]. Effect of initial substrate moisture content on SCP production (expressed in term of crude protein) was shown in figure 3. Increasing moisture content in the substrate in the range of 65% to 75% increased the crude protein production, and optimum production (21%) occurred at the moisture content of 75%. The increasing of moisture content from 75% to 85% resulted in the reduction of crude protein production. The higher moisture content (> 75%) may result in reducing oxygen mass transfer and preventing the growth fungus as a result of a reduction in substrate porosity and change in the structure of substrate particles [10-12]. However, at low moisture content will hinder the growth of the microorganism. Therefore it is required to determine the optimal moisture content because each solid substrate has its favourable growing condition for acceptable protein production [13-15].

![Figure 3](image1.png)

**Figure 3.** Effect of initial moisture content on SCP production for 12 days at temperature 30°C, pH 5 and RH 95%

![Figure 4](image2.png)

**Figure 4.** Effect of inoculum size on SCP production for 12 days at 30°C, and RH 95%
3.4. Effect of Inoculum Size

The effect of inoculum size on the SCP production was shown in Figure 4. The SCP production increase with increasing addition of inoculum to the solid substrate from the ratio of 1:0.5 to 1:1, but addition more inoculum caused SCP production decreased. The addition of inoculum to the solid substrate with a ratio higher than 1:1 may result in an increase of moisture content in the solid substrate, consequently more void space on solid substrate filled with water, causing a decrease of oxygen mass transfer and prevented cell growth sequentially to crude protein yield [16-20].

4. Conclusion

This work shows that rice straw, coffee husk, corncob and bagasse are the potential carbon source for producing single cell protein in solid state fermentation by a local fungus of *Trichoderma reesei*. Besides urea and NPK fertilizer are prospective nitrogen source for single cell protein production for their low-cost and obtainable in the local market. The highest crude protein content (22%) was produced by *Trichoderma reesei* in solid state fermentation with solid substrate consisted of rice straw pulp and urea with C/N ratio of 20:1 at fermentation process conditions; temperature 30 °C, initial medium pH 5, moisture content 75% and inoculum size 1:1.

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