Utilization of pineapple peel and rice washing water to produce single cell proteins using *Saccharomyces cerevisiae*

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Abstract. Single cell protein (SCP) is an alternative for meeting protein needs in the future. The wastes such as pineapple skin and rice water washing can be utilized to produce SCP. These two materials are abundant in Indonesia. The consumption of pineapple and rice in Indonesia is very high. The rice is the main food for Indonesian people. The aim of this study is to determine the best ratio of pineapple skin juice and rice washing water as a growing medium to produce single cell proteins. This study was performed in a completely randomized design with two factors. The first factor was the ratio of pineapple skin and rice washing water. The ratios between pineapple skin and rice washing water were 1:1, 2:1 and 1:2. The second factor was fermentation times (8, 24, 32, 48, 56, 72 and 80 hours). The analysis carried out included pH, cell dry weight and protein content. The best treatment is obtained by the treatment of pineapple skin and rice washing water in ratio of 1:2 when it is fermented for 56 hours. This media produced 0.4752 grams of cell dry weight with the protein content at about 289.08 ppm.

Keywords: Single cell protein, pineapple peel, rice washing water, saccharomyces

1. Introduction

Protein is one source of energy that is needed to build and to maintain the cells. The development of science makes humans look for alternatives to meet the needs of protein in the future. One alternative obtained is single cell protein (SCP) which has high protein content. SCP is a biomass product sourced from microbes that generally produce protein [1]. SCP has many applications in food and feed industries. It has high protein content, high percentage of essential amino acids and other nutrients [2,3,4,5]. In general, the protein contents in the single cells for protein production should be between 39 and 73% [2,6]. SCP production is based on the incidence of starvation in the 60s in several places in the world. It was related to economic, social problems and an increasing of the world's population that was imbalanced with an increasing of food production. One of the efforts made to face that condition was development of protein from microorganisms as an alternative food source to replace meat protein.

SCP production has some advantages. First, its production does not require a large area. Second, it does not produce waste. The third is that it has fast production processes and large proliferation [7]. SCP can be produced from many species of microorganisms (Table 1). These include algae fungi and bacteria. It is convenient to use fungi and bacteria for production of SCP when grown on inexpensive waste material. Their rapid growth and high protein content have made them the prime candidates for...
use as sources of SCP [8]. *Saccharomyces cerevisiae* is one of microorganisms commonly used to produce SCP. *Saccharomyces cerevisiae* contains lots of protein, carbohydrates, and fats which are very beneficial for humans and animals in completing their nutritional needs. In addition, *Saccharomyces cerevisiae* is easy to grow in various media containing carbon, nitrogen, oxygen, sulfur, calcium, vitamins, minerals and water [9]. The production of the microbial biomass is done either by a submerged or solid state fermentation process. After fermentation, biomass is harvested and may be used as a protein source or be subjected to processing steps like washing, cell disruption, protein extraction and purification [10]. In general, high production rates and protein yields as well as ease of production control makes SCP more attractive as a protein source compared to conventional plant and animal sources [11].

Table 1. Single cell protein production from fungi [12].

| Organism used                     | Substrate                      |
|-----------------------------------|--------------------------------|
| Aspergillus niger AS 101          | Corn cobs                     |
| Aspergillus niger, Sporotrichum pulverulentum | Maize and Cotton stalk     |
| Candida krusei SO1 & Saccharomyces spp. LK3G | Sorgum hydrolysate    |
| Candida tropicalis ceppo 571      | Sulfite waste liquor          |
| Chaetomium cellulolyticum         | Cellulosic wastes             |
| Chrysosporium strophila           | Lignin                        |
| Fusarium graminearum              | Starch hydrolysates           |
| Marine yeast                      | Pawn shell wastes             |
| Mixed cultures of yeasts          | Dairy wastes                  |
| Paecilomyces variolii             | Sulfite liquor                |
| Penicillium cyclopium             | Whey                          |
| Penicillium roqueforti, Penicillium camemberti | Citrus fruit peel |
| Pichia pastoris                   | Methanol                      |
| Saccharomyces cerevisae           | Molasses, Stillage            |
| Schwanniomyces occidentalis       | Starch                        |
| Scytalidium acidophilum           | Waste paper                   |
| Trichoderma album                 | Not disclosed                 |
| Trichoderma reesei & Kluyveromyces marxianus | Beet-pulp         |
| White rot fungi                   | Sugarcane bagasse             |
| Yeast                             | Plant origin liquid waste     |

In producing an SCP, a substrate or media is needed for the growth of microorganisms. In recent years, many studies have used waste as a growth media to produce SCP. One waste that can be used is pineapple skin waste. Pineapple skin is widely used because the level of consumption of pineapple fruit is high enough. The choice of pineapple skin waste as a growth medium is certainly based on the nutrition on it. It contains carbohydrates that are easy to digest. It also contains enzyme, which plays a role in protein digestion [13]. Pineapple skin contains 14.22% dry matter, 81.90% organic matter, 8.10% ash, 0.56% nitrogen, 3.50% crude protein, and 3.49% crude lipid [14].

Rice is a high carbohydrate food. Rice is also the main food of the Indonesian people which will produces waste in the form of rice washing water. Rice washing water contains many organic compounds such as carbohydrates [15]. Rice washing water also contains gluten and vitamins such as niacin, riboflavin and thiamine. It also contains several minerals such as Ca, Mg, and Fe which are needed for fungal growth [16]. Therefore, rice-washing water has potential to be used as a media in the production of SCP. The aim of this study is to determine the best ratio of pineapple skin extract and rice-washing water in the production of SCP using *Saccharomyces cerevisiae*. 
2. Methodology
The instruments to produce SCP were knives, blenders, filters, autoclaves, beaker glass, measuring cups, micropipettes, analytical balance, incubators, and laminar airflow. While the instruments for analysis were pH meters, centrifuge, ovens, UV-Vis spectrophotometers, and cuvettes. Materials used to make SCP were pineapple skin waste taken from traditional market in Bandung, rice washing water taken from household near to our department, distilled water, bread yeast, potato dextrose agar media, sucrose, bovine serum albumin standard solution (1, 2, 3, 4, 5 ppm), biuret solution (CuSO$_4$.5H$_2$O and KNaC$_4$H$_4$O$_6$), MgSO$_4$, (NH$_4$)$_2$SO$_4$, KH$_2$PO$_4$, NaCl, CaCl$_2$, 10% NaOH solution, and CH$_3$COOH solution. This research was done in several steps as follow:

2.1. Isolation of Sachharomyces cerevisiae from bread yeast
A total of 1 gram of bread yeast was put into a test tube then added 9 ml of sterile distilled water ($10^{-1}$) and shaken until homogeneous. Then a $10^{-2}$ dilution was carried out by mixing 1 ml of the previous solution with 9 ml of sterile distilled water. The dilution was carried out up to $10^{-4}$. Furthermore, it was bred in potato dextrose agar (PDA) with sloping agar technique. Then the culture was incubated at 30 °C for 48 hours.

2.2. Producing a starter solution
Nutrients used for the growth of Sachharomyces cerevisiae included 17 grams of sucrose; 0.78 grams of (NH$_4$)$_2$SO$_4$; 0.39 grams of KH$_2$PO$_4$; 0.195 grams of MgSO$_4$; 0.039 grams of NaCl; and 0.039 grams of CaCl$_2$. The ingredients were mixed in 78 ml of distilled water and then the degree of acidity was adjusted to reach pH 5. Then it was sterilized at 121 °C for 15 minutes. The nutrient solution was cooled and then inoculated the S. cereviceae from taste buds $10^{-4}$. The solution was fermented for two days at 30 °C.

2.3. Making a growing media
Growing media was made from pineapple skin extract and rice-washing water in three comparisons that were 1: 1; 2: 1; and 1: 2. Pineapple skin waste used was 850 grams which mixed with 850 ml of distilled water. The rice washing water used was taken from the first washing process. First, the pineapple skin was washed and then mixed with distilled water. Then it crushed using a blender. After that, it was filtered using a filter cloth. Pineapple skin extract and washing water were mixed in the beaker glass with the following ratio:

a. Growing medium 1 consisted of 350 ml pineapple skin extract and 350 ml rice washing water (1: 1), called as P1 in this study.

b. Growing medium 2 consisted of 467 ml pineapple skin extract and 233 ml rice washing water (2: 1), called as P2.

c. Growing medium 3 consisted of 233 ml pineapple skin extract and 467 rice washing water (1: 2), called as P3.

All the growing media were added with nutrients which included 1.5 grams (NH$_4$)$_2$SO$_4$; 0.7 grams KH$_2$PO$_4$; 0.38 grams MgSO$_4$; 0.07 grams of NaCl; and 0.07 grams of CaCl$_2$. After that, the media was added with CH$_3$COOH 1 N and was sterilized at 121 °C for 15 minutes.

2.4. Fermentation
The media was then fermented at 30 °C and 100 ml solution at 8, 24, 32, 48, 56, 72 and 80 hours was taken from each media.

2.5. Sampling
Sampling was carried out at 8, 24, 32, 48, 56, 72 and 80 hours. Before the sample was taken, the sample was shaken until it was homogeneous. Then 100 ml was taken each time. Sampling was done by pouring as much as 100 ml of media into a measuring cup. After that, 100 ml of sample was put into the beaker glass for analysis.
2.6. Analysis
The analysis included pH value, cell dry weight, and protein content. Protein content were carried out using the biuret method in accordance with the AOAC (1995) procedure.

3. Result and discussion

3.1. pH
The highest pH value was obtained by growing media P3 (Figure 1). The lowest pH value was obtained by growing media P2. Previously, three growing media were conditioned at pH 4.5. The analysis results showed that the pH value of the three growing media was in the range of 3.77 to 4.47, which means it was in the normal range. The normal limit of growth for *Sachharomyces cerevisiae* was at pH 2.5-8.5 [9]. The changes in pH in the three growing media did not inhibit the growth of *Sachharomyces cerevisiae*.

![Figure 1](image)

*Figure 1. Effect of growth media and fermentation time on pH values.*

The pH value of the three growing media decreased when entering 24 hours of fermentation. This was due to the formation of organic acids such as lactic acid, acetic acid, and pyruvic acid [17]. The butyric acid and other fatty acids had little effect on decreasing the pH [18]. After 24 hours, the pH continued to increase until the 72nd hours. The pH then decreased again when entering 80 hours. This condition showed that *Sachharomyces cerevisiae* was still actively growing. The pH optimum for *Sachharomyces cerevisiae* was at 4-4.5 [19]. Yeast also grew well in the pH range of 3.0 to 6.0 [20]. Protein disassembly will occur when a media has a pH exceeding the optimum pH [9]. This condition was because the media did not have an adequate source of carbon so that the metabolic process would produce metabolites resulting from protein degradation, which caused an increase in pH.

3.2. Cell dry weight
Based on Figure 2, the largest cell dry weight was produced from P3 growing media. Whereas the smallest dry cell weight was obtained from P2 growing media. This showed that P3 growing media contained higher nutrition than the other two growth media. Therefore, the addition of rice washing water as a growing medium to produce PST was quite effective. This was due to the higher carbohydrate content in rice washing water, which was 85-90% compared to pineapple skin which only contained 10.54% carbohydrates [21].
Figure 2. Effect of growth media and fermentation time on cell dry weight

In addition, when compared to other nutritional content, rice washing water contains more nutrients than pineapple skin. The highest nutrient content of rice is in the epidermis. During rice washing, about 80% vitamin B1, 70% vitamin B3, 90% vitamin B6, 50% manganese (Mn), 50% phosphorus (P), 60% iron (Fe), fiber and essential fatty acids dissolve in water.

Enzymes contained in *S. cerevisiae* cell membranes such as protease, carboxypeptidase, aminopeptidase and invertase played a role in utilizing nutrients in growing media [22]. The highest cell dry weight was produced at 56 hours. This indicates that at that time *Sachharomyces cerevisiae* maximally used a protein, carbon and minerals to synthesize components of cell. Synthesis of these cell components increased the cell dry weight [9]. Whereas at the 72nd and 80th hours, the dry weight of the cell decreased. This was because the nutrients contained in the media would decrease with the increasing fermentation time.

3.3. Protein content

Based on Figure 3, the highest protein content of *Sachharomyces cerevisiae* cells was produced by P3, while the lowest was produced by P2. This was related to the availability of nutrients in a growing media. Rice wash water provides more carbohydrate nitrogen, vitamins and minerals for the proliferation of *Sachharomyces cerevisiae*. Rice washing water contains abundant nutrients consisting of starch, protein gluten, cellulose, hemicellulose, sugar and vitamins [16]. Microorganisms which produced SCP could grow in wastes that contained high carbon and nitrogen. High nutrient containing sugar could provide high energy for the metabolic process of *Sachharomyces cerevisiae*.

The higher nutrients in a media would be able to increase the protein content of SCP. The increase in protein content could be caused by two things. First was by increasing yeast biomass. Second was by increasing yeast cells which acted as a SCP agent. This due to the fact that *Sachharomyces cerevisiae* consists of 50-52% crude protein, 30-37% carbohydrates, 4-5% fat and 7-8% minerals [23]. The highest levels of *Sachharomyces cerevisiae* cell protein produced from P3 occurred at 56 hours. Meanwhile, the highest protein content of *Sachharomyces cerevisiae* cells in P1 and P2 occurred at 72 hours. The protein content in SCP increased in line with the increase in the concentration of carbon sources in the growing media [24]. It could be said that at 56 hours in P3 and 72 hours in P1 and P2 growing media, *Sachharomyces cerevisiae* was utilizing excess carbon sources to continue to multiply which increased protein levels.
Figure 3. Effect of growth media and fermentation time on protein content.

In addition to nutrition, fermentation time also affected cell protein levels. The shorter fermentation time would produce *Sachharomyces cerevisiae* cells that contain low protein levels. This was because of media conversion was not optimal. Based on Figure 3, protein levels decreased when fermentation time entering into 80 hours. Prolongation of fermentation time could cause a decrease in protein of SCP which was caused by auto biodegradation to meet its energy needs in connection with the availability of nutrients in a media that was declining.

4. Conclusion

Three growing media show that pH agrees with the pH for the development of *Sachharomyces cerevisiae*. The highest cell dry weight and protein content were produced by growing media consisting of pineapple skin extract and rice washing water with a ratio of 1:2 (P3) at 56 hours. The present finding reveals that pineapple waste and rice washing water can be used as effective alternative carbon source for SCP production.

5. References

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