**PRODUCTION OF SINGLE CELL PROTEIN FROM BANANA PEEL WASTE IN BATCH FERMENTATION USING *SACCHAROMYCES CEREVISIAE***

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Abstract

Through engineering the fermentation process, it is hoped that new data can be obtained that will explain the ability of Saccharomyces cerevisiae to maximize the production of single-cell protein (SCP). SCP microorganisms have a high protein content, making them suitable for use as a human protein source as well as food additives in the cattle and fishing industries. The goal of this experiment is to see if the microbe Saccharomyces cerevisiae can generate SCP from kapok banana peel waste. Some of the process variables used in this study include the variation in nutrition, fermentation time, and the effect of pH variations on SCP production. Where the variation in pH used is 3; 3.5; 4; 4.5; 5; and 5.5. As for the nutrients used, namely (NH4) 2SO4 and KH2PO4 with a variety of nutrients, namely 0; 0.3; 0.6; 0.9; and 1.2 grams. Then the fermentation time was varied to 1,2,3,4 days. This study also analyzed the growth of microorganism cells using wet weight and dry weight with variations in pH and nutrition. The variation in nutrition is the same as the variation in the previous analysis of protein content, and the fermentation time is 1,2,3,4,5,6, and 7. In the analysis of protein content with Kjeldahl protein, the obtained optimal pH is 4.5 and the optimal protein content is 0.6 grams. As for the fermentation time, the optimal protein content is obtained on the 4th day. For the growth of microorganisms, the optimal pH is obtained at a pH value of 4.5 with optimal nutrition of 0.6 grams, and the optimal fermentation time is obtained on the 7th day.

**Keywords:** Single cell protein; Banana peel waste; Yeast; Saccharomyces cerevisiae; Batch fermentation

1. INTRODUCTION

The growing global population may provide challenges in terms of food consumption, particularly in terms of expanding the usage of protein as a source of food for people and the cattle sector. Climate change, land scarcity, and natural catastrophes are all important issues in the production of conventional proteins in many nations (Huang *et al.*, 2013). Other issues that must be considered when producing protein from plants and animals include the fact that it takes a long time and has relatively high production costs. Bioprocess technology is an intriguing alternative to employing single-cell bacteria to produce proteins (Nasution, Feliatra and Effendi, 2021). As a result, this study is extremely relevant and significant, and it is expected to provide a solution to the problem of traditional protein deficiency (Hülsen *et al.*, 2020). A single cell protein (SCP) is a dried cell of microorganisms that contains protein biomass and may be utilized as a protein source for humans and cattle. SCP is a microbe-derived biomass product with a high protein content. Saccharomyces cerevisiae is a yeast microorganism capable of producing huge quantities of SCP (Nasseri *et al.*, 2011).

Saccharomyces cerevisiae is generally grown on a medium containing Yeast Extract Peptone Dextrose (YEPG) and Yeast Extract Peptone Glycerol (YEPG) (YEPG) (LaTurner *et al.*, 2020). Temperature, pH, oxygen demand, water, and nutrients are all important variables that impact SCP products when using fermentation methods. The protein content of the protein generated throughout the fermentation process will be determined using the Kjeldahl nitrogen technique. The kjeldahl nitrogen technique is a method for determining protein content that has been widely utilized. (Magalhães *et al.*, 2018). According to recent literature research, the SCP creation process is divided into various stages. Preparing microbial growth media, choosing and maintaining microorganisms, selecting carbon sources, sterilizing, fermentation processes, separating and purifying products are the basic process stages carried out in this study (Rasouli *et al.*, 2018). The sample is next analyzed to determine the product composition (protein), cell content, and organic substrate content. The bacteria to be utilized are first chosen, then injected into the medium. Aeration is necessary during the fermentation process if the procedure is carried out under aerobic conditions (Jabart *et al.*, 2020).

 The goal of this research is to see if the bacteria Saccharomyces cerevisiae can manufacture SCP using organic waste as a carbon source. Carbon is derived from a variety of organic waste leftovers from indigenous crops, such as kepok banana peels. Because banana peel waste is rich in nutrients, it is an excellent source of substrate for SCP synthesis (Petersen *et al.*, 2020). As far as we know, there have been relatively few studies and research on the synthesis of microbial protein utilizing local fruits as a low-cost protein source (Zhou *et al.*, 2019). It is intended that this research can be utilized as a reference to improve the SCP manufacturing process and give the greatest option for overcoming the reliance on protein synthesis from plants and animals (Huang *et al.*, 2013); (Kustyawati, Sari and Haryati, 2013). As information, there are very few studies and research on the production of microbial protein using local fruits as a cheap alternative protein source. From this research, it is hoped that it can be used as an additional reference to optimize the SCP production process so that it can provide the best solution to overcome the dependence on protein production from plants and animals. (Patelski *et al.*, 2015).

**2. MATERIALS AND METHODS**

**2.1 Protein and microorganisms sources**

Microorganisms have a greater protein content than fat content. Bacteria have a protein level of 50-65 percent based on cell dry weight, yeast 45-55 percent, while fungi and algae have a protein content of 40 percent. According to the findings, 100 pounds of yeast produces 250 tons of protein in 24 hours. In a year, pond algae may produce 20 tons of dry weight protein per acre (Maryana, Anam and Nugrahani, 2016). Because yeast has a bigger size, it can readily generate protein. It has several benefits in creating protein biomass. Yeast also has a low nucleic acid content, a high lysine level, and can thrive in acidic environments. Yeast can utilise a variety of sugars as a carbon source, which allows it to be employed in a variety of traditional food processing methods. Saccharomyces cerevisiae is the most widely utilized yeast for microbial protein production. Yeast may survive on a variety of sugar-based carbon sources, including molasses, whey, glucose hydrolysis from carbs, and liquid sulfate waste from fruits and skin (Wardani and Agustini, 2017); (Spalvins, Ivanovs and Blumberga, 2018). Saccharomyces cerevisiae offers a number of advantages, including the ability to tolerate higher acidic conditions and the ability to be separated without coagulation using a centrifuge. Saccharomyces cerevisiae grows best at a pH range of 3.5 to 5.5 and at a temperature of roughly 25 oC to 30oC. Saccharomyces cerevisiae possesses round and oval morphological features, with a cell width of 1-5 m and a cell length of 5-30 m. (Muniz *et al.*, 2020). Saccharomyces cerevisiae was employed as a source of microorganisms in this work to generate SCP. Figure 1 depicts the several microorganisms that may be utilized to make SCP.



Figure 1 Various Microorganisms used for PST Production

**2.2 Date and Location**

Some material relating to the study's subject was acquired to help make doing research in the lab simpler. To get specific and up-to-date literature that is closely connected to the title of the study, more investigation through libraries and the internet is required. The procedures for conducting the research are as follows: material preparation, SCP production process, and product separation fermentation process follow basic rules using some of the best references. This research was conducted by the Microbiology Laboratory of the Chemical Engineering Department, Faculty of Engineering, Syiah Kuala University. For the preparation of materials and the maintenance of microorganisms, Saccharomyces cerevisiae was carried out at the Soil and Plants Laboratory, Faculty of Agriculture, Syiah Kuala University.

**2.3 Materials and equipment**

 The equipment used in this study consisted of glassware such as Erlenmeyer, flask, test tube, glass beaker, measuring cup, Petridis, pipette funnel, spatula, blender, cellulose acetate membrane 0.2 m; waterproof cotton, aluminum foil, stainless steel pan, shaking incubator, filter paper, knife, analytical balance, centrifuge, and aluminum foil. The equipment used for the SCP process includes autoclaves, incubators, ovens, shaker tables, and centrifuges. Other supporting equipment includes digital pH/mV meters, digital scales, spectrophotometers, clean benches and thermometers. The main ingredients needed are kepok banana peel waste and the microorganism Saccharomyces cerevisiae. The chemicals used in solid or liquid form include glucose, aquadest, (NH4)2SO4, KH2PO4, DNS reagent, buffer solution for pH and cellulose acetate.

**2.4 Research procedures and stages**

The production of SCP from kepok banana peel waste is divided into 4 stages, namely:

**Material preparation**

A blender is used to crush the kepok banana peel waste. The kepok banana peel is cleaned before mixing. The results are filtered to remove all but the juice. The kepok banana peel extract is then cooked in an autoclave for 1 hour at 1 atm pressure and 121oC temperature. This solution is referred to as the fermentation medium once it has been cooled to room temperature.

**Making a starter**

A total of 22.4 grams of glucose was dissolved in 100 ml of distilled water. The pH of the solution was made in 5 variations, then added 0.5 grams of nutrients in the form of (NH4)2SO4 and KH2PO4. Then sterilized by heating using an autoclave for 1 hour. After the cold solution, put the yeast Saccharomyces cerevisiae into the batch fermenter. Then, followed by shaking for the fermentation process for 2 days.

**Fermentation process**

Enter the fermentation medium into the Erlenmeyer and add nutrients in the form of (NH4)2SO4 and KH2PO4 with several variations. A solution of fermentation media that has varied pH value is then sterilized for 1 hour using an autoclave. In the pH solution that has varied, optimal nutrients are added. After the solution has cooled, add the starter and shake it in the batch fermenter using 4 variations of fermentation time. When testing time variations, also use the optimal pH value.

**Wet and dry weight test**

Media that has fermented for 1-7 days. At the time of testing the wet and dry weight, 10 ml of the sample is taken every day, then centrifuged for 10 minutes at 1500 rpm. Then, the sample is filtered using filter paper and weighed to get the wet weight, then dried at a temperature of 130 ᵒC to dry and then weighed.

1. Results and discussion

**3.1 Autoclave Temperature Calibration**

Microbial growth is the process of a microbial cell component's number or form changing on a regular basis. The microbial cell divides into two daughter cells once it has grown to a size that is bigger or almost twice that of the previous components. The doubling time is the amount of time it takes for one cell to grow and mature before dividing into two cells. The process of an organism's cell mass development is heavily influenced by external factors and cellular nutrition. Microorganisms' growth is evaluated in terms of the number of cells produced rather than the size of the cell mass.

Figure 2. Autoclave Temperature Calibration for Feasibility of Sterilization

Figure 2 shows the results of the autoclave's medium sterilization tests. The calibration results show that the autoclave equipment's maximum temperature is 121 oC. The media and equipment sterilization time lasted for 15 minutes, starting from the 45th minute to the 60th minute. It is recommended that, in order to avoid mishaps caused by excessive pressure and temperature.

**3.2 Standard curve of glucose and yeast growth**

The HK Sigma technique is used to determine glucose in Figure 3. Sugar concentrations ranging from 5 to 55 mg/l are determined using this technique. The sample must be diluted if the sugar content is more than 55 mg/l. The sugar concentration value to be examined is multiplied by the dilution factor.  The microbes used here are Saccharomyces cerevisiae obtained from the Bioprocess Laboratory. The subcultures are stored at 4 oC and the function of the subcultures is to maintain the media and keep it durable.  To determine the protein content of Kjeldahl, 0.51 g of the sample was weighed and then put into a 100 ml Kjeldahl flask. Add 2 grams of a mixture of selenium and 25 ml of concentrated H2SO4. Using an electric heater or a fire burner, heat for 2 hours until the solution boils and becomes clear and greenish.

Figure 3. Determination of the Glucose Standard Curve using the HK Sigma Method

After the solution is cooled, it is diluted in a 100 ml volumetric flask. 5 ml of the solution is pipetted and put into a distiller. 5 ml of 30% NaOH and a few drops of Phenolphthalein Indicator are added and distilled for about 10 minutes. Mix 10 mL of a 2% boric acid solution with the indicator. After that, the cooler's tip is washed with distilled water and titrated with 25 mL of 0.01 N HCl solution, with the blank determined.

Calculation:

Protein content = (𝑉1 − 𝑉2) 𝑥 𝑁 𝑥 0,014 𝑥 𝑓𝑘 𝑥 𝑓𝑝 𝑥100% 𝑤

Description:

w = Snippet weight

V1 = The volume of HCl 0.01 N used for titrating the sample

V2 = Volume of HCl used blank titration

N = Normality HCl

Fk = Conversion factor for protein from:

 - food in general : 6,25

 - milk and its products : 6,38

 - peanut butter : 5,46

Fp = Dilution factor

**3.3 Effect of pH on protein content**

In this study, Saccharomyces cerevisiae was the microbe employed to make single-cell protein from banana peel. The pH value was changed from 3 to 5.5 to examine the influence of pH on protein content in the fermentation batch, namely 3; 3.5; 4; 4.5; 5; and 5.5. The nutrients employed are (NH4)2SO4 and KH2PO4 at 0.5 grams each, with a 48-hour fermentation period (2 days). Figure 4 depicts the impact of pH changes on the product's protein content. The higher the pH value, the more protein is generated, as seen in Figure 4. In Figure 4, protein content is 0.9671, 1.2773, 1.9716, 2.6517, 2.1758, and 1.6468 percent.

Figure 4. Effect of pH on protein content

The maximum level of protein production is produced at pH 4.5, then begins to decrease significantly due to the osmotic pressure of the solution. The higher the osmotic pressure of the solution, the more Saccharomyces cerevisiae die as a result of cell wall rupture, lowering the protein concentration of the final product (Pawignya, 2011).

**3.4 The effect of pH on microbial cell weight in the product**

Figure 5 shows the effect of changes in pH values on the growth of the microorganisms Saccharomyces cerivisae. The pH variation utilized in this experiment is the same as the pH value used to determine the protein content of foods. The following graph depicts the influence of pH on yeast growth. Figure 5 depicts a clear relationship between pH changes and microbial cell weight. In batch fermentation, the higher the pH, the faster Saccharomyces cerevisiae grows.

Figure 5. Relationship of pH variation to microbial cell weight

Figure 5 shows that the greatest microbial growth occurs at a pH of 4.5. At pH levels ranging from 3.5 to 5.5, the rise in microbial cell weight was observed. Meanwhile, the microbial development of Saccharomyces cerivisae begins to decelerate around pH 5.0 to 5.5. The weight of microbial cells obtained in Figure 6 is 2.19; 2.48; 2.56; 2.67; 2.45; and 2.23 grams. This is because the growth of microbial cells is extremely tolerant of variations in the pH value of the environment. In this investigation, the optimum pH value was found to be 4.5. Changes in pH have a direct proportionate influence on the protein content of the fermentation batch.

**3.5 Effect of addition of nutrients on protein levels**

Figure 6 shows the effect of adding nutrients to protein content to evaluate the effect of adding nutrients to protein content.  The addition of nutrients (NH4)2SO4 and KH2PO4 into batch fermentation is varied into 5 variations, namely 0; 0.3; 0.6; 0.9 and 1.2 grams with a fermentation time of 48 hours (2 days). The pH value of 4.5 was chosen since it is the pH value under ideal conditions. Figure 6 shows that the addition of 0.6 grams of nutrients results in the maximum protein content, followed by a steady reduction in protein content with the addition of 0.9 to 1.2 grams of nutrients. This is due to the initial conditions of Saccharomyces cerevisiae microbes requiring a lot of nutrients for the microbial growth process. However, the addition of nutrients in excess can disrupt the balance of yeast growth metabolism in batch fermentation. In Figure 6, it can also be seen that the percentage of product protein content in batch fermentation is respectively 1.0641; 1.6515; 2.2540; 1.9152; 1,7354%.

Figure 6. Relationship of the amount of nutrients (grams) to protein content

**3.6 Effect of the addition of nutrients on microbial cell growth**

The main purpose of this study was to see the effect of adding nutrients on yeast growth. Nutrients are substances that are indispensable for bacterial growth, such as nitrogen, carbon, sulfur, phosphate, potassium, and others. Each microbe requires certain types of nutrients for growth, and nitrogen is the main source of nutrients in culture media and is needed by almost all microbes.  In Figure 7, it can be seen that the maximum yeast growth occurred by adding 0.6 grams of nutrients and the pH value was 4.5. Figure 7 shows the weights of each microbial cell, which are 1.78, 1.95, 2.07, 2.04, and 1.95 grams, respectively. Figure 7 depicts the effect of nutrient additions (NH4)2SO4 and KH2PO4 on yeast development.

Figure 7. Relationship of addition of nutrients (NH4)2SO4 and KH2PO4 to yeast growth

The nutrients used are 0; 0.3; 0.6; 0.9 and 1.2 grams with a fermentation time of 48 hours. In Figure 7, it can be seen that bacterial growth is increasing because the supply of nutrients for microbial cell growth is sufficient (Nurmalasari and Maharani, 2020).

**3.7 Effect of variation in fermentation time on product protein content**

Figure 8 shows the influence of fermentation duration on protein concentration in batch fermentation. The substrate utilized in this study is organic waste from local fruits, namely kapok banana peels. The goal of this experiment is to examine how different fermentation times affect the protein content of the product generated by the bacterium Saccharomyces cerevisiae. In this study, they observed changes in fermentation time starting from the first day to the fourth day with a nutritional weight of 0.6 grams and a pH of 4.5. The nutritional weight and pH values utilized are the weight and pH of the nutrients in ideal conditions.

Figure 8. The relationship of fermentation time (days) to protein content

Figure 8 depicts the influence of fermentation time on the product's protein concentration. From the figures, it can be seen that the longer the fermentation time, the higher the protein content. This is because in batch fermentation, the longer the fermentation period, the more yeast grows or develops.

**3.8 The effect of variation in fermentation time on yeast growth**

Figure 9 describes the effect of fermentation time on yeast growth in batch fermentation. The microbiological growth of Saccharomyces cerivisae was studied in this study for seven days, from the first to the seventh. The chosen pH value is 4.5, and the nutritional weight is 0.6 grams.

Figure 9. The relationship between fermentation time and yeast growth

Figure 9 shows that the trend of yeast growth is increasing from day one to day five. Microbial division is highly rapid and steady at this phase, allowing microbial cells to have the most metabolic processes. However, after day 5, the growth of Saccharomyces cerivisae bacteria began to slow down. This is due to the fact that the amount of nutrients in batch fermentation is decreasing, causing most microorganisms to die (Petersen *et al.*, 2020).

1. Conclusion

The determination of glucose concentration and standard dry biomass can be used for related studies in bioprocesses using various carbon sources and Saccharomyces cerivisae as microbes. The Kjeldahl protein determination procedure takes a long time and is complicated, so it is recommended to test protein content using other methods. Kepok banana peel waste can be utilized as an alternate carbon source for single-cell protein production. According to the findings of this study, the optimum conditions for producing the maximum protein content are at pH 4.5, with nutrient levels of (NH4)2SO4 and KH2PO4 of 0.6 grams each, and the best fermentation time is 4 days. Meanwhile, the optimum conditions for wet and dry weight are obtained at pH 4.5, nutrients in the form of (NH4)2SO4 and KH2PO4 are 1.2 grams, and the best fermentation duration time is 5 days.

1. Acknowledgment

This research was funded by Syiah Kuala University, Ministry of Research, Technology and Higher Education (Lector Based Research, No. 522/UN11/SPK/PNBP/2019).

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