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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 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 $(NH_4)_2SO_4$ and KH_2PO_4 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.

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 et al., 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).

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Saccharomyces cerevisiae is generally grown on a medium containing Yeast Extract Peptone Dextrose (YEPD) and Yeast Extract Peptone Glycerol (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). Figure 1 depicts the several microorganisms that may be utilized to make SCP.

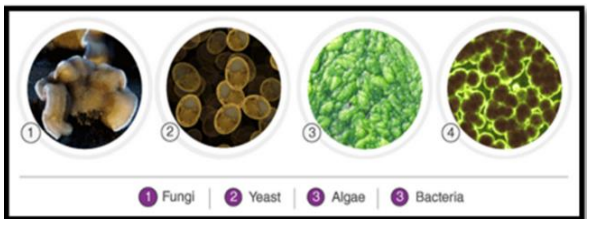

Figure 1. Various Microorganisms used for PST Production.

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 banana peel waste. 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). 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).

MATERIALS AND METHODS

Materials

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 local banana peel waste and the microorganism Saccharomyces cerevisiae. The chemicals used in solid or liquid form include glucose, aquadest, $(NH_4)_2SO_4$, KH_2PO_4, DNS reagent, buffer solution for pH and cellulose acetate.

Methods

In general, this research procedure is divided into 5 main stages, namely: preparation of materials and equipment, making a starter, fermentation process, separation process, and product analysis. The fixed variables selected in this study were microbial yeast (*Saccharomyces cerevisiae*), solvent (aquadest), and carbon source (banana peel waste). The independent variables selected in this study were the initial pH of the media, the source of nutrients KH_2PO_4 and $(NH_2)SO_4$ and the fermentation time. This research was conducted on three main factors. The first factor varied the pH into 6 variants, namely 3; 3.5; 4; 4.5; 5; and 5.5. The second factor is the variation of the fermentation time, namely 1, 2, 3, and 4 days. The third factor is varied nutrition, namely

$(NH_4)_2SO_4$ and KH_2PO_4 with 5 variants, namely 0; 0.3; 0.6; 0.9; 1.2 grams. The data obtained will be analyzed for protein content and cell weight. The research procedures and stages are as follows:

Material preparation

During the material preparation stage, a blender was used to crush the local banana peel waste before it is cleaned and mixed well. Then, it was filtered to remove the residue from the juice. After that, the local banana peel extract was cooked in an autoclave for an hour at the pressure of 1 atm pressure and temperature of 121°C. The solution was finally cooled down to room temperature, where it was also referred to as the fermentation medium.

Making a starter

The second stage involved the making of a starter. A total of 22.4 grams of glucose was dissolved in 100 ml of distilled water and was divided into 5 solutions according to its pH variation. Then, 0.5 grams of nutrients in the form of $(NH_4)_2SO_4$ and KH_2PO_4 was added. This was followed by sterilizing the solution by heating it using an autoclave for an hour. It was then waited to be cooled prior to add the Saccharomyces cerevisiae yeast into the batch fermenter. The fermenter was shaking well to allow the fermentation process to occur within 2 days.

Fermentation process

The fermentation medium was inserted into the Erlenmeyer flask and nutrients are added in the form of $(NH_4)_2SO_4$ and KH_2PO_4 with several variations. Then, the fermentation media with varied pH value was sterilized for an hour using an autoclave. The optimal nutrients were also added in those variety pH solution. After the solution had been cooled, the starter was added and the solution was shaken in the batch fermenter using 4 different fermentation time for comparison. Here, the optimal pH value was utilized while varying the time.

Separation of fermented cells

After the fermentation process was complete, the entire media was separated to obtain the composition of cells and proteins using a centrifuge at a speed of 10 thousand rpm for 10 minutes. The supernatant (liquid) was stored for analysis of the remaining carbon source content. Then the cells were washed once with ditiled water and followed by repeated centrifugation. Then the cells were dried using an oven at 80 °C to obtain a constant dry cell weight. In the end, samples were taken to analyze the content and composition of cells and proteins.

Wet and dry weight test analysis

Product analysis includes wet and dry weight tests. The dry cell weight content and the percentage of protein content in the cells were analyzed using the Kjeldahl method. While the reduction of glucose from carbon sources can be analyzed using the DNS method. Once the media has been fermented for about 1-7 days, testing is carried out. During the testing, 10 ml of the sample is taken daily and then centrifuged for 10 minutes at 1500 rpm. After that, the sample is filtered using a filter paper and weighed to obtain the wet weight before it is dried at temperature of 130 °C and is weighed again to find the dry weight of the media. The schematic of the research stages, which include preparation of materials and equipment, making a starter, fermentation process, separation process, and product analysis, can be seen in Figure 2.

RESULTS AND DISCUSSION

Autoclave Temperature Calibration

Microbial growth is the process of a microbial cell component's number or form changing on a regular basis. Microbial cells divide into two new cells after growing to a size that is larger or almost double the previous component. 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 (Wardani & Agustini, 2017).

Figure 3 shows the results of the autoclave's medium sterilization tests. The calibration results show that the autoclave equipment's maximum temperature is 121 °C. 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.

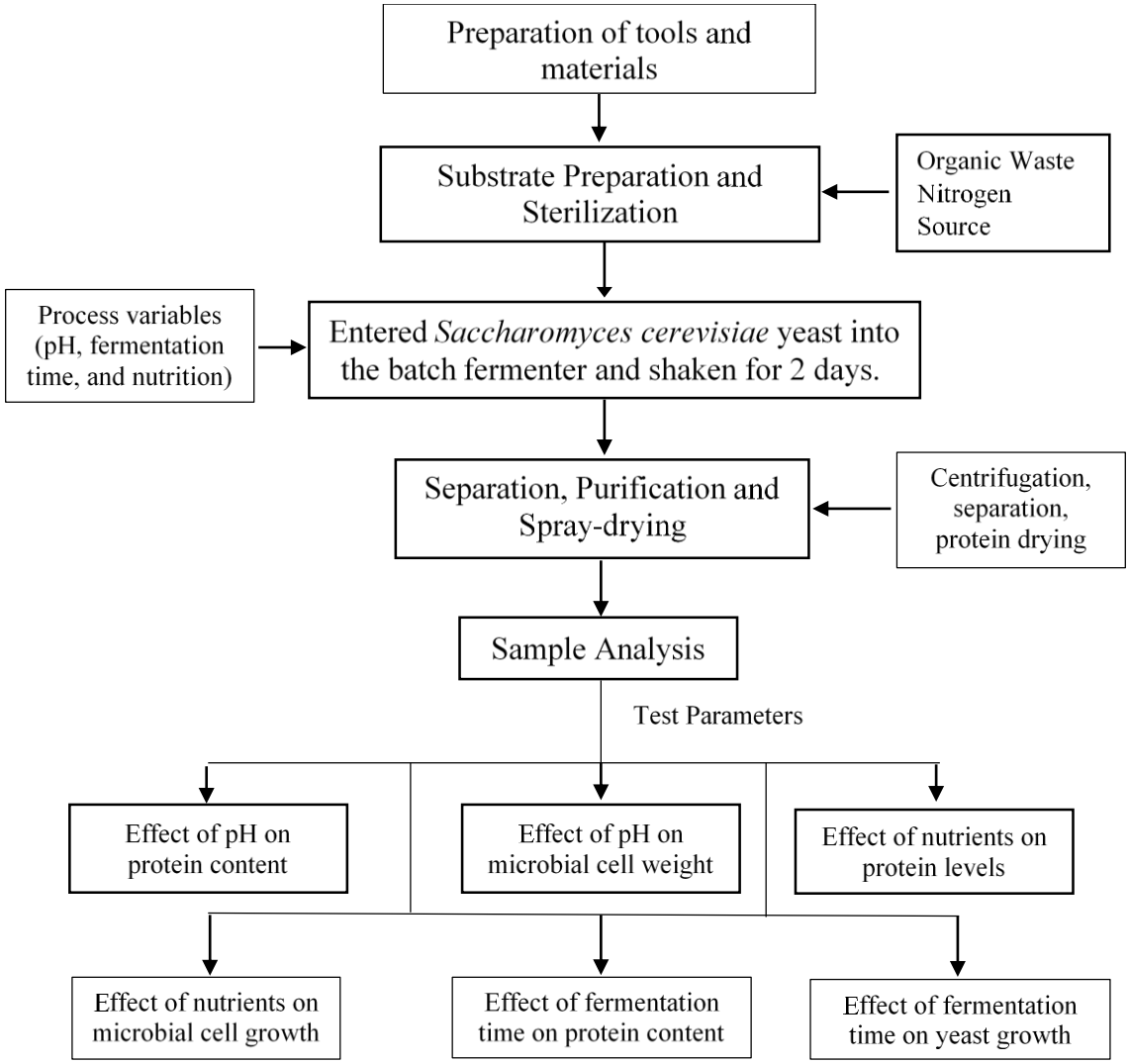

Figure 2. Flowchart of research procedures and SCP production scheme.

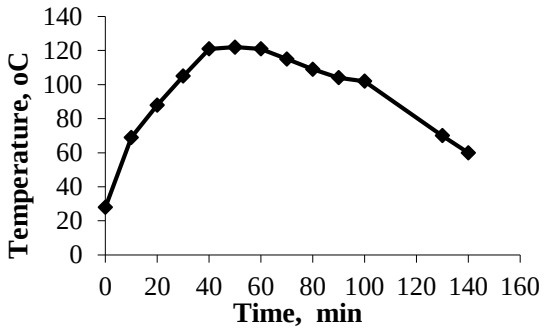

Figure 3. Autoclave Temperature Calibration for Feasibility of Sterilization.

Effect of pH on protein content

pH is one of the important parameters in the fermentation process because it is directly related to the growth rate of microorganisms in the bioreactor. The initial pH value for the initial growth of the media is around pH 3–pH 5 (Wardani and Agustini, 2017). According to Maryana et al., (2016), in the early stages of fermentation, the appropriate acidity level was positively correlated with the growth rate of Saccharomyces cerevisiae. In addition, the relatively low acidity can inhibit the growth of other contaminating bacteria, so that it can increase the rate of growth of the yeast *Saccharomyces cerevisiae*. 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 $(NH_4)_2SO_4$ and KH_2PO_4 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 produced, as shown in Figure 4, where the maximum protein production level is produced at

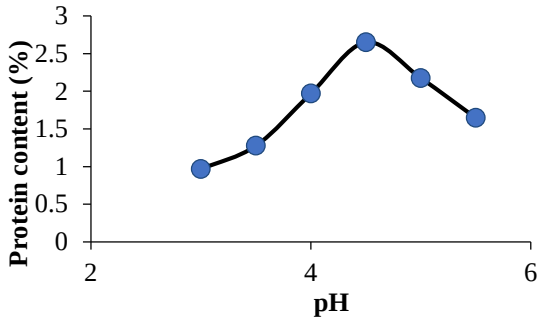

Figure 4. Effect of pH on protein content.

pH 4.5. This is because these conditions are very suitable for the environment needed for the growth of these bacteria. At a pH value of pH 3 to pH 4.5, *Saccharomyces cerevisiae* was able to convert the substrate into protein significantly, but after pH 5, the protein concentration decreased. This was due to the protein denaturation process, which means the biological activity of *Saccharomyces cerevisiae*. The decrease in the level of protein production is also caused by the osmotic pressure of the solution, which causes changes in the molecular structure and solubility due to the breakdown of the cell wall. This event resulted in more Saccharomyces cerevisiae dying due to the rupture of the cell wall, thereby reducing the protein concentration of the final product (Kustyawati et al., 2013).

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 microorganisms. The pH variation used in this experiment is the same as the pH value used to determine the protein content of food. The following graph illustrates the effect of pH on yeast growth in a bioreactor. Figure 5 shows a clear relationship between changes in pH and the weight of microbial cells. The occurrence of changes in the pH value is related to the kinetics of the growth of microorganisms in the bioreactor. The lag phase is the initial phase in which the cells inoculated with the medium are still at the adaptation stage to the environment. In this phase, the number of microbial cells in the product is still low (Muniz et al., 2020).

In batch fermentation, the higher the pH value, the faster *Saccharomyces cerevisiae* grows in the bioreactor. Figure 5 demonstrates that microbial growth was best at pH 4.5. An increase in microbial cell weight was found at pH levels ranging from 3.5 to 5.5. At pH 3, the increase in microbial cell weight in the product began to appear at pH 3. At this pH, the growth of *Saccharomyces cerevisiae* began in the bioreactor. This second phase is also called the exponential phase, where cell division and microbial metabolic reactions begin to increase. In this phase, microbial cell division occurs very quickly and constantly, so that it affects the total weight of microbial cells in the product (Spalvins et al., 2018).

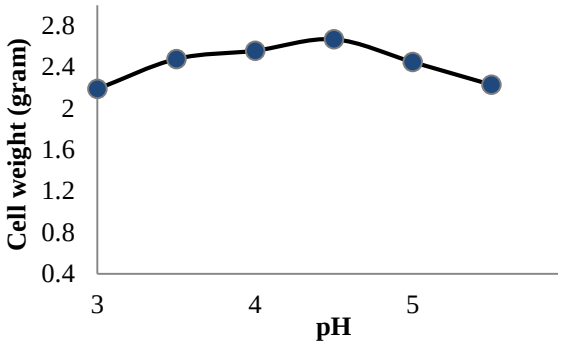

Figure 5. Relationship of pH variation to microbial cell weight.

In Figure 5, it can be seen that the microbial growth of *Saccharomyces cerivisae* has started to slow down in the pH range of 5.0 to 5.5. The growth of the number of microbes has begun to decline. The reproduction of new cells has been balanced with the number of dead cells. This phase is also called the stationary phase because the growth rate of *Saccharomyces cerivisae* in the bioreactor is zero. In this phase, the kinetics of microbial growth in the bioreactor decreased sharply due to the reduced amount of nutrients in the medium. An increase in the buildup of toxin metabolism in the bioreactor also causes the death of a number of cells. The weight of microbial cells obtained in Figure 5 is 2.19, 2.48, 2.56, 2.67, 2.45, and 2.23 grams. In this investigation, the optimum pH value was found to be 4.5. Changes in pH have a direct proportional effect on the total microbial cell weight of the fermented batch. This is because the growth of microbial cells is very tolerant of variations in environmental pH values (Maryana et al., 2016).

Effect of addition of nutrients on protein levels

Figure 6 shows the effect of adding nutrients to the medium and the correlation to the total protein content in the bioreactor. The addition of nutrients $(NH_4)_2SO_4$ and KH_2PO_4 into batch fermentation was varied into five 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 because it is the pH value under ideal conditions. The protein content in the bioreactor is related to the availability of nutrients in the medium. The availability of nutrients in the medium determines how fast the microbial growth rate of *Saccharomyces cerevisiae* will be. The higher the nutrient content of the medium with various compounds such as nitrogen, vitamins, sugars, and minerals for the metabolic process of Saccharomyces cerevisiae, the higher the possibility of producing protein levels in the reactor (Magalhães et al., 2018).

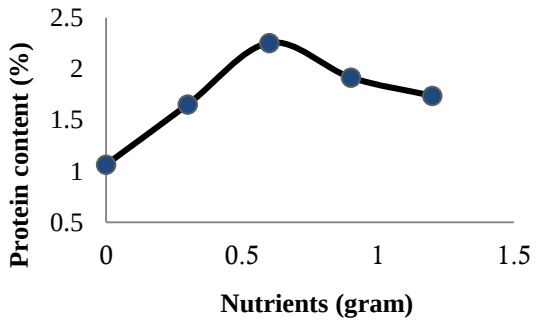

Figure 6. Relationship of the amount of nutrients (grams) to protein content.

In Figure 6, it can also be seen that the percentage of product protein content in batch fermentation was 1.0641; 1.6515; 2.2540; 19152; and 1,7354. Figure 6 shows that the addition of 0.6 grams of nutrients can produce maximum protein content, but when the addition of 0.9 to 1.2 grams of nutrients, the level of protein production in the reactor gradually decreases. This is because the initial conditions of *Saccharomyces cerevisiae* microbes require a lot of nutrients for the microbial growth process. However, the addition of excessive nutrients can disrupt the balance of yeast growth metabolism in batch fermentation.

Effect of the addition of nutrients on microbial cell growth

The main objective of this study was to examine the effect of adding nutrients on the growth rate of *Saccharomyces cerevisiae* in a bioreactor. Nutrients are compounds that are indispensable for bacterial growth, such as nitrogen, carbon, sulfur, phosphate, potassium, and others. Each microbe requires certain types of nutrients for its growth, and nitrogen is the main source of nutrients in the culture media required by all microbes. During the breeding period, *Saccharomyces cerevisiae* utilizes the nutrients in the medium for cell growth. An increase in the amount of cell mass indicates an increase in microbial growth in the bioreactor. The higher the cell growth rate in the reactor means the higher *Saccharomyces cerevisiae* requires nutritional elements such as C, H, O, N, S, P, K, and various minerals such as Fe, Mg, Na, and Mn. Bacterial growth increases and is constant because the supply of nutrients for microbial cell growth is sufficient (Maryana et al., 2016).

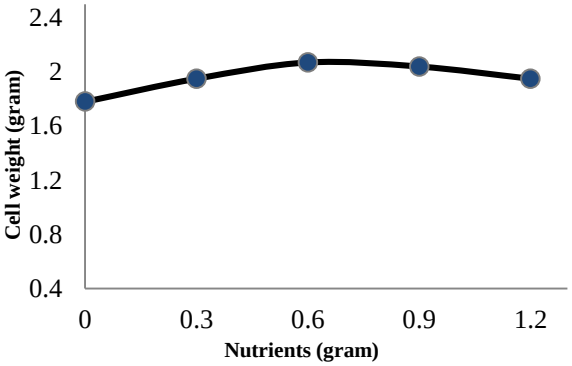

Figure 7. Relationship of addition of nutrients $(NH_4)_2SO_4$ and KH_2PO_4 to yeast growth.

In Figure 7, it can be seen that the maximum yeast growth occurred with the addition of 0.6 grams of nutrients and a pH value of 4.5. Figure 7 shows the weight of each microbial cell, namely 1.78, 1.95, 2.07, 2.04, and 1.95 grams, respectively. Figure 7 illustrates the effect of the addition of nutrients $(NH_4)_2SO_4$ and KH_2PO_4 on the development of yeast. The nutrients used are 0; 0.3; 0.6; 0.9; and 1.2 grams with a fermentation time of 48 hours.

Effect of variation in fermentation time on product protein content

Figure 8 shows the effect of fermentation time on protein concentration in batch fermentation. The substrate used in this study is organic waste from banana peels. The purpose of this experiment was to examine the effect of the difference in fermentation time on the protein content of the product produced by the bacterium Saccharomyces cerevisiae. In this study, we observed changes in fermentation time starting from the first day to the fourth day with a nutritional weight of 0.6 grams at pH 4.5. Nutrient weights and pH values used are weights and pH values under ideal conditions.

Figure 8 shows that the lag phase lasts for 0–1 days, during which time *Saccharomyces cerevisiae* is still adapting to the environment. The figure shows that the effect of fermentation duration on the protein product was not substantial throughout this time period. Then, during the log phase, which lasts 1–4 days, *Saccharomyces cerevisiae* bacteria interact with the substrate, causing the protein production process to accelerate.

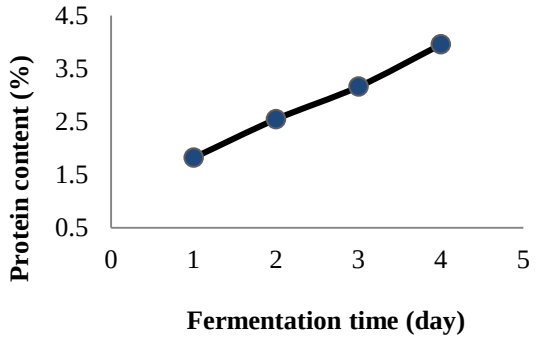

Figure 8. The relationship of fermentation time (days) to protein content.

Protein production began to diminish after entering the stationary phase, which occurs around 4.5 days and is the optimum time for protein production in the reactor. After that time, protein production began to decline due to a decrease in the amount of substrate in the medium. The accumulation of protein formed and the effect of protein degradation are also two of the inhibiting factors for bacterial growth in bioreactors. From the figure, it can be concluded that the longer the fermentation time, the higher the protein content. This is because, in batch fermentation, the longer the fermentation period, the more Saccharomyces cerevisiae will grow or develop in the bioreactor (Rasouli et al., 2018).

The effect of variation in fermentation time on yeast growth

Figure 9 describes the effect of fermentation time on yeast growth in batch fermentation. In this study, the time period for the growth of *Saccharomyces cerevisiae* in the bioreactor was observed for seven days, from the first day to the seventh day. The pH value chosen is 4.5, with a nutritional weight of 0.6 grams. Based on the research results, the maximum production growth of *Saccharomyces cerevisiae* can be achieved on the fifth day with an ambient temperature of around 30 ^{o}C. From the observations, it was also found that temperatures that were too low or too high could reduce the efficiency of the fermentation process.

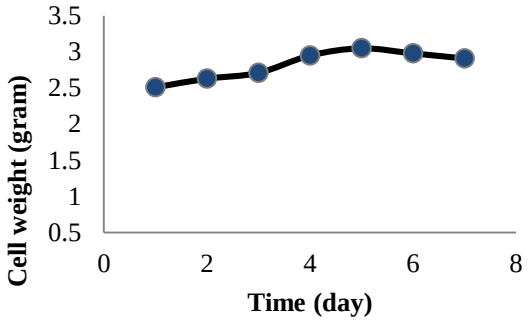

Figure 9. The relationship between fermentation time and yeast growth.

Figure 9 shows that the growth trend of yeast increased from the first day to the fifth day. Microbial division is very fast and stable in this phase, allowing the microbial cell to carry out the most metabolic processes. On days 1–5, Saccharomyces cerevisiae can develop exponentially because new cells divide at a steady pace. Based on the literature study, it was found that the appropriate time for the fermentation process is about 1–6 days. Variations in fermentation time are required to identify the appropriate fermentation time by analyzing the influence of each time variation on the growth rate of Saccharomyces cerevisiae. According to the observations, the growth of *Saccharomyces cerevisiae* began to decelerate after the fifth day. Microbial growth in the bioreactor begins to experience a decline in population and enters a slow growth phase. This is because the amount of nutrients in batch fermentation is decreasing and there is an accumulation of toxic waste products, causing most of the microorganisms to die (Petersen et al., 2020).

CONCLUSION

Determination of glucose concentration and standard dry biomass can be used for related studies in bioprocesses using various carbon sources and *Saccharomyces cerevisiae* as microbes. The Kjeldahl protein determination procedure is time-consuming and complicated, so it is recommended to test the protein content using another method. Banana peel waste can be used as an alternative carbon source for single-cell protein production. Control of the appropriate acidity level was positively correlated with the growth rate of

Saccharomyces cerevisiae and single cell protein production in bioreactors. A relatively low or high acidity level can inhibit the growth rate of the yeast *Saccharomyces cerevisiae*. From the observations, it can be concluded that the administration of a substrate or nutrient concentration that is too high will increase the osmotic pressure, thereby reducing the efficiency of the fermentation process. Based on the results of the study, the optimum condition to produce maximum protein content was at pH 4.5, with nutrient content of $(NH_4)_2SO_4$ and KH_2PO_4 of each 0.6 grams, and the best fermentation time was 4 days. The optimal pH level is 4.5, with nutrients of $(NH_4)_2SO_4$ and KH_2PO_4 weighing in at 1.2 grams each and fermentation time of 5 days. From the results of the study, it can be concluded that the length of fermentation time, the number of microorganisms used, operational conditions, substrate concentration, and pH all have a major effect on single-cell protein production.

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