PRODUCTION OF (SACHAROMYCES CEREVISIAE) (FNCC-3049) IN THE FLOUR OF BANANA SKIN CULTURE IN THE AEROBIC CONDITION

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Abstract.

Sacharomyces cerevisiae (FNCC-3049) have been produced in the flour banana skin culture in the aerobic condition at room temperature. The parameters studied were the ratio of starter to the volume of medium, to the number of cells generated. The research was conducted through several steps including: (i) the breeding, (ii) preparation of banana flour skin, (iii) hydrolysis, (iv) inoculation, (v) fermentation and (vi) separation of the product. Analysis of cell numbers using haemacytometer. Growth of cells is done by inserting inoculants into each 200 ml of sterile fermentation culture at a various ratio of starter/culture. Air flowed into the culture and the fermentation performed for 24 hours. Analysis of cell number is conducted for every 3 hours until 24 hours. After 24 hours, cells were separated by centrifugation and dried at 40^{o}C and then weighted. At incubation time of 9 hours the number of cells found = $10^{6.11}$. In these conditions obtained dry weight of the product is 51.9052 grams.

Keyword: S. Cerevisiae, banana skin, aerobic fermentation.

I.Introduction.

Sacharomyces cerevisiae is an important additive in the food industry and industries that produce wine and beer (alcoholic beverages) [1]. The results have shown that *S.*

cerevisiae can be grown in several types of media such as glycerol, vegetable waste such as cabbage and fruit waste, [2] [3] and materials containing sugars such as molasses [4]. The content of total sugar, reducing sugar, and starches for the bananas skin in the range of 7.96, 6.85%, and 7.83% weight and contain proteins that can be used as a medium for growing cells of microorganisms is one example of the kind of baker's yeast *(Saccharomyces cerevisiae).*

Saccharomyces cerevisiae was chosen for its ability to use various types of sugar [5], has the highest digestibility properties when compared with other types of microorganisms [6], which is more tolerant of acidic environment with pH ranging from 4.5 to 5, so the process can take place in the net without having to sterile media [5]. Another report mentioned that the pH optimum growth of *Saccharomyces cerevisiae* is 3.5 to 4.5 [7], large cell diameter of about 0.0005 cm, so it can be separated from the growing medium by centrifugation without the need for coagulation phase [7], easily available and commonly used in the manufacture of bakers yeast [8]. By looking at some of the considerations above, the authors try to make bakers' yeast with the growing medium is a banana skin.

The characteristics of *Saccharomyces cerevisiae* cells are oval or round. Fixed cell shape, so it can help for identification. Cell diameter of approximately 0.0005 cm [9] and facultative microorganisms [10] [11].

Factors affecting the growth of *Saccharomyces cerevisiae* in the fermentation process are: (i) water. As with other living beings, the *Saccharomyces cerevisiae* requires water to life, grow, and reproduce. For those reasons, during the fermentation process must be available water in sufficient quantities. *Saccharomyces cerevisiae* can life in aw (water activity) in the range of 0.88 to 0.99, but also can life at aw 0.68 to

0.78 [7], (ii) Nutrients (element or compound required by yeast in order to grow and multiply), namely: C, N, F, Mg and vitamins. In the aerobic process conditions, the ratio of the elements C: N: P is 100:5:1. Optimum growth temperature of *Saccharomyces cerevisiae* is 30^{o}C, whereas the minimum temperature is (9 -11) ° C and maximum temperature is (35-37) ^{o}C [7].

Sufficient aeration is one of the most important factor in the production of yeast. The purpose of aeration is to supply free oxygen into the fermentation medium during the process. The amount of oxygen depends on the process of fermentation. If the process is anaerobic, it does not need free oxygen and the resulting product is alcohol. Aerobic process, requiring free oxygen and generate cells. In the anaerobic process, which lasted only glycolysis metabolism and will produce 2 moles of ATP, whereas the aerobic process, glycolysis and the Krebs cycle, both can take place in the cell and produce 38 moles of ATP. The more ATP is produced, the more cells are produced [8] [12]. Aeration is done by using a spurger to create the bubbles, to enlarge the surface contact between the air with the media [13].

II.Materials and Method

The methodology describes the raw materials, auxiliary materials, equipment and the experiment. In this paper only describes the process of fermentation to produce cells in the form of active dry cell.

2.1 Materials.

Raw materials for cell growth media is green banana skin, pure cultures (*Sacharomyces cerevisiae*) (FNCC-3049), culture media starter (bean sprouts, sugar, agar, ammonium

sulfate, hydrochloric acid 37 wt.%, Natrium hydroxide (NaOH) 99 wt. %). Auxiliary materials for sterilization is (alcohol 70%, paraffin liquid and sterile cotton).

2.2 Equipment.

Balance, pH sticks, microscopes, glass cover, haemocytometer, vacuum oven, thermometer and filter paper (Whatman 93). Process equipment such as knives, blenders, electric stove, stirrer, filter, autoclave, test tubes, pipettes, needle Ose, a set of glassware, shakers, aerator, flow meter, centrifuges and counter.

2.3 Experiment.

2.3.1 The process to growth of *Saccharomyces cerevisiae* in a sterile medium (inoculation medium).

The composition of medium for cell inoculation are: 12.5 g of a Flour Banan Skin and 2.5 g for sprouts in the form of powder into a glass beaker that has been sterilized and then dissolved with aquadest until the volume of solution is 125 ml and boil for 15 minutes to obtain homogeneous solution. Furthermore, the solution is filtered and cooled. Into the solution was added 7.5 g of sugar and reboil to obtain the culture media. The solution then inserted into the reaction tubes that have been sterilized and then covered with cotton that has been soaked in paraffin. Test tube containing sterile solution then inserted into the autoclave at the temperature of 121^{o}C for 15 minutes. Subsequently cooled to room temperature and turned in to solid. Next, to the solid media planted each two ose of pure cultures of *Saccharomyces cerevisiae* using streak plate method, and then incubated at room temperature for 48 hours. After 48 hours obtained the sub-master's culture of *Saccharomyces cerevisiae*.

2.3.2 Preparation of Starter

Starter made by inoculating 1 ml of pure cultures of *Saccharomyces cerevisiae* that has been dissolved into sterile medium in the 5 pieces of sterile Erlenmeyer, each of which contained the media that have the pH in the range of 3, 3.5, 4; 4.5; 5. Then incubated while shaken with a shaker at room temperature. Every 3 hours the number of cells were analyzed for up to 24 hours. The graph of he number of cells against the incubation time describe the pattern of growth of *Saccharomyces cerevisiae*. In general cases, the growth pattern consist of several phases of growth namely: lag phase, exponential phase, stationary phase and death phase. From these growth charts we could determine the optimum of pH and the optimum of incubation time, resulting in the highest of the number of *Saccharomyces cerevisiae* cells.

2.3.3 Materials Preparation.

Preparation of raw materials includes the production of starch and the process of hydrolysis of banana skin. Banana skin that is still quite fresh washed and peeled the outside then cut into thin pieces. After it is weight and dried under the sun to constant dry weight with water content in the range of (10-12) wt.%. The banana skin that have been dried mashed with the blender to obtained powder banana skin. The banana skin then put into sterilized jars and sealed, then stored in a dry.

The process of hydrolysis conducted with mix the powdered of banana skin (1636.36 g) and aquadest (163.64 ml) then added 883.41 g of sugar and 83.95 g of ammonium sulfate, 180 ml of 10 wt.% of HCl. The mixture then heated to boiling for 30 minutes while stirring to produce slurry then cooled to room temperature. The hydrolysis take place during heating of the slurry. The slurry then filtered, obtained filtrate. The filtrate

be used for starter media and fermentation media. The pH of the starter and fermentation mediua is a just with NaOH 99%. The varaition of pH of the starter media in the range of (3, 3.5, 4; 4.5; 5). The starter media is divided into 25 pieces in the sterile Erlenmeyer, 40 ml for every erlenmeyer. While the fermentation medium is divided into 4 pieces of a sterile Erlenmeyer, 200 ml for each erlenmeyer. Furthermore, all the Erlenmeyer is closed with sterile cotton wrapped paper and sterilized in the autoclave at a temperature of 121^{o}C for 15 minutes and then cooled to room temperature. The result is a sterile starter media and fermentation media.

2.3.3 Fermentation.

The process to produce a starter is not described in this paper. Starter is a cell of S. cerevisiae that had been cultured in the medium of a banana skin in a small volume in order to adjust cell growth environment at this stage of fermentation. When cells were cultured directly in the fermentation media, the possibility of cells cannot grow optimally. Another goal will be achieved is to determine the optimum pH of the cell growth of S. cerevisia. So in the next process is the process of fermentation, the pH of the media shall be in accordance with the optimum pH of the starter. In the fermentation process the volume ratio of starter and fermentation media was varied.

The volume ratio of starter/media was varied between (10% v 20% v 30% v, and 40% v). The volume of each medium are 200 ml. Air before entering the media of fermentation are sterilized by passing to a 10 wt.% of iodine solution. Sterile media containing starter of *S. Cerevisiae* were incubated with air flowed. The incubation carried out for 24 hours, which is taken 1 ml of solution for every 3 hours for analysis of the cell number. The number of cell plots against time of incubation to obtain the cell growth curve of *S. cerevisiae*. Growth curve is used to determine the optimum

incubation time and pH that produces the highest cell number. Cells that generated then separated from fermentation broth using a centrifuge.

2.3.4 Harvesting Products (*Saccharomyces cerevisiae* in the form of active dry yeast).

After fermentation is complete, the cell (solid) is separated from the liquid fermentation broth) using centrifuged with the speed of 2000 rpm for 10 minutes. The solid of biomass is filtered with filter paper (Whatman 93). Filter paper was dried at 110^{o}C and weighted until constant weight before its used. The cell on filter paper weighed using electrical balance and the residue that sticks to the filter paper dried in vacuum oven at temperature of 40^{o}C to obtain bakers yeast in the form of active dry yeast (dry product).

2.4. Product analysis.

Methods of analysis number of cells of *Saccharomyces cerevisiae* appropriate with the standard of microbiology lab prepared by [14]. First of all prepare haemacytometer tools and glass lid, and then cleaned with alcohol for sterile. By using a needle loop, put the sample of material that has been diluted, in the middle of haemositometer. After it closes the section with a glass lid. Haemositometer then placed on a microscope and counted the number of cells in each box. Next determine the average number of cells in 32 boxes and the analysis was repeated for 3 times. The means were called Y, so that can determine the number of cells per ml of volume. Given: Haemocytometer have a box size: the depth of 0.1 mm, area 0.0025 mm^2, the volume of 25 x 10^{-5} mm^3. Each 1 ml = 1000/25. x10^{-5} = 40 x10^{-5}. So that the number of cells per ml = 40 x10^{-5}x Y x dilution. Dilution = 1:100

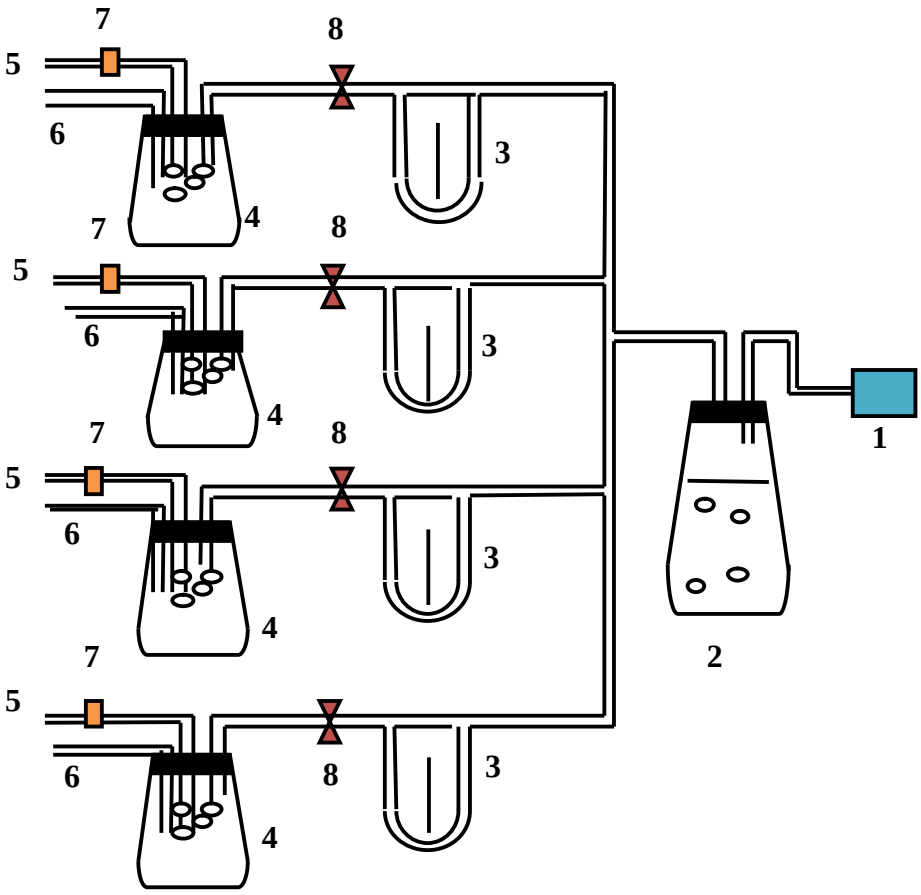

Figure 1. The equipment of fermentation: (1). Aerator, (2) Erlenmeyer fill with 10 wt.% of iodide solution , (3) Flow meter, (4) Erlenmeyer with sterile media, (5) Sampler, (6) Air effluent (7) Clamp, (8) Ccontrol valve

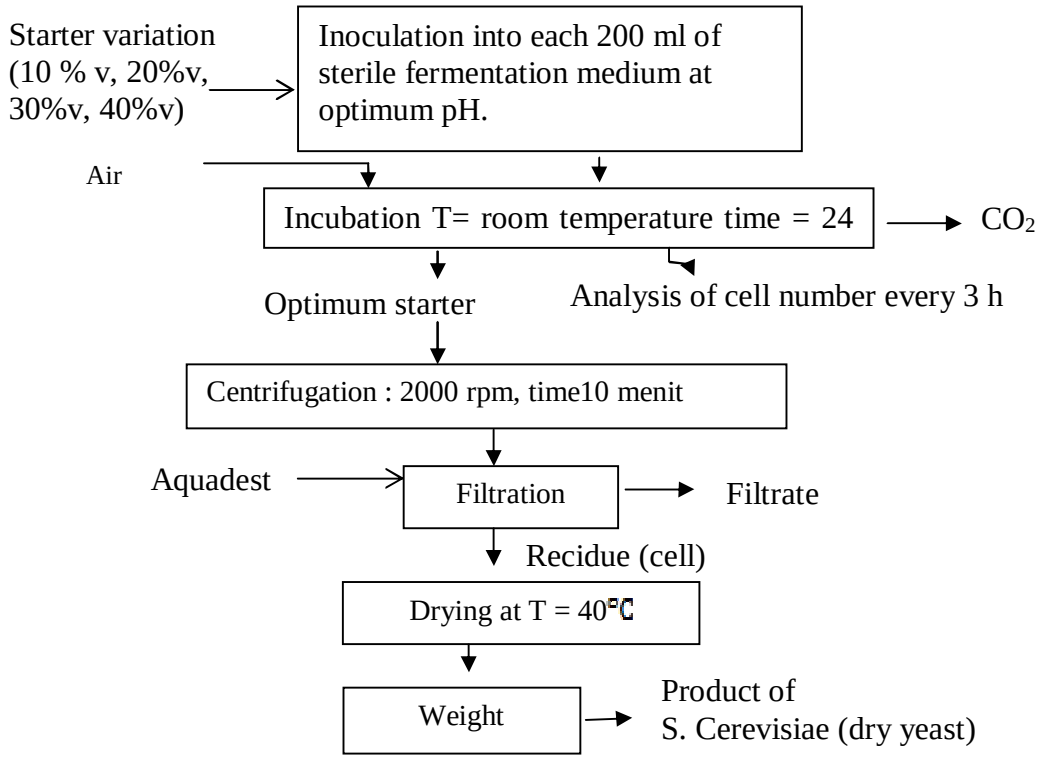

Figure 2. Flow diagram of fermentation and product separation of *S. Cerevisiae.*

III. Results and Discussion.

Optimum conditions in the process of cell growth in the starter media. Two ose of original cells of *S. Cerevisiae* inserted into the breeding media. Media that has been planted with seedlings placed on a shaker instrument. The temperature at room temperature. Cell growth was analyzed every 3 hours to 24 hours. The number of cell was analyzed using a haemocytometer and the data of the number of cell is shown in Table 2 and 3 at various pH between (3, 3.5, 4; 4.5; 5).

Table 2. Log number of cells of Saccharomyces cerevisiae/ml in the breeding of starter media at various of pH.

pH	Incubation time (h)							
	3	**6**	**9**	**12**	**15**	**18**	**21**	**24**
3	5.82	5.84	5.86	5.88	5.88	5.84	5.82	5.8
3,5	5.89	5.9	5.91	5.92	5.94	5.94	5.92	5.89
4	5.91	5.94	5.94	5.9	5.89	5.89	5.86	5.85
4,5	5.94	5.98	5.99	6.01	6.01	6	6	5.96
5	6.07	6.07	6.08	6.07	6.04	6.02	6.01	6

Table 3. Log number of cells of Saccharomyces cerevisiae/ml in the fermentation stage in the optimum pH at various starter ratio/medium.

% Volume of starter	Incubation time (h)							
	3	**6**	**9**	**12**	**15**	**18**	**21**	**24**
10%v	6.07	6.1	6.11	6.09	6.08	6.08	6.07	6.02
20%v	6.09	6.1	6.1	6.06	6.05	6.03	5.99	5.98
30%v	6.08	6.09	6.08	6.08	6.07	6.04	5.98	5.98
40%v	6.09	6.09	6.07	6.07	6.04	6	6	5.99

Table 2 shows that the highest number of cells obtained at pH = 5. Furthermore, the fermentation process in the medium of a banana skin with a volume of 200 ml performed at pH = 5. Variation of the % volume of the starter/media between (10%, 20%, 30% and 40%).

Table 3 shows the number of cells in each % volume of starter and the incubation time of 3 hours to 24 hours. Table 3 shows that at pH=5 and the incubation time of nine hours obtaine the highest number of cells/ml was $10^{6.08}$. Based on literature review, the pH optimum for growth of Saccharomyces cerevisiae is 4.5 - 5, while the pH optimum obtained from the results of this experiment is 5. Optimum pH differences may be caused by different growth media composition with the media that usually use for S. Cerevisiae fermentation as well as monosaccharide as a source of food for *S. Cerevisiae. S. cerevisiae* grown in glucose medium showed a lower

optimum pH of 4.5. The other reason when viewed from the optimum pH of enzyme produced by *Saccharomyces cerevisiae* cells in the fermentation process, namely for the enzyme amylase of 4.8 and for the enzyme invertase at 5, so that the number of cells of *Saccharomyces cerevisiae* obtained an optimum at pH = 5. Because the enzyme amylase and invertase role is to degrade starch in banana flour.

Table 3, shows that for 10 % starters, stationary phase is reached at 9 hours. The number of cells/ml equal to $10^{6.11}$ while for 40 % of starters, stationary phase is reached faster, ie at 3 h. The number of cells/ml equal to $10^{6.09}$. Based on these data, it can be concluded that the greater the % volume of the starter, then the time to reach the stationer phase is faster. After the stationer phase, that a decline in the number of cells of *Saccharomyces cerevisiae*. This is because for the 40% is not proportional to the amount of nutrients available, meaning that the limited amount of nutrients result in a decrease in the number of cells. Lack of availability of oxygen, which is due to increased in the % volume of the starter, causing the amount of oxygen required is not sufficient. So it can be concluded that 10% starter is optimum. The dry weight of cell at 10% volume of starter and at pH=5 is 51.9052 grams.

IV. Conclusion.

The calculation of the number of cells at any incubation time of 3 hours up to 24 hours found that the best condition at breeding starter and fermented at pH = 5 and 10 % volume of starter produce 51.9025 grams of dry weight of cells.

V. References

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