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Characterization of Nata de Coco Produced by Fermentation of Immobilized *Acetobacter xylinum*

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Abstract

Nata de coco is coconut water fermented foods by the bacteria *Acetobacter xylinum*. In general, the production of nata de coco is done by direct inoculation into liquid medium. Immobilization of cells is a technique used to trap the cells into a matrix. The use of immobilized cells for the production of nata de coco is one alternative to the product resulted in a cell-free nata. This study used immobilized *Acetobacter xylinum* to produce nata on coconut water medium. Immobilization technique used is to trap *Acetobacter xylinum* in Ca-alginate matrix. Immobilized cells were then used for the fermentation of nata de coco repeatedly. Factors examined included repeatability fermentation, time consuming on establishment of nata, nata thickness, viability of immobilized cell. From the results obtained that immobilized cell still produced nata up to two replications fermentation. The average time for producing nata was 11 days, with an average thickness of 0.8 cm. While the rate of formation of nata equation y = 0,077x -0.086. After two replications fermentation, cell viability of immobilized cell was still high.

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Keywords: immobilization; *Actobacter xylinum*; nata

1. Introduction

Nata de coco is a product of fermentation culture of *Acetobacter xylinum* in coconut water medium enriched with carbon and nitrogen through a controlled process. In such conditions, these bacteria produce enzymes that can be

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compiled into a thousand chain sugars or cellulose fibers. Of the millions of biomass grown in the coconut water, will produce millions of pieces of cellulose threads that eventually appear solid white to transparent, called nata.

The production of nata de coco is still done by inoculating *Acetobacter xylinum* directly into the culture medium of coconut water. This way is always a little left over to use as a starter culture for the next fermentation. This method has the advantage of easy and cheap. However, this method also has the risk of which culture is used as a starter culture become susceptible to contamination and death, especially when the storage before being used for the next fermentation if handled inappropriately.

In addition, the use of nata de coco is not only limited in the field of food, but to the field of medicine and pharmacy, for example, on an open wound healing process. Therefore, it is necessary a fermentation method to produce nata containing little or free from biomass. One way is by immobilization of *Acetobacter xylinum* for fermentation of nata de coco.

Immobilization of cells defined by Chibata (1978) as a method for confining or physically placing of microbial cells in a particular space in which the cells still have the catalytic activity and can be used continuously and repeatedly. This immobilized cell state that can be in a state of growth, rest (resting) and or the state of autolysis. In some cases, the microbial immobilized cells were dead, but still show the activity of the enzyme. Advantages include cell immobilization technique can be used in continuous system, can be used repeatedly on a batch system, can be used for excretion of secondary metabolites, can protect from interference turbulent flow and can prevent the interfacial inactivation (Tramper, 1990 in Champagne et al, 1994).

Cell immobilization technique used in this study is the technique of cell entrapment in calcium alginate gel matrix. Method of forming beads were made in this study is the extrusion method or methods droplets. Mechanical entrapment in calcium alginate gel matrix is technically very simple and easy to implement and is suitable for immobilization of viable cells (Groboilot et al, 1994; Tampion and Tampion, 1987). In this study used immobilized *Acetobacter xylinum* for nata de coco fermentation. In this research will be characterizing of the process of formation of nata. This technique has advantages including several starter cultures were trapped in the matrix can be used repeatedly, and from some of the literature immobilization of cells can increase of the production of metabolites. So in this study will be the potential for increasing production of nata using immobilization

2. Material and Method

2.1. Material

Biomass is produced by growing in nutrient broth medium for 24 hours. Separation of biomass is done by centrifugation. As for the fermentation process, the material used as a medium is coconut water fortified with the addition of ZA as a source of nitrogen.

2.2. Immobilized Acetobacter xylinum

Deposition of biomass *Acetobacter xylinum* centrifugation results are put into a solution of sodium alginate. A number of 50 ml alginate solution prepared by mixing 1.5 gram alginate with 50 ml of distilled water. Then sterile 0.1 M $CaCl_2$ prepared by mixing 1.47 grams of $CaCl_2$ into the Erlenmeyer flask that had contained 100 ml of distilled water for sterilization. For the next period of the cell precipitate in a solution of sodium alginate mixed in a solution of sodium alginate as a trapper. Period of cell-alginate mixture that has been mixed evenly subsequently dropped into 100 ml of sterile $CaCl_2$ solution (in a glass beaker with a concentration of 0.1 M) were mixed on a magnetic stirrer (Termolyne) on the speed with scale 1. To avoid the diversity of beads then chosen burette drops with a capacity of 20 ml and ± 0.18 mm aperture size. Droplets of cell-alginate mixture in $CaCl_2$ solution will soon form a bead or beads immobilized cells, so automatically the time of *Acetobacter xylinum* cells already trapped in Ca-alginate gel matrix. To form a solid beads immobilized cell, then left for ± 15 minutes in a solution of $CaCl_2$. Beads then immobilized cells obtained were then rinsed (2 times) with a 0.1% sterile peptone water.

2.3. Fermentation

A total of 100 ml of coconut water mixed with 10 g of glucose, 2 g of ZA, and 2 mL of acetic acid. This solution then heated to a temperature of 80 ° C and continued cooling to 30 ° C. The fermentation process is done by mixing coconut water medium with beads containing cells of *Acetobacter xylinum* in a container and then given a cover.

3. Result and Discussion

Acetobacter xylinum grown in nutrient broth medium for 24 h at room temperature were able to show good growth (Fig. 1), then characterized by the formation of a thin layer of nata and sediment of biomass.

Fig. 1. Acetobacter xylinum in Nutrient broth medium

The calculations show the amount of biomass is ready for the immobilization of $3{,}5x10^7$ CFU/mL. Then do the immobilization process that begins with the stage of separation of biomass continued trapping process in alginate matrix. *Acetobacter xylinum* trapped in the alginate matrix in the form of beads. The average number of beads ranges between 400-450 beads/50 ml of alginate (Fig. 2).

Fig. 2. Beads containing Acetobacter xylinum

Growth parameters of immobilized *Acetobacter xylinum* fermentation were observed in the decrease in pH and the rate of formation of nata. The first fermentation time required to achieve a thickness of 0.8 cm was 11 days with a pattern of decrease in pH as in Fig. 3. A decrease in pH up to day six and there is no longer a decrease up to day 11. After the first fermentation is over, then proceed with the second fermentation using immobilized cells of the same. Even though the fermentation of immobilized *Acetobacter xylinum* a bit longer than its free cell, it shows that the immobilized cell still can release microbial cellulose to form nata. The results showed that the pattern of the second fermentation is not much different from the pattern of pH. After day 6 the pH remains stable until day 11.

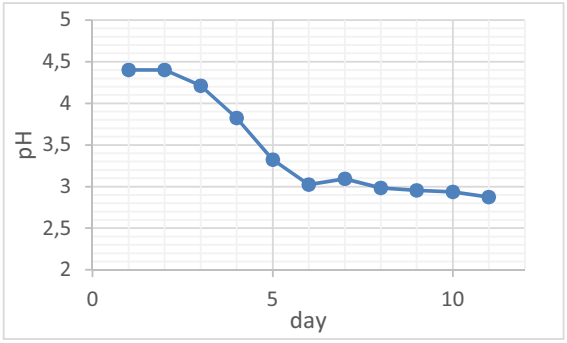

Fig. 3. The change of pH during fermentation of Acetobacter xylinum immobilized

The pattern of formation nata until second replications of nata fermentation also showed no difference. Nata thickness of 0.8 cm is achieved for 11 days (Fig. 4). So the use of immobilized cells through 2 replications can still be done.

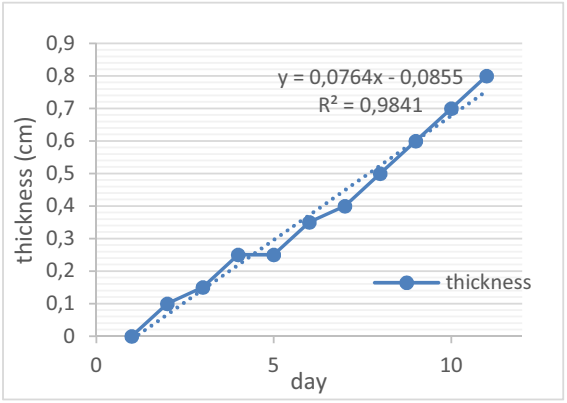

Fig. 4. Average velocity pattern of nata's formation

The number of cells were counted before the second fermentation is $3,47x10^7$ CFU/mL, almost no decrease in the number of cells from the beginning of the first fermentation $3,5x10^7$ CFU/mL. This means that the process of immobilization of *Acetobacter xylinum* can maintain viability while maintaining the production of nata. Visually, the results showed no difference with nata nata produced in general (Fig. 5).

Fig. 5. Nata de coco produced by immobilized Acetobacter xylinum

4. Conclusion

From the results obtained that immobilized cell still produced nata up to two replications fermentation. The average time for producing nata was 11 days, with an average thickness of 0.8 cm. While the rate of formation of nata equation y = 0,077x -0.086. After two replications fermentation, cell viability of immobilized *Acetobacter xylinum* was still high.

References

Chibata, I., 1978. Immobilized Enzymes. Kodansha Ltd., Japan.

Champagne, C.P., C. Lacroix dan I. Sondini- Gallot, 1994. Immobilized cell Technologies for the Dairy Industry in Stewart, G. G. and I. Russell (Eds). Special Issue on Immobilized Cell Technology in Food Processing Crit. Rev. in Biotech. Vol. 14/Issue 2, CRC Press.

Groboillot, A., D. K. Boadi, D. Poncelet dan R. J. Neufeld, 1994. Immobilized of cells for Application in the Food Industry in Stewart, G. G. dan I. Russell (Eds). Special Issue on Immobilized Cell Technology in Food Processing Crit. Rev. in Biotech. Vol. 14/Issue 2. CRC. Press.

Tampion, J. dan M. D. Tampion, 1987. Immobilized Cells : Principles and Applications, Cambridge University Press, Cambridge.