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BIOMASS PRODUCTION AND SINGLE-CELL PROTEIN (SCP) ENCAPSULATION OF BACTERIA *Bacillus cereus* SN7

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ABSTRACT

Bacillus cereus SN7 is a bacterium with potential applications in producing Single-Cell Protein (SCP). SCP can be an alternative to protein derived from traditional sources like agriculture, fisheries, and animal husbandry. Encapsulation is a process used to stabilize cells, aiming to potentially enhance probiotics' viability and stability throughout production, storage, and handling. This study aims to make capsules from *B. cereus* SN7 single-cell protein to protect them from contaminants. This study used a survey method, which carried out in-situ processing of bacteria, as well as conducting experiments on the formation of bacterial encapsulation, testing the effectiveness of encapsulation, and observing the viability of *B. cereus* SN7 bacteria by counting the number of colonies. *B. cereus* produced biomass with a total dry weight of 1.3708 g/mL. The alginate encapsulation experiments can be formed into capsule beads that protect bacteria. The best encapsulation process is an alginate-based coating, which is indicated by the ability of the alginate to withstand temperature, pH, and salinity.

Keywords: Biomass, Encapsulation, *B. cereus* SN7

1. INTRODUCTION

Bacillus cereus is a gram-positive bacterium that produces endospores and is tolerant to heating and preservation through drying. According to Feliatra et al.[1], one of the bacteria in the Bacillus genus that may be a source of protein production is *B.cereus*, and this is evident from several previous studies which stated that *B.cereus* is a probiotic that can inhibit several pathogenic bacteria and also a heterotrophic bacterium that can degrade organic matter. Toxic material in the environment, especially in the waters.

Bacillus cereus SN7 bacteria is a *B. cereus* strain that can be utilized to manufacture SCP. According to Feliatra et al.[1], five species of B. cereus have the potential to be developed as SCP, and one of these five species is *B. cereus* SN7. Informed *B. cereus* SN7 has the potential to be developed as a biological control agent, especially in the field of aquaculture. According to Maryana et al.[2], the main components of SCP are amino acids and minerals. SCP can be used as a substitute for protein from conventional sources such as agricultural, fishery, and livestock products.

Biomass cells produced from *B. cereus* SN7 bacterial SCP carried out an encapsulation process to protect the bacterial biomass yield. According to Trimudita & Djaenudin[3], encapsulation aims to stabilize cells, potentially increasing the viability and stability of probiotics during production, storage, and handling. Encapsulation is a technology for wrapping the core material in micro-sized capsules that can release their contents in a certain environment[4].

The advantages of the encapsulation process are that it can produce large quantities of encapsulation, it is easy to

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form capsules, and it has good durability. The encapsulation process has various advantages: being more resistant, stable to the external environment, durable, and lighter[4]. Apart from being protected by the core substance, encapsulation can also release cells with a controlled release rate under specific conditions. It allows the diffusion of small molecules (cells, metabolites, and substrates) across the membrane[5].

2. RESEARCH METHOD

Time and Place

This research was conducted from September to December 2022 at the Marine Microbiology Laboratory, Chemical Oceanography Laboratory, and Marine Biology Laboratory, Department of Marine Science, Faculty of Fisheries and Marine, Universitas Riau.

Methods

The study employed a survey method to conduct in-situ bacteria processing, experiments on bacterial encapsulation formation, assessment of encapsulation efficacy, and observation of *B. cereus* SN7 bacteria viability through colony counting.

Procedures

Media Used for Bacterial Growth

Modified media materials used in this study are sago liquid waste and chicken egg white. Liquid waste is taken in the last holding pond. As much as 50 mL of Sago liquid waste is mixed with other micronutrients such as Vitamin B_{12} 25 mg, K_2HPO_4 0.5 g, and KH_2PO_4 0.75 g as a buffer solution. Modified liquid waste media was sterilized using an autoclave at 121°C, 2 atm pressure for 15 minutes. The modified liquid waste media that has been sterilized is then added with 50 mL of chicken egg white as a source of protein, which has been pasteurized separately.

The standard McFarland scale used is 0.5 with a composition of 0.05 ml of 1% $BaCl_2$ plus 9.95 mL of H_2SO_4 with an estimated number of bacteria in suspension of 1.5×10^8 /mL[6].

The Biomass Production of *B. cereus* SN7

Biomass production of *B. cereus* SN7 was carried out by adding *B. cereus* SN7 bacteria whose turbidity level had been equalized with McFarland's solution and into a 500 mL Erlenmeyer bottle which has been filled with suspension (sago liquid waste, chicken egg white, vitamin B_{12}, K_2HPO_4, KH_2PO_4, and distilled water), then installed using an aerator for 12 hours.

The results of bacterial subculture using sago liquid waste are then put into a centrifuge containing bacteria for 10-15 minutes. Falcon-containing bacteria were centrifuged at 6000 rpm for ten minutes[7]. It will produce a supernatant and a precipitate of bacterial cells underneath. The formed supernatant was discarded and placed in the oven at 100°C for 15 minutes. After drying, the falcon is cooled in a desiccator, and then its dry weight is weighed using a digital balance. The dry weight obtained is reduced by the weight of the previous falcon so that the actual dry weight of the bacteria is obtained.

Encapsulation Process

The encapsulation process was carried out by putting 5 mL of *B. cereus* SN7 bacterial cell biomass into a solution of 50 ml of distilled water and 2 g of sodium alginate, which had been sterilized using an autoclave. Then, the sodium alginate solution mixed with bacterial biomass was put into an emulsion solution of 100 mL of 0.1 M $CaCl_2$ and 100 mL of 1% acetic acid added with 0.20 g of chitosan by dripping using a 5 mL syringe while rotating with a magnetic stirrer. Each emulsion solution was observed for the resistance of the encapsulation beads every 15-minute intervals for 75 minutes. Furthermore, the effectiveness of the beads that can survive well in one of the emulsion solutions is tested.

The effectiveness test was carried out to see the resistance of bacterial

encapsulation under certain conditions. This effectiveness test looks at the resistance of *B. cereus* SN7 encapsulated bacteria to temperature (cold 10°C, room temperature 27°C, and hot temperature 40°C), pH (acidic water pH 5, neutral water pH 7, and alkaline water pH 8), and 0.9% $NaCl_2$.

Observation of *B. cereus* SN7

This measurement of bacterial growth was determined based on the statement from Feliatra et al.[8], namely *B. cereus* bacteria has a generation time value of 18 minutes and a growth rate constant of 2.27 per hour, so measurements of the growth parameters of *B. cereus* are carried out in a short time and can obtain a bacterial growth curve that includes all growth phases bacteria. Bacterial growth was measured using the total plate count (TPC) method to measure bacterial cells. Bacterial capsule beads, left in the emulsion solution for 15, 30, 45, 60, and up to 75 minutes, were counted using a colony counter. The results of the number of colonies obtained were then entered into the bacterial calculation formula based on research conducted by Tyas[9], namely:

$$\text{CFU} = \frac{1}{Volume \times \sum Faktor\ Pengenceran} \times \sum coloni$$

Bacteria treated with encapsulation are worried about contamination, so the encapsulation results must be tested for gram staining on the bacteria inside. The process of the bacterial Gram staining test was carried out by growing the bacteria into NA media first utilizing encapsulated beads which had been left in the emulsion solution for 75 minutes, taken and rinsed using sterile distilled water with as much as three grains, then put into the NA media aseptically near Bunsen. Furthermore, the bacteria growing in the NA media were subjected to a Gram stain test. Then, the shape of the bacteria and its color were observed using a microscope; if it is blue, it means Gram-positive, while red means it is Gram-negative.

3. RESULT AND DISCUSSION

B. *cereus SN7* Bacterial Cell Biomass Production

Biomass production of *B. cereus* SN7 bacterial cells obtained each falcon's wet and dry weights. The wet and dry weight results in one production were 1.7933 g/mL wet weight and 1.3708 g/mL dry weight.

The growth of bacteria in sago liquid waste suspension media plus chicken egg whites yielded relatively few results, with a dry weight yield of 1.3708 g/mL for 12 hours. It can be estimated that the causes that affect the acquisition of *B. cereus* SN7 bacterial biomass are temperature and oxygen supply during the culture process. The temperature used during culture is room temperature, which tends to be unstable so that it can affect bacterial growth. However, according to Suriani et al.[10], the optimal time for bacterial growth is 37-40°C. Oxygen supply is needed for aerobic bacteria such as *B. cereus* SN7. The use of aerators in the culture process is also relatively unstable. According to Ferdiansyah[11], a limited oxygen supply can result in reduced growth of microorganism cells.

Encapsulation Process Results

The encapsulation process of *B. cereus* SN7 bacteria used a 0.1 M $CaCl_2$ emulsion solution and an emulsion solution of a mixture of chitosan plus 1% acetic acid in 100 mL. The test results for making bacterial encapsulation are shown in pictures in Figures 1 and 2.

Based on Figures 1 and 2, each emulsion solution has an effect showing the resistance of alginate in the formation of the bacterial capsule of *B. cereus* SN7. The effects seen in experiments using two types of emulsion solutions look different. Figure 1, using a 0.1 M $CaCl_2$ emulsion solution left for 75 minutes, shows changes in the dipped capsule beads, and it appears that lysis occurs. Figure 2 uses an emulsion solution of a mixture of chitosan plus 1% acetic acid in 100 mL, showing the shape of

the capsule beads that appear to have solidified.

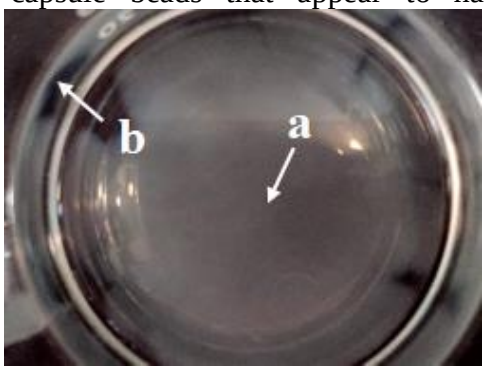


Figure 1. Results from 0.1 M $CaCl_2$ Solution for 75 minutes; a. Alginate form that Occurs Lysis; b. Beaker as a Container.

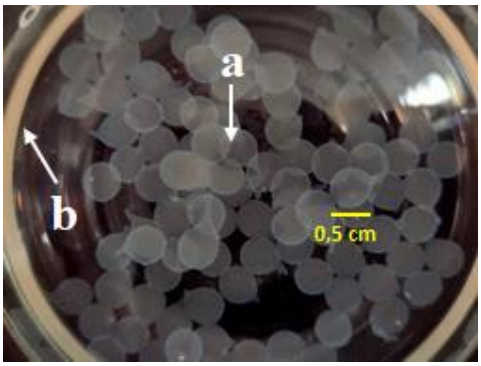
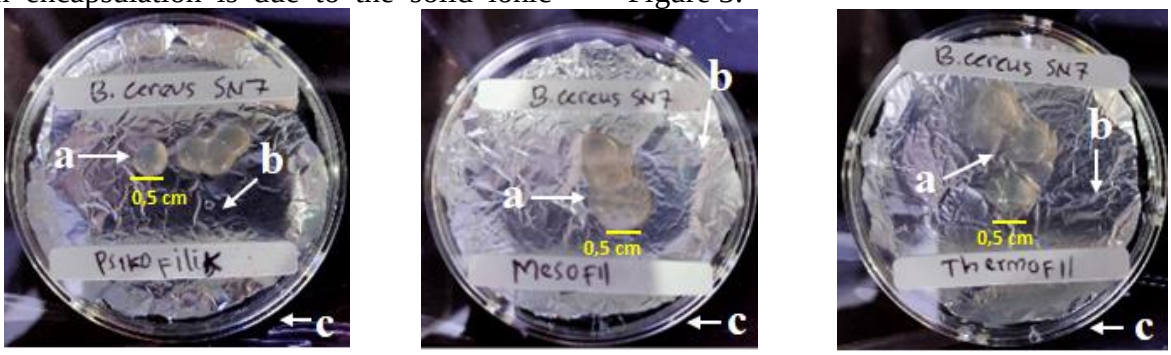


Figure 2. The Results of Chitosan Solution Plus Acetic Acid for 75 Minutes; a. The Form of Alginate, which forms Capsule Beads, b. Beaker as a container

The test results for making bacterial capsules showed that the effect of using an emulsion solution that could form capsule beads was in a solution of chitosan plus 1% acetic acid, which was tested for 75 minutes. Capsule beads formed using a syringe can form condensed dots, so the process of forming the bacterial capsule of *B. cereus* SN7 is considered successful. If the alginate capsule is made, the longer the capsule is soaked using a chitosan emulsion solution plus 1% acetic acid, the cross-linking of calcium ions with alginate polymers results in a denser texture. According to Hidayah & Djaenudin[12] the increase in stability and efficient protection in encapsulation is due to the solid ionic interaction between alginate and chitosan. Add 1% acid content into the chitosan emulsion solution to adjust the acid content in the alginate so that the alginate is not easily diluted.

Encapsulation Effectiveness Test Results

The effectiveness of bacterial encapsulation against temperature shows different resistance results at specific temperatures. The temperature treatment was carried out at a cold temperature of 10°C, at room temperature of 27°C, and a hot temperature of 40°C for 75 minutes. The results of the resistance of bacterial encapsulation to temperature can be seen in Figure 3.



(i) (ii) (iii)

Figure 3. Test Results for Cold Temperature 10°C (i), Room Temperature 27°C (ii), Hot Temperature 40°C (iii); a. Capsule Beads, b. Aluminum Foil, c. Petri dish.

Based on Figure 3 (i), (ii), and (iii), changes are experienced in the encapsulation results at a specific temperature for 75 minutes. The effectiveness of capsules at different temperatures determines the feasibility of

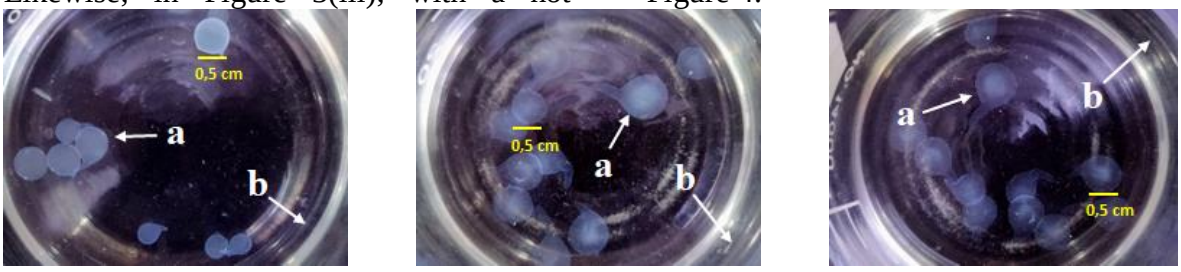
capsule beads during the storage process; capsule beads damaged at certain temperatures cannot be used for capsule storage. If there is damage to the capsule beads, they will not be able to protect the bacteria inside.

In Figure 3(i), the results of the capsules tested at 10°C cold did not appear to experience changes and damage to the bacterial capsule beads, whereas in Figures 3 (ii) and (iii), the bacterial capsule beads experienced changes such as damage to the beads-capsule beads, some look broken, and have a color change. In Figure 3(ii), using a room temperature of 27°C, the bacterial capsule beads appear soft and sticky to each other; this is caused by the unstable room temperature and changes in the surrounding environment, which will reduce the effectiveness of encapsulation. Likewise, in Figure 3(iii), with a hot temperature of 40°C, the encapsulation spheres have changed to yellow, and some of the beads have broken.

The temperature test results showed that the encapsulated beads had a stable shape at cold temperatures. So, a cold temperature of 10°C is the best place to store the results of bacterial encapsulation so it can last longer. According to Yulinery & Nurhidayat[13], it cannot withstand high temperatures in the encapsulation process. The optimal temperature for encapsulation resistance is lower than 37°C. Meanwhile, according to Putri et al.[14] allowed to stand at 10°C for encapsulation to obtain stable conditions.

Results of pH Treatment

The results of the resistance of bacterial encapsulation to pH can be seen in Figure 4.



(i) (ii) (iii)

Figure 4. pH 5 Test Results (i), pH 7 Test Results (ii), pH 8 Test Results (iii): a. Capsule Beads, b. Beaker as a Container.

Based on Figure 4, the results obtained at pH treatment for 75 minutes showed that encapsulation resistance was longer lasting at pH 5 (acid). Bacterial encapsulation shows resistance to pH <7 (neutral), but at too low a pH, it can cause a decrease in the number of cells in the encapsulation. This follows the statement of Ngatirah & Ulfah[15] that the effect of high acid will reduce the number of cells.

Treatment result $NaCl_2$ 0.9%

The 0.9% $NaCl_2$ test results on bacterial encapsulation were put into 0.9% $NaCl_2$ for 75 minutes. The bacterial encapsulation beads looked bright, and the membrane looked increasingly thick. The results of the resistance of bacterial encapsulation to 0.9% $NaCl_2$ solution can be seen in Figure 5.

Based on Figure 5, a 0.9% $NaCl_2$ solution is an alternative to equalize acid levels in the stomach. This is in line with the statement of Trimudita & Djaenudin[3] that the use of NaCl solution is a pH range commonly observed in the stomach.

The persistence of the capsule form from the 0.9% $NaCl_2$ solution, which still contains acid levels, causes the encapsulation beads not to be damaged. This is because the alginate used as an encapsulation material is made of components containing acid, so the beads that are put into 0.9% $NaCl_2$ solution can retain the alginate component that is already in the form of encapsulated beads.

According to Rasyid[16], alginate is a linear copolymer consisting of two monomeric units: D-mannuronic acid and L-guluronic acid. Each acid content contained in the solution and encapsulation beads does not affect the quality of alginate and damages the encapsulation too quickly.

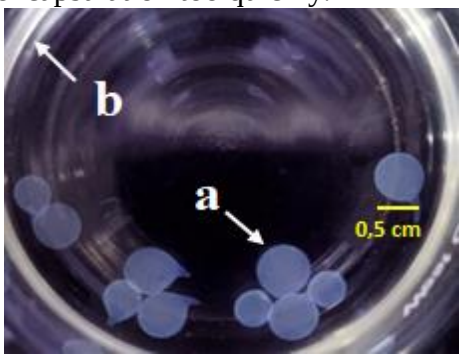


Figure 5. Test Results Using 0.9% $NaCl_2$ Solution: a. Capsule beads, b. Beaker as a container.

Results of Viability Test of *B. cereus* SN7 Encapsulated Bacteria

The results of the bacterial viability test were obtained by measuring the growth of bacterial cells and performing gram staining of bacteria. The results of *B. cereus* SN7 cell growth in encapsulation can be seen in Table 1.

Table 1. Number of *B. cereus* SN7 Bacterial Cells in Encapsulation

Encapsulation Time (minute)	TPC (CFU/mL)
15	85×10^2
30	109×10^2
45	76×10^2
60	158×10^2
75	124×10^2

Based on Table 1, the results obtained based on the time variation show the number of bacterial cells in every 15-minute interval, which shows the number of bacterial cells fluctuating (fluctuating). According to Respati et al.[17], this is caused by bacteria that experience death and then experience growth again after death. Based on previous research, several factors cause bacterial growth and viability.

Based on the statement of Utami[18], growth factors and viability of probiotic bacteria are strongly influenced by nutrient intake, temperature, pH, water, and oxygen. The ability of bacteria to grow back is due to a source of nutrients from other bacterial cells that have died so that bacteria can utilize the new nutrient sources as a material for the metabolism of these bacterial cells. The bacterial bodies undergo decomposition to be used as an energy source for the remaining surviving bacteria to grow again[17].

The following results of Gram staining can be seen in Figure 6.

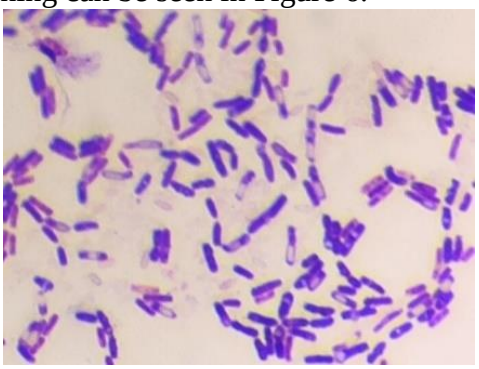


Figure 6. Gram Staining Results of *B. cereus* SN7

Figure 6 shows a Gram stain with a positive result, shown in purple, and the visible form of bacteria is bacilli. Gram staining of bacteria aims to determine whether the bacteria that have been encapsulated are contaminated or not. The bacteria that the encapsulation process has carried out can survive. This was evident from the Gram staining results, which showed that the *B. cereus* SN7 used was the same as the results of the previous Gram stain. This also shows that there is no contamination during the encapsulation process. If the bacteria are contaminated, gram bacteria will appear red (negative), or if there is a mixture of bacterial forms, then the encapsulation process is considered not aseptic. The gram test is positive if the cells are purple and negative if the cells are red. *B. cereus* will show a stem shape and purple color (Gram-positive)[13].

4. CONCLUSION

Based on the research that has been done on the production of B. cereus SN7 biomass, the total dry weight of the bacteria is 1.3708 g/mL. The alginate encapsulation experiments can be formed into capsule beads that protect bacteria. After testing the capsule's effectiveness and the bacteria's viability, it was determined that it is very effective for the production process or further applications. The best encapsulation process is an alginate-based coating, which is indicated by the alginate's resistance to temperature, pH, and salinity.

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