

***Bacillus cereus* SN7 Encapsulation process as a substitute protein source in fish feed**

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Abstract. *Bacillus Cereus* SN7 bacteria can inhibit the growth of pathogenic bacteria. Encapsulation is a technology for wrapping the core material in micro-sized capsules that can release their contents in a certain environment. The research aimed to encapsulate the biomass yield as a protection for *B. cereus* SN7 bacteria from contaminants. This study used an experiment method, namely conducting experiments on the formation of bacterial encapsulation with 2 sources of emulsion: CaCl₂ 0,1 M and chitosan mixture, 1% acetic acid in 100 ml⁻¹, then tested the effectiveness of encapsulation by looking at the viability of *B. cereus* SN7 bacteria by counting the number of colonies pH, temperature and physiological aspect of the bacterium. The results of encapsulation studies carried out using emulsion of chitosan mixture, 1% acetic acid in 100 ml⁻¹ can be formed into capsule beads that can protect bacteria. Meanwhile, using citosan could not last for 75 minutes. After testing the effectiveness of the capsule and its bacterial viability, it is very effective for the production process or for further applications. then the best storage temperature is 10⁰C, pH 5 and stable immersion in physiological solutions.

1 Introduction

Feed is an important factor affecting the growth of farmed fish. Approximately 86.5% of the production costs of aquaculture businesses are allocated to purchase feed. Good quality and efficient feed determine the success of a farming business (Craig, 2019). In 2019, the Ministry of Maritime Affairs and Fisheries estimated that the need for feed will reach 14 million tons. However, the high demand for feed, the difficulty in obtaining quality types of feed and the high selling price of feed are often obstacles. In addition, the amount of feed demand which continues to increase from time to time was not able to suppress the decline in feed prices. This is presumably due to the difficulty in obtaining sources of quality feed raw materials and the limited feed industry in Indonesia.

Several types of high protein products suitable as feed have begun to be produced on an industrial scale (Overland et.al, 2013). Currently, the search for feed protein sources that

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contain high protein continues to be developed in the form of finished products that can be marketed. One of the efforts made is the utilization of *B. cereus* as a source of protein in feed (Subharanjani et.al, 2015). PST is a dry cell product of microorganisms that are cultured on a large scale for use as a source of protein in food or feed (Nyman, 2016; Sisan et. al, 2013). (Feliatra et.al) Heterotrophic bacteria are able to inhibit the growth of pathogenic bacteria in fish including: *Vibrio alginolyticus*, *Aeromonas hydrophila* and *Pseudomonas* sp. The ability to inhibit these pathogenic bacteria is thought to be due to the *B. cereus* bacteria producing antibiotic compounds. *B. cereus* SN7 bacteria is a *B. cereus* strain that can be utilized in the manufacture of PST. Encapsulation aims to stabilize cells which has the potential to increase 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 (Mustopa et.al, 2018). The encapsulation process has various advantages, namely more resistant, stable to the external environment, more durable, and lighter. In encapsulation, apart from being protected by the core substance, it is also able to release cells with a controlled release rate under specific conditions and allows the diffusion of small molecules (cells, metabolites, and substrates) across the membrane (Hidayah et.al, 2021). This study aimed to determine a suitable emulsion material for encapsulating *B. cereus* SN7 bacterial and the effectiveness of the *B. cereus* SN7 capsule against pH temperature and physiological solutions.

2 Methodology

This study used an experimental method: encapsulation with two different emulsions: 1 using 0.1 M CaCl₂ emulsion solution. 2 using a mixed emulsion solution of chitosan plus 1% acetic acid in 100 ml. The encapsulation process was carried out by providing 5 ml of *B. cereus* SN7 bacterial cell biomass into a solution of 50 ml of distilled water and 2 g of sodium alginate that 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 CaCl₂ 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 durability of the encapsulated beads every 15 minute interval for 75 minutes.

Furthermore, beads that could survive well in one of the emulsion solutions were tested for effectiveness. The effectiveness test was carried out to see the resistance of bacterial encapsulation under certain circumstances. In this effectiveness test, we looked at the resistance of encapsulated bacteria *B. cereus* SN7 with temperature (cold temperature 10°C, room temperature 27°C, and hot temperature 40°C), pH: (acidic pH 5), (neutral pH 7), and (alkaline pH 8), and immersion with physiological solution (NaCl 0.9%). Bacterial capsule beads were immersed in the emulsion solution for 15, 30, 45, 60, up to 75 minutes and then the shape of the resulting beads was observed. To ensure the level of non-contamination, bacteria that have been treated with encapsulation are tested for gram staining. The bacterial gram staining test process was carried out by growing bacteria into NA media first encapsulated beads that had been allowed to stand in the emulsion solution for 75 minutes and were taken and rinsed using sterile distilled water as many as 3 grains and then inserted into NA media aseptically near Bunsen. Furthermore, the bacteria that grew on the NA media were tested for gram staining. Data analysis was done descriptively.

3 Results and discussion

Encapsulation of *B. cereus* SN7 using 0.1 M CaCl₂ emulsion solution (pic. 1) and using a mixed emulsion solution of chitosan plus 1% acetic acid in 100 ml (pic.2).

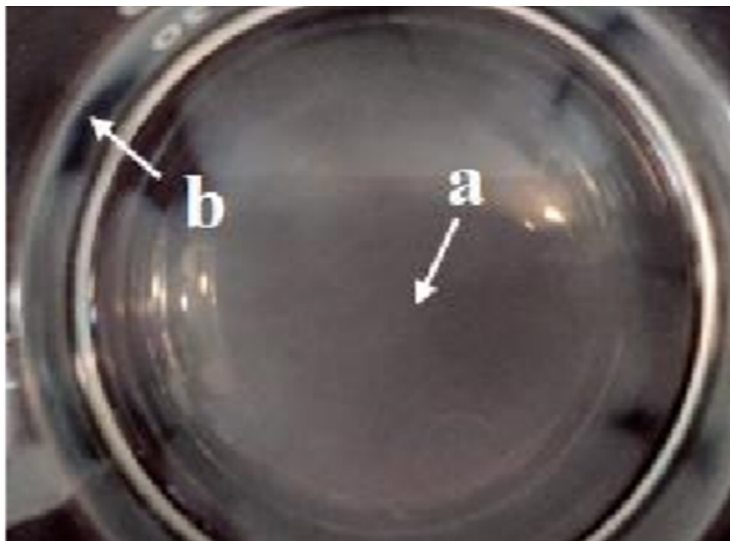


Fig 1. The result of 0.1 M CaCl_2 solution for 75 minutes: a shape of the lysed alginate, b. The beaker as storage vessel.

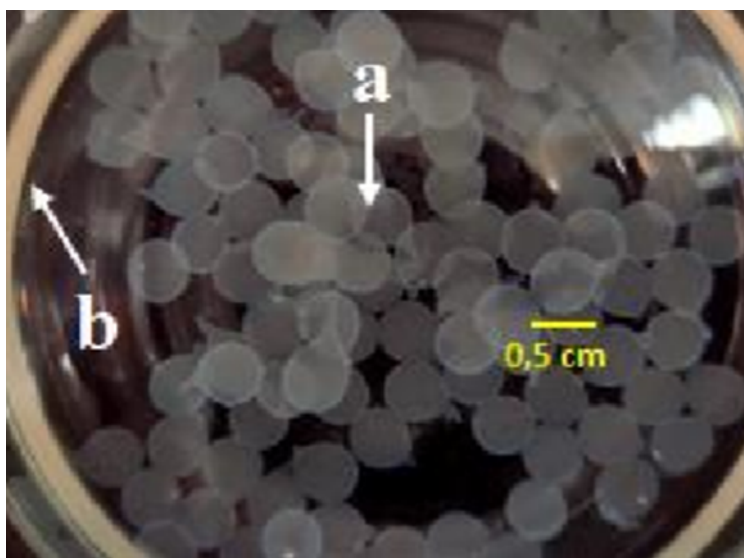


Fig. 2. Results of chitosan plus acetic acid solution for 75 minutes: a. Shape of alginate-formed capsule beads, b. Beaker as a storage vessel

The durability of the encapsulated beads differed between the two emulsions used. The 0.1 M CaCl_2 emulsion solution that was allowed to stand for 75 minutes showed changes in the capsule beads that were dripped and appeared to be lysed. While using a mixed emulsion solution of chitosan plus 1% acetic acid in 100 ml the shape of the capsule beads had solidified, so that the process of forming capsules of *B. cereus* SN7 bacteria was successful. Alginate capsules were made, if the longer the capsule is soaked using chitosan emulsion solution plus 1% acetic acid, the cross-linking of calcium ions with alginate polymers produces a denser texture. Increased stability and efficient protection in encapsulation is due

to the strong ionic interaction between alginate and chitosan. The addition of 1% acid content to the chitosan emulsion solution to adjust the acid content in the chitosan emulsion solution.

3.1 Encapsulation effectiveness

The effectiveness of the *B. cereus* SN7 bacterial capsule was carried out by looking at the durability of the capsule beads that had been formed. The effectiveness of bacterial capsule resistance was tested by treating it with temperature, pH, and 0.9% NaCl solution.

The capsules tested at the cold temperature of 10°C did not experience changes and damage to the bacterial capsule beads, while storage at 27°C and 40°C the bacterial capsule beads experienced changes such as damage to the capsule beads, some were broken, and experienced changes in their color. At 27°C room temperature storage, the bacterial capsule beads appear to be mushy and sticky to each other, this is caused by room temperature which is unstable to changes in the surrounding environment so it will reduce the effectiveness of encapsulation (Putri et. Al, 2020). Likewise, storage at 40°C showed that the encapsulated spheres changed color to become more yellow and there were broken beads. At a cold temperature of 10°C is the best place to store the results of encapsulated bacteria can last longer. (Ngatirah and Ulfah, 2007) The encapsulation process cannot withstand high temperatures, the optimal temperature for encapsulation resistance is lower than 37°C. At a temperature of 10°C encapsulation gets a stable condition.

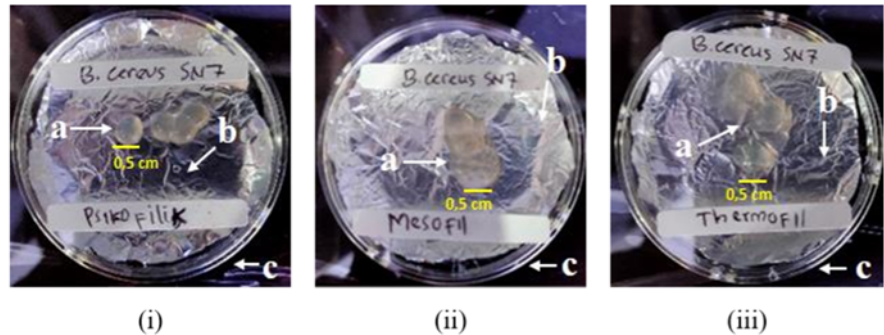


Fig. 3. Test results of cold temperature 10°C (i), room temperature 27°C (ii), hot temperature 40°C (iii):

The effectiveness of capsules to different temperatures determines the feasibility of capsule beads during the storage process, capsule beads that are damaged at certain temperatures cannot be used as capsule storage. Some researcher (Putri et. Al, 2020) reported that the increase in stability and efficient protection in encapsulation is due to the strong ionic interaction between alginate and chitosan. The results of the Effectiveness Test and encapsulation of *B. cereus* SN7 bacteria were carried out by looking at the resistance of the capsule beads that had been formed.

Testing with different pH, namely: pH 5 (acid), pH 7 (neutral), and pH 8 (base) for 75 minutes showed that the encapsulation resistance of pH 5 (acid) did not cause damage to the capsule beads, instead the membrane of the bacterial capsule beads seemed to thicken and look brighter in color. Whereas in the pH 7 (neutral) test, the capsule membrane seemed to be getting thinner with a fading color, as well as what happened in the pH 8 (alkaline) test was damage to the capsule beads occurred. Capsule beads become wrinkled with increasingly faded colors and some beads are broken. Other (Yulinery and Nurhidayat, 2012) mentioned that the effect of high pH will reduce the damage to the shape of the beads.

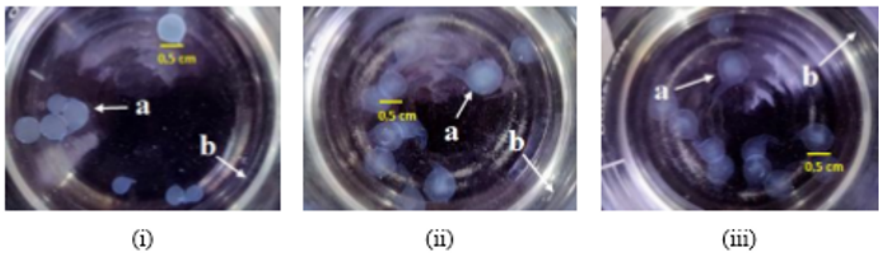


Fig. 4. pH 5 test results (i), pH 7 test results (ii), pH 8 test results (iii): a. Capsule beads, b. Beaker as storage vessels.

Testing resistance to the physiological solution (NaCl 0.9%) for 75 minutes, bacterial encapsulation beads look bright with membranes that look increasingly thickened. NaCl 0.9% solution is an alternative to equalize acid levels in the stomach, (Trimudita and Djaenudin (2021). The use of NaCl solution is the pH range commonly observed in the stomach. The survival of the capsule shape from 0.9% NaCl solution which still contains acidic levels causes the encapsulated beads to not be damaged. This is because the alginate used as an encapsulating material is a material made of acid-containing components, so the beads inserted into 0.9% NaCl solution can maintain the alginate component that is already in the form of encapsulated beads. (Rasyid, 2005). Alginate is a linear copolymer consisting of two monomeric units, namely D-mannuronic acid and Lguluronic acid. The respective levels of acid contained in the solution and the encapsulated beads did not result in a decrease in the quality of the alginate and damage to the encapsulation too quickly.

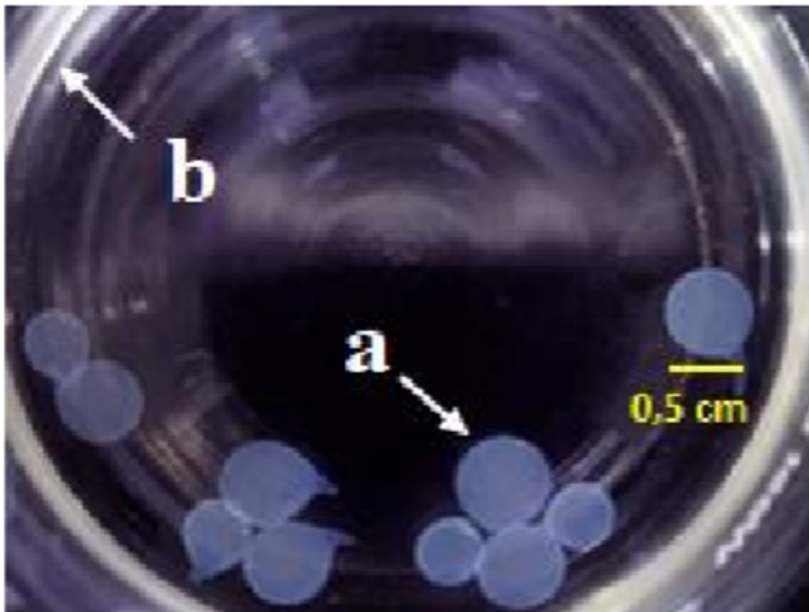


Fig. 5. Test results using 0.9% NaCl2 solution: a. Capsule beads, b. Beaker as a storage vessels.

Gram staining results of *B. cereus* SN7 (Figure 6) showed gram staining with positive results shown in purple color and the shape of bacteria seen is bacillus. The bacteria that have been encapsulated survive. This is evident from the gram staining results which show the *B. cereus* SN7 used is the same as the previous gram staining results. This also shows that there

is no contamination in the encapsulation process. If there is a contamination of bacteria, it will be seen that the bacterial gram is red (negative) or there is a mixture of bacterial forms, then the encapsulation process is considered not aseptic.

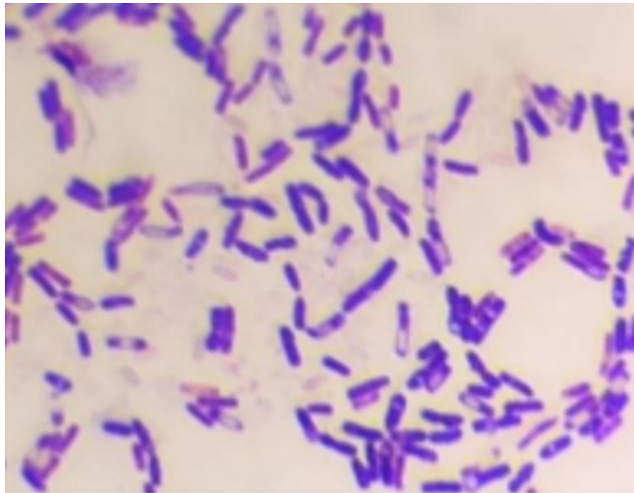


Fig. 6. Gram stain results of *B. cereus* SN7

4 Conclusion

1. The encapsulation research process carried out using alginate by using a mixed emulsion solution of chitosan plus 1% acetic acid can form into capsule beads that can protect bacteria.
2. Test the effectiveness of capsules and bacterial viability, the best is at a temperature of 100 C and pH 5 and able to survive the capsule on immersion with physiological solution.
3. No Gram stain result change of *B cereus* SN7 bacteria and no contamination occurred.
4. Further suggestions are suggested to the use of *B cereus* SN7 capsules as a protein source of protein substitutes of fish diet.

Acknowledgments

The authors wish to thank the LPPM University of Riau and the Ministry of Research, Technology and Higher Education (RISTEKDIKTI), for funding this research through the applied research grant with Contract Number: 1313 /UN.19.5.1.3/AL.04/2023.

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