The 4th International Conference on Agriculture and Environmental Sciences (ICAES) 2023
Volume 2024
http://dx.doi.org/10.11594/nstp.2024.3901

NST Proceedings
OPEN ACCESS

Conference Paper

Analysis of Encapsulation Efficiency *Bacillus* sp. Based Variations in Sodium Alginate Concentration in the Beads

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ABSTRACT

The use of biological agents such as Bacillus sp. Bacteria have started to be widely used by farmers as a new form of control. Bacillus sp. needs to require a special method in its application because it has certain living conditions, and Bacillus sp. is susceptible to environmental stress like other microorganisms. Apply Bacillus sp. as a biological control agent in the field, it needs to be formed into a formulation to be more effective and efficient when applied. Liquid formulations often used cannot maintain the viability of bacteria in the soil. The bioencapsulation formulation in the form of microcapsules is considered more effective in maintaining the viability of bacteria in the soil. This study aims to determine the effect of concentration on the encapsulation efficiency of Bacillus sp. inside the microcapsule. Beads are made using the extrusion method by combining a sodium alginate suspension of 1%, 1.5%, and 2% concentration and a bacterial suspension and dropping it into a calcium chloride solution. The encapsulation efficiency test was carried out once by extracting the bacteria on the beads, growing them in the growth medium, and then observing them. The results of the encapsulation efficiency test with a concentration of 1%, 1.5%, and 2% had values of 1%, 0.36%, and 1.35%, respectively. The difference in the results of this encapsulation efficiency indicates that there is an effect of sodium alginate concentration on the value of encapsulation efficiency.

Keywords: Bacillus sp., efficiency encapsulation, sodium alginate, beads

Introduction

Control of plant pathogens is an important challenge in modern agriculture. Biological agents, such as the bacterium *Bacillus* sp., have received increasing attention as a more sustainable alternative to synthetic chemicals. However, using biological agents often faces challenges, especially regarding the application formulation. Liquid formulations, which are traditional formulations, have limitations regarding storage stability and risk of contamination (Tu et al., 2015). Therefore, developing more effective and innovative formulations is an urgent need.

Bioencapsulation formulations are considered more effective and innovative as a biological agent application. Bioencapsulation involves trapping biological agents, such as *Bacillus* sp., in a protective matrix, such as beads or microscopic particles, which protects against adverse environmental conditions and contamination risks. Previous research has demonstrated several advantages of bioencapsulation formulations, as described by Huq et al. (2013); and Schoebitz et al. (2013) that beads can protect against extreme conditions, such as changes in temperature, humidity, and light, which generally affect the survival and performance of *Bacillus* sp. in the field. Additionally, beads can reduce the risk of cross-contamination with other microorganisms, often a problem in liquid formulations.

One of the important challenges in developing bioencapsulation formulations is ensuring that the biological agents, in this case *Bacillus* sp., can be encapsulated efficiently and that the resulting beads

How to cite:
Yuningsih, H., Nashriana, Febriani, I et al. (2024). Charges and payments of compensation for state financial losses as an effort to obliteration of corruption crimes. *The 4th International Conference on Agriculture and Environmental Sciences (ICAES) 2023*. NST Proceedings. pages 7-14. doi: 10.11594/ nstp.2024.3901

can adsorb *Bacillus* sp. well. Encapsulation efficiency refers to how well the microorganism can be encapsulated in the coating material (Kurniasari, 2016), while the ability of the beads to adsorb *Bacillus* sp. is related to the capacity of the beads to retain the microorganisms within them, despite the great potential of bioencapsulation formulations in increasing the effectiveness of using *Bacillus* sp., there is not much information available about the extent to which beads from these formulations can adsorb *Bacillus* sp. and the factors that influence this ability.

Material and Methods

Preparation of Bacillus sp. suspension

The preparation of the bacterial suspension is carried out using distilled water. It involves the first step of rejuvenating the bacteria for one day or 24 hours before the bacteria will be used. After rejuvenation, the bacterial isolates were diluted in distilled water separately to obtain the desired population density. In this situation, the bacterial population density reached 10^8 CFU/ml, and 10 ml of this solution was taken as an inoculum source.

Preparation of bioencapsulation solution

Bioencapsulation consists of making a sodium alginate solution and a $CaCl^2$ solution. Sodium alginate was dissolved in distilled water using three different concentration treatments, namely 1%, 1.5%, and 2%. $CaCl^2$ was dissolved in distilled water with a concentration of 3%, and both solutions were sterilized using an autoclave at 120 ^{O}C for 20 minutes.

Making beads

The previously prepared biopolymer solution was mixed with a suspension of *Bacillus* sp. in a ratio of 10:1 according to the method described by (Khan et al., 2013). Then, a syringe slowly injected this mixture into a 3% CaCl2 solution.

Wash beads

Beads were cleaned from contamination on the surface by washing them using a sterile 0.85% NaCl solution. Beads were washed by rinsing and filtered using a sterile 0.85% NaCl solution three times before analysis (Ratnasari et al., 2014).

Encapsulation efficiency test

One gram of beads is crushed with a scalpel until coarse, then placed in a test tube containing 10 ml of sterile distilled water. After that, the tube was vortexed for 5 minutes. The results of the vortex process were taken as much as 1 ml using a micropipette and implanted into NA media, then incubated for 24-48 hours (Pringginies, 2020).

The encapsulation efficiency of *Bacillus* sp. in beads is measured by calculating the population growth of the colony according to the formula by (Tu et al., 2015).

$$\% = \frac{B\ x\ 100}{S}$$

Note:

B = Number of bacteria in beads (CFU ml^{-1})

S = The number of bacteria added to the suspension (CFU ml^{-1})

Results and Discussion

Encapsulation efficiency (EE) is an important element affecting a material's encapsulation ability (Çabuk & Harsa, 2015). The encapsulation process of *Bacillus* sp. with different concentrations of sodium alginate showed the following results (Table 1).

Table 1. Efficiency encapsulation results

Concentration	EE%
1%	1
1.5%	0.36
2%	1.35

A concentration of 2% has the highest EE value of 1.35%. According to Elnashar et al. (2010) the higher the concentration of sodium alginate, the more places it will provide for Ca^{2+} to bind result in forming a more compact gel membrane with a smaller pore size. However, the highest value, 1.35%, can be considered low compared to similar studies. Khimmakthong et al. (2020) arried out encapsulation of *Bacillus* subtilis bacteria using sodium alginate+gelatin, showing EE values ranging from 90-93% and carried out encapsulation using sodium alginate, sodium alginate+gelatin, sodium alginate+chitosan, sodium alginate+gelatin+ chitosan showed EE value results in sequence, namely 54.02%, 62.68%, 52.53%, 70.09%. This difference in EE values can be caused by differences in the method to obtaining EE values. The method used by Khimmakthong et al. (2020) is to dissolve the beads in a Phosphate-buffered saline (PBS) solution, while in this study a coarse crushing method was used. The choice of the coarse crushing method compared to the PBS solution is because the PBS solution is a buffer solution that is often used in biological applications (Michnik et al., 2022). However, the buffer solution has a specific pH and composition, so the PBS method is not used because the core material of these beads is microorganisms that are sensitive to changes in pH and temperature.

The comparison of EE values between the use of a single material, sodium alginate, is lower than multiple materials due to the nature of the sodium alginate material, which has a porous surface. According to Peretz et al. (2013), sodium alginate is a porous material. Encapsulation efficiency is influenced by several parameters, one of which is polymer concentration. Efficiency will increase in direct proportion to increasing polymer concentration (Hapsari et al., 2022). This can be proven when the concentration of sodium alginate increases from 1.5% to 2% and the efficiency value increases from 0.36% to 1.35%. The high concentration of sodium alginate will cause viscosity, and the increased viscosity affects the formation of a faster density, reducing the bead wall (Gayo, 2016). However, increasing the concentration only sometimes shows positive results because by increasing the concentration from 1% to 1.5%, the efficiency value decreases from 1% to 0.36%. This is thought to be because a greater concentration of sodium alginate causes density, so *Bacillus* sp. is more difficult to enter, and cause *Bacillus* sp. there are fewer coated ones.

Conclusion

The sodium alginate concentration significantly affects the encapsulation efficiency (EE) of the beads, with a concentration of 2% giving the highest EE value of 1.35%. Increasing the concentration of sodium alginate can increase the EE because it provides more places for binding Ca2+ ions, which helps form a more compact gel membrane with smaller pores. Other parameters, such as solution viscosity, also influence encapsulation efficiency. Increasing the concentration of sodium alginate can increase the viscosity and affect the formation of beads with a faster density. However, an increase that is too large may reduce the EE value due to the difficulty of the material getting into the beads. Overall, the results of this study indicate that the encapsulation efficiency is greatly influenced by various factors, especially the concentration of the polymer as well as the viscosity of the solution.

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