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(REVIEW ARTICLE)

# Prospective bioencapsulation to improve the quality of phosphate-solubilizing biofertilizers for crop production: A review

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## Abstract

Farmers commonly use chemical phosphorus fertilizer to increase crop yields, but long-term use may harm the environment. Biofertilizers specifically phosphate solubilizing microbes (PSMs), are used for restoring soil fertility in crop production. PSMs contribute to plant phosphorus supply by increasing phosphorus availability in the soil through organic acid and phosphatase enzyme secretion. However, the conventional biofertilizer formulation is often limited by a short shelf-life followed by a decreased microbial effectiveness. In recent years, bioencapsulation formulation have received more attention because they offer products that have a longer shelf-life, protect microbes in harsh environments, and maintain the effectiveness of biofertilizers. This literature review focuses on the latest bioencapsulation technology, the impact of bioencapsulation on phosphate-solubilizing biofertilizers, and the potential for developing bioencapsulation-based biofertilizers for sustainable agriculture. Precise formulation is crucial in bioencapsulation, influencing the quality and performance of biofertilizer inoculants. Bioencapsulated biofertilizers offer advantages such as improved nutrient management, enhanced plant stress tolerance, disease suppression, and innovative agricultural waste management. Despite longer shelf life and adaptability to various crops, further research is needed to address scalability, cost-effectiveness, and standardized encapsulation techniques. In conclusion, bioencapsulation innovations have the potential to promote sustainable and productive crop production.

**Keywords:** PSMs; Biofertilizers formulation; Polymers; Additives

## 1. Introduction

Agricultural productivity is an important factor in meeting the world's food demand, which is increasing as the world's population grows. Plant productivity is limited by low P availability in fields. A lack of phosphorus in crops may delay photosynthesis, resulting in lower total chlorophyll and plant biomass, along with changes in metabolism and carbohydrate translocation [1]. Chemical phosphorus fertilizers have played an important role in increasing crop yields, but their overuse has led to negative environmental consequences [2]. Biofertilizers have emerged as a sustainable solution to this problem, leveraging the natural interactions between plants and beneficial microorganisms [3].

Phosphate exists in soil as an unavailable form in amounts ranging from 95 to 99% [4]. Using phosphate solubilizing microbes (PSMs) is a single approach to maximize the non-available form of P remains in the soil after fertilization. PSMs are able to increase P availability in soil by secreting organic acids and phosphatase enzymes [5]. This allows PSMs to be used to reduce the phosphorus fertilizer needs. Several PSMs are reported not only solubilizing phosphate but also release phytohormones such as IAA and GA3 (Gibberellic Acid), used to stimulate cell elongation at the plant's growing tip [6]. PSMs with their unique ability to enhance phosphorus accessibility for plants, offer great potential for sustainable agriculture.

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However, biofertilizers formulation has limitations related short shelf life and decreased effectiveness of inoculants [7]. Biofertilizers formulation is a critical step in utilizing the potential of microbial inoculants for sustainable agriculture, driven by the need to improve their viability, stability, and efficient delivery to target plants [8]. Microbial inoculants must be carefully formulated to face environmental challenges, ensuring their survival during storage, transportation, and field application [9]. Conventional carriers for biofertilizers can be classified into two categories which are solid and liquid formulas. Solid carriers are typically made up of natural or synthetic materials that provide a protective environment for the beneficial microbes, while liquid carriers are solutions that contain the microbes in a liquid form [10].

Both liquid and solid formulations have advantages and disadvantages, necessitating careful consideration of the specific requirements of the target crop as well as the environmental conditions. Bioencapsulation, a technique that involves encapsulating microorganisms within protective matrix [11], has a promising strategy for resolving these challenges and improving biofertilizer stability and performance. bioencapsulation acts as a shield against harsh environmental conditions, protecting microbial cells during storage, transportation, and application [12]. This encapsulation not only extends the shelf life of biofertilizers but also ensures that microbes are delivered to the rhizosphere in a precise way, optimizing their interaction with plant roots and subsequent phosphorus solubilization [13,14]. Bioencapsulation has the potential to PSMs compatibility with other biofertilizer components in addition to addressing the challenges of PSMs survival and delivery. Through bioencapsulation, synergistic combinations of PSMs with nitrogen-fixing bacteria and other beneficial microorganisms can be achieved, promoting a holistic approach to plant nutrition [15].

Furthermore, bioencapsulation can significantly reduce the environmental impact of biofertilizer application. The controlled release of microbial reduces the risk of nutrient leaching, ensuring that fertilizers are used efficiently by the target crops while minimizing pollution to the environment [16]. The use of bioencapsulated biofertilizers is not limited to traditional agriculture, it also holds promise for more sustainable farming practices such as organic and precision agriculture. The flexibility of bioencapsulation techniques allows for customized formulations customized to specific crop needs and soil conditions [11]. In conclusion, the bioencapsulation of phosphate-solubilizing microbes represents a game-changing approach to improving the quality and efficiency of biofertilizers in crop production. This review summarizes current knowledge on bioencapsulation techniques, offering insights into potential applications and paving the way for future research of phosphate-solubilizing biofertilizers aimed at sustainable and resilient agricultural practices.

## 2. Bioencapsulation materials and methods for biofertilizers development

Cell immobilization and encapsulation have been widely used to achieve protective cells due to limited exposure to harmful environmental factors, relatively stable cells, and potentially enhanced viability and stability [17]. Formulating biofertilizers using bioencapsulation methods is a promising avenue that addresses the challenge of preserving microbial viability and enhancing the efficiency of nutrient release. The materials used for encapsulation are selected by factors such as the desired release kinetics, environmental compatibility, and the specific needs of the target crop [18]. The carrier materials commonly used for bioencapsulation are polymers such as alginate, chitosan, maltodextrin, etc.

Alginate, chitosan, maltodextrin, or any other polymers are used for encapsulation have various benefits in terms of biocompatibility, controlled release, and resistance to external factors. Alginate has been shown increase the viability, maintain microbial activity, and protect microbes from environmental stress [19]. As a carrier material, chitosan can protect against extreme conditions such as low pH and high temperature [20]. Maltodextrin can also guarantee microbial stability and viability at high temperatures, such as encapsulation with spray drying process [21]. These numerous advantages can be utilized to improve the shelf life and long-term effectiveness of biofertilizers.

Bioencapsulation methods also play a crucial role in protecting and delivering microbes for various applications. Several encapsulation techniques, such as extrusion (cross-linking), spray drying, freeze drying, and emulsion, are commonly used to encapsulate microbes within protective matrix.

- Extrusion (cross-linking) is a method that involves forcing a mixture of microbes and a matrix material through a small opening to form spherical particles [22]. Cross-linking agents are then used to chemically cross-link the matrix material, creating a stable structure that encapsulates the microbes. This method is often used with materials such as alginate or other polymers to protect microbes from harsh environmental conditions [23].
- Spray drying is a widely used encapsulation technique that involves atomizing a mixture of microbes and a matrix material into a hot air stream [24]. The rapid evaporation of the solvent results in the formation of dried

microcapsules containing the encapsulated microbes. This method is suitable for heat-sensitive microbes (such as thermophilic) and allows for the production of free-flowing powders that can be easily stored and transported [25].

- Freeze drying, also known as lyophilization, is a method that involves freezing a mixture of microbes and a matrix material and then removing the frozen solvent under vacuum [26]. This results in the formation of dried microcapsules containing the encapsulated microbes.
- Emulsion is a method of dispersing a mixture of microbes and matrix material in a continuous phase to form droplets that are then stabilized to prevent coagulation [27]. The droplets are then solidified to form microcapsules containing the encapsulated microbes. This method is often used with materials such as lipids to protect microbes from environmental stresses.

Furthermore, the addition of additives has become integral to improving the quality of bioencapsulation products. Cross-linking agents, such as calcium chloride for alginate encapsulation, enhance structural stability [12]. Stabilizing agents like glycerol are usually used to improve their shelf life and stability [28]. The addition of other materials, such as clay minerals, helps to create a better microcapsule structure for microbial attachment [14,29]. There are many other additives might be used for bioencapsulation to achieve the ideal outcomes. These additives aim to enhance the physical and chemical properties of the encapsulating materials, ultimately ensuring the efficacy and reliability of biofertilizers in sustainable agricultural practices. Table 1 summarizes several previous studies on bioencapsulation with various carrier materials and methods applied to PSMs inoculants.

**Table 1** Various Bioencapsulation material and methods for Phosphate-Solubilizing Biofertilizer

| **Microbes** | **Carrier** | **Additives** | **Encapsulation Methods** | **References** |
|---|---|---|---|---|
| *Pseudomonas putida & P. kilonensis* | Sodium alginate | Natural char & nano clay | Extrusion (Cross-linking) | [14] |
| *Bacillus* spp. | Maltodextrin & alginic acid sodium salt | Rock Phosphate | Spray drying | [24] |
| *Azosprillum brasilense & Pseudomonas fuorescens* | Sodium alginate | Montmorillonite & halloysite | Extrusion (Cross-linking) | [29] |
| *Kosakonia radicincitans* | Amidated pectins | Maltodextrin, sorbitol, & monosodium glutamate | Extrusion (Cross-linking) | [30] |
| *Azotobacter vinelandii* | Sodium alginate | - | Extrusion (Cross-linking) | [31] |
| PSMs | Sodium alginate | - | Aerosol - Freeze drying | [32] |
| *Trichoderma harzianum* | Sodium alginate & sodium carboxymethyl cellulose | Trehalose & xylitol | Extrusion (Cross-linking) | [33] |
| *B. megaterium* | Sodium alginate | - | Extrusion (Cross-linking) | [34] |
| *Bacillus subtilis & Pseudomonas fluorescens* | Itaconic acid (IA), Tert-butyl hydroperoxide (tBHP), sodium alginate, & maltodextrin | - | Spray drying | [35] |
| *Bacillus licheniformis* | Sodium alginate & chitosan | Rice starch | Extrusion (Cross-linking) | [36] |
| *Bacillus velezensis* | Sodium alginate | Gelatin | Extrusion (Cross-linking) | [37] |

| | | | | |
|---|---|---|---|---|
| *Bacillus megaterium* | Sodium alginate | Cassava starch | Extrusion (Cross-linking) | [38] |
| *Pseudomonas* spp. | Laponite | - | Extrusion (Cross-linking) | [39] |
| *Bacillus pumilus* & *Glycyrrhiza uralensis* | Sodium alginate | Trehalose & kaolin | Extrusion (Cross-linking) | [40] |

## 3. Benefits of Bioencapsulation to Enhance Biofetilizer Inoculant Quality

A longer period of shelf life is one of the primary benefits of using bioencapsulation in biofertilizer production. Microorganisms coated in protective shells are more resistant to environmental stressors like temperature fluctuations and UV radiation [11]. This extended shelf life ensures that the biofertilizer retains its efficacy for a longer period, making storage, transportation, and application in a variety of agricultural settings easier. Several previous studies have proven that bio encapsulation has the potential to increase the viability of biofertilizers inoculant. Quynh *et al.* [38] reported the density of *B. megaterium* cells does not seem to change after 7, 30, 90, or 180 days in the beads-based biofertilizer. After 6 months of storage, the density of *B. megaterium* was still high, indicating that the biofertilizer was possibly stored for longer time. Compared to their previous study, the density of *B. megaterium* decreased from 8,6 x $10^8$ CFU $g^{-1}$ to 2,6 x $10^8$ CFU $g^{-1}$ when the carrier was created from a combination of cassava starch and rice bran. This shows that the bioencapsulation formulation can maintain the microbial population alive longer compared to conventional solid carriers.

The presence of *Azotobacter vinelandii* at various sampling times after inoculation on 7, 14, and 28 days was found to have a much higher microbial population in the alginate-Na bead treatment compared to the liquid inoculum [31]. Kapishon *et al.* [35] also reported the storage stability and viability of encapsulated microbes tested on spray-dried *B. subtilis* stored in a refrigerator at 4 °C for a long period of time. The encapsulated bacteria were viable with only a minor net loss in viability, with 83% and 78% of the *B. subtilis* surviving storage without additives at 3 and 6 months, respectively. Moreover, encapsulated *Trichoderma harzianum* showed increased mycelial growth rate on saline solid medium and viability in saline solution by using trehalose-doped shell structured hydrogels as the microbial carrier [33]. Furthermore, after 180 days of encapsulated *T. harzianum* incubated in the mixtures of chemical-organic fertilizers with NPK levels of 5:5:5, the shelf-life of *T. harzianum* increased significantly from log 5,72 CFU $g^{-1}$ to log 7,30 CFU $g^{-1}$. This finding was probably related to offering valuable guidance for improving agricultural microbe shelf-life and developing agricultural practices.

Bioencapsulation also enables biofertilizer formulations to be customized by encapsulating multiple strains of microorganisms or incorporating additional components such as nutrients, growth-promoting substances, or microbial enhancers [10]. This adaptability enables the development of tailored biofertilizers to address specific soil and crop requirements, increasing their overall efficiency and impact on agricultural productivity. Kadmiri *et al.* [29] evaluated viable cell numbers in the alginate-based capsules combined with the montmorillonite and halloysite minerals. The halloysite-alginate formulations performed better than the montmorillonite-alginate formulations in terms of survival stability, with up to 6 x $10^{14}$ CFU $g^{-1}$ and 0,66 x $10^{14}$ CFU $g^{-1}$, respectively. It should be noted that both formulations used the same culture at the same initial concentrations.

*Bacillus pumilus* encapsulation based on alginate, kaolin, and trehalose has been shown to keep a viable number of bacteria above $10^8$ CFU $g^{-1}$ for 6 months [40]. The optimized microcapsule structure forms a dense wrinkled surface, allowing for slow and controlled release of *B. pumilus*, the rough interior of the microspheres allows attachment of *B. pumilus* in microcapsules. The other research, Safari *et al.* [14] reported the mixture of nano clay (montmorillonite) and alginate in freeze-drying encapsulation also guaranteed the viability of *Pseudomonas putida* and *P. kilonensis* (up to $10^8$ CFU $g^{-1}$). Mineral clay such as montmorillonite and kaolin have a high specific surface area, porosity, and its ability to adsorb organic compounds and microorganisms, providing an ideal environment for bacteria growth and proliferation, making it a favorable carrier [41].

Safari *et al.* [14] also reported nano clay alginate-based encapsulation increased phosphatase activity of both isolates, but it was not significantly different from natural char alginate-based encapsulation in *P. kilonensis* isolates. This assumed to be influenced by the high P content in the matrix and the isolate's ability to solubilize phosphate. The ability of bacteria to solubilize phosphate was also directly related to the bacterial population in safari [14] research findings, as they observed the highest solubility index for both bacterial species found in the alginate nano clay carrier compared to other carriers. Jokkaew *et al.* [34] reported *B. megaterium* cells encapsulated in alginate were able to rapidly dissolve phosphorus from swine wastewater-derived struvite, resulting in soluble phosphorus concentrations of 400 mg P/L in 2 days, compared to 12 days for those without cells. This finding allows the development of phosphate-solubilizing biofertilizer products utilizing agricultural waste.

The precise formulation in encapsulation plays a critical role in determining the quality of the biofertilizer produced and has a significant impact on the performance of biofertilizer inoculants. The formulation includes a precise combination of microbial strains, carrier materials, protective agents, and other critical components, serves as the basis for the biofertilizers efficacy and stability. A well-designed formulation not only ensures the viability and activity of beneficial microorganisms but also improves their resistance to environmental stressors. When used in agricultural contexts, this leads to increased efficiency in nutrient mobilization and plant nutrient uptake.

## 4. The Effects of Using Bioncapsulated Biofertilizers on Crop Production

Encapsulated biofertilizers have shown great potential in enhancing crop production by providing a controlled and targeted release of beneficial microbes, which can improve nutrient uptake, plant growth, and stress tolerance. Zhang *et al.* [40] reported that incorporating *B. pumilus* microcapsules significantly increases available nutrient levels in *Pharbitis nil* rhizosphere soil, especially under salt and/or drought stress and their effect is significantly greater than liquid inoculant. This effect is caused by *B. pumilus* dominating the soil around the roots of *P. nil*, which may happen because the encapsulated microbes are released slowly and can survive longer in the soil. The presence of *B. pumilus* and microcapsule components may promotes the formation of bacterial groups, increases their metabolism, and increases enzyme and organic acid production [42]. As a result, phosphorus solubilization in the soil is encouraged, causing the rate of phosphorus accumulation to increase. Furthermore, nitrogen-fixing microorganisms such as *B. pumilus* contribute at nitrogen fixation, improving nitrogen status of the soils.

Conde-avila *et al.* [31] reported addition of encapsulated *A. vinelandii* in tomato increased root size by 69%, this could be related to auxins like IAA, which are found in *A. vinelandii* are responsible for increasing root length by stimulating root cell division and differentiation, as well as nutrient absorption [43]. Although encapsulating *A. vinelandii* in alginate-Na beads limits its spatial distribution in the soil, it does not affect its metabolic activities, allowing its release and establishment during the first phenological stage of crop[31]. Similarly, treatment with encapsulated *Bacillus licheniformis* enriched with alginate-chitosan nanoparticles (CNPs) resulted in a significant increase in shoot length in *Capsicum annuum* seedlings compared to the control [36]. The plant growth promoting properties such as IAA, ACC deaminase, phosphate solubilization, and nitrogen fixation, may have favored the observed changes in shoot length.

Using modified pectin beads to immobilized bacterial cells shows promise in promoting plant colonization by endophytic bacteria. When the beads are dried, they may take longer to absorb water leads formation of pores within the gel matrix, which can improve internal moisture for the multiplication of *K. radicincitans* cells, this promotes higher radish yields when compared to free-living cell treatment [30]. The establishment of bacteria can be successful through indirect mechanisms provided by the formulation. Pectin as a major component of plant cell walls may act as an environmental factor that stimulates bacterial biofilm formation during plant colonization [44]. The addition of maltodextrin and amyloglucosidase in the modified pectin beads may also provide an extra carbon source for bacterial growth [45].

Encapsulated biofertilizers also can be combined with other nutrients, such as nitrogen, phosphorus, and potassium, to create multiple-nutrient products that can replace or supplement chemical fertilizers. This combination can lead to more sustainable and efficient nutrient management practices, reducing the reliance on inorganic fertilizers and promoting environmentally friendly agriculture. The results show that cabbage inoculated with beads-based biofertilizer in addition to NPK grew and yielded better than cabbage treated with NPK alone. The use of the beads-based biofertilizer stimulated plant growth and resulted in a yield that was 13,07% higher than the control. Even after reducing the recommended NPK dosage by 20%, the cabbage yield was still 12,36% higher than the control [38].

The application of a *Pseudomonas* spp. consortium encapsulated in laponite was found to improve growth in *Vigna unguiculata* plants, and the formulation has the potential to increase crop yield and improve soil fertility [39]. Laponite clay creates a microenvironment suitable for the safe distribution, preservation, and multiplication of the plant growth promoting rhizobacteria (PGPR) to plants. Moreover, Kadmiri *et al.* [29] investigated the application of *Azosprillum brasilense* encapsulated in alginate with the addition of both clay mineral types significantly improved plant growth parameters when compared to the control. The plant's height increased by 25%, and root and shoot biomass increased by more than 100% when compared to control plants. Nonetheless, the montmorillonite-alginate formulation of this strain had a lower effect than the halloysite-alginate formulation. These results confirmed the interaction between bacteria and matrix composition, which affects release behavior, survival stability, and thus the effect of biofertilizers on plants.

In addition, bioencapsulation can also create a controlled release of biological agents to suppress the growth of plant diseases. Alginate-gelatin encapsulated *B. velezensis* had the greatest ability (93.66%) to reduce *Phytophthora drechsleri* infection in pistachio plants, compared to a disease control rate of 75% in plants treated with free inoculum [37]. Futhermore, pistachio plants applied with encapsulated bacteria grew significantly faster than control plants, as measured by shoot length and fresh and dry weight of shoots and roots. This was due to the high production of auxin by *B. velezensis* isolates in the study may promoted the growth of pistachio plants.

Bioencapsulated biofertilizers provide numerous benefits to crop production, including improved nutrient management, enhanced plant stress tolerance, disease suppression, and great innovation for agricultural waste management. These innovations have the potential to promote environmentally friendly and sustainable agriculture practices, resulting in increased crop yield and quality.

## 5. Challenges and Opportunities Developing Bioencapsulated Biofertilizers

The development of bioencapsulated biofertilizers presents several challenges and opportunities (Table 2).

**Table 2** Bioencapsulation formulation challenges and opportunities

| Challenges | Opportunities |
|---|---|
| Developing an optimal formulation for bioencapsulation is a complex process. The matrix material should be biocompatible, stable, and able to protect the microbes from environmental stresses. | Bioencapsulation can improve the efficiency of biofertilizers by protecting the microbes from environmental stresses and releasing them in a controlled manner which can lead to better uptake and utilization by plants. |
| Ensuring the viability of microbes during the encapsulation process is crucial. Encapsulation methods such as spray drying, freeze drying, and emulsion can help maintain the viability of microbes, but optimization of these methods is essential. | Bioencapsulation allows for the targeted delivery of biofertilizers to specific locations, such as rhizosphere zones, where they can have the most significant impact on plant growth and health. |
| Scaling up the production of bioencapsulated biofertilizers can be challenging, as it requires efficient processes and appropriate equipment. | Bioencapsulated biofertilizers can be combined with other types of fertilizers, such as inorganic fertilizers, to provide a balanced nutrient supply to plants. |
| The cost-effectiveness of bioencapsulated biofertilizers is an important factor, as high production costs can limit their commercialization. | The use of bioencapsulated biofertilizers can contribute to agricultural waste management by converting organic waste materials into valuable fertilizers. |

## 6. Conclusion

Current problems related to PSMs-based biofertilizers (such as short shelf life) need improvements in formulation techniques. Bioencapsulation solves these problems by improving inoculant shelf life, protecting inoculant against harsh conditions, and maintaining precise delivery to the plant. Bioencapsulated biofertilizers not only have a longer shelf life, but they also enable for modified formulations that combine multiple strains or additional nutrients, improving adaptability for various crops and soils. Although the benefits of bioencapsulation in improving biofertilizer quality are obvious, further research is required to address issues such as scalability, cost-effectiveness, and the development of standardized encapsulation techniques. In this sector, prospects for the future include the incorporation of nanotechnology for more advanced encapsulation methods, the exploration of novel carrier materials, and the optimization of formulations for specific crop types and soil conditions. Bioencapsulation innovations have the potential to encourage a more sustainable and productive strategy for crop production.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

None of the authors has any conflict of interest.

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