

# ENCAPSULATED FORMULATIONS FOR MICROORGANISMS IN AGRICULTURE AND THE ENVIRONMENT

Bashan, Y., L. E. de-Bashan – The Bashan Institute of Science, Alabama, USA and CIBNOR, Mexico

## INTRODUCTION

Inoculation of plants with microorganisms to enhance crop yields or native plants has been practiced for several decades now (Bashan et al., 2014; Calvo et al., 2014). Two factors predominate in the success of inoculation: effectiveness of the bacterial isolate and the technology of application. Technologies of microorganism encapsulation in these areas are experimental, and no commercial encapsulation products currently exist (Nussinovitch, 2010). The first formulation of encapsulation with alginate, which is the most common experimental polymer in agriculture, is already 30 years old (Bashan, 1986). Technologies of encapsulation related to agricultural and environmental fields are lagging behind the advances in pharmaceuticals and nanotechnology. Consequently, the main goal of encapsulation in agricultural and environmental fields is to incorporate it into commercial products and to make the products acceptable to farmers as a routine practice, rather than creating new approaches or polymers. This should largely consist of an adaptation of current techniques to current agricultural and environmental needs.

## MACROBEAD INOCULANTS

In 2016, alginate derivatives, frequently combined with adhesives, nutrients, surfactants, stabilizers, dispersal materials, bulk materials and cryo-protectants, are the preferred experimental polymers for most encapsulations of microorganisms for agricultural and environmental uses. Alginate formulations

are currently used for the application of biological control agents, bacterial growth promoters, mycorrhizal fungi, and mushroom cultivation. The advantages of alginate formulations for these purposes are their nontoxic nature, biodegradability, availability at low cost (US\$2 per kg for a Chinese product), slow release of the entrapped microorganisms into the soil—which can be accomplished by variations in the polymeric structure, and approval for human use by the US Food and Drug Administration.

While alginate formulations may have solved many of the difficulties associated with other agricultural inoculants, application of macro-alginate beads as inoculants has two unsolved disadvantages:

1. an additional treatment during sowing is needed by the grower, even if the inoculant is planted by seeding machines;
2. microorganisms released from the inoculant need to migrate through the soil to the plants.

Under agricultural practices, when beads are loosely mixed with seeds and sown together, the beads might land up to several millimeters or

even few centimeters away from the plant. The bacteria released from the beads must move through the soil, facing competition with and predation by the native microflora. Consequently, the future of macrobeads in agriculture is uncertain.

## MICRO-ALGINATE BEADS

The microbead concept (50–200  $\mu\text{m}$  or even smaller) was developed to overcome the two difficulties of macrobeads (Bashan et al., 2002; Figure 1). The idea is that if the beads are small enough, yet still capable of encapsulating a sufficient number of bacteria, it would be possible to produce a “powder-like” formulation. At the seed-handling facility, the seeds are coated with this “bead powder” and sold to the farmer as “improved seeds”. Today, seeds coated with fertilizers, fungicides, or hormones are commonplace and universally accepted by most farmers. In developed countries with large-scale agricultural practices, pre-coating seeds with microbead inoculants would eliminate the need for an additional expensive field treatment and provide

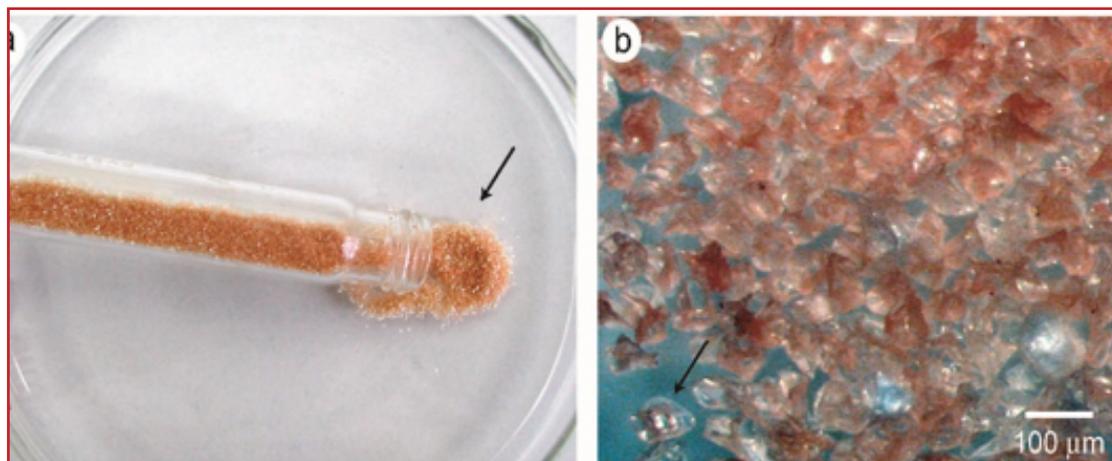


Figure 1. (a) Dry alginate microbead inoculant containing the plant growth-promoting bacterium *Azospirillum brasilense* currently being used in agriculture and the environment. (b) Single microbead (arrow).

## ARTICLE

the ultimate convenience and incentive to farmers. Application of formulations in micro-alginate beads to inoculate plants in the soil has been successful on several occasions (de-Bashan et al., 2002).

## FUTURE IMPROVEMENTS IN MICRO-ALGINATE BEADS

Currently, food, pharmacology, nanotechnology, and cosmetics represent much larger research fields for the use of encapsulation than its uses on land. Consequently, several technical improvements derived from these fields to make the polymer more suitable for the encapsulation of biological materials have been proposed. Although these encapsulations are unrelated to plant inoculants, they may provide insights for future developments. Based on experiments over the last three decades, alginate seems to be the most promising polymer. However, because of the relatively limited published research data on alginate beads related to land use, and even if the material is currently inexpensive compared to all other polymers, it is premature to predict whether alginate will displace traditional inoculants in the crop inoculation industry or will remain in the domain of industrial and environmental microbiology.

## INOCULANTS WITH OTHER POLYMERIC MATERIALS

Ironically, although commercial alginate preparations are not yet available for agriculture, several other polymers that are used in industrial and environmental microbiology may serve as substitutes when the microorganism fails to adapt to alginate preparations. All of these materials are experimental and use both macro- and microbead formulations. They include chitosan, carboxymethylcellulose-starch, ethylcellulose, modified starch, and commercial film-forming "methacrylic acid copolymer". However, because very limited information is available on these potential carriers, it is impos-

sible to predict their future as vehicles for bacterial inoculants.

## DRIED POLYMERIC CARRIERS

A main bottleneck in the production of any inoculant for agricultural and environmental improvements is shelf life, which should be increased rather than maintaining a high bacterial count because the number of bacteria eventually decreases during storage. From commercial and agricultural standpoints, longer survival of bacteria in polymeric preparations makes dry formulations extremely attractive. Such experimental formulations exist and have a shelf life of 1 to 14 years, superior, by far, to any existing commercial microbial inoculant.

## CONCLUSIONS AND PERSPECTIVES

Even though numerous experimental results indicate that this is perhaps the future of inoculants, no encapsulated formulation of microorganisms has obtained industrial approval. Immobilization of microorganisms is a large emerging field in pharmaceuticals, nanotechnology, medicine, aquaculture, and cosmetics (Bioencapsulation Innovations, <http://bioencapsulation.net>). Many different and efficient immobilization techniques have been developed for these fields, but almost none of these technologies has been tested in the inoculant field apart from direct polymerization of beads from a few polymers. Many of these emerging technologies from other fields merit testing in the agricultural inoculant industry.

## REFERENCES

- Bashan, Y. Alginate beads as synthetic inoculant carriers for the slow release of bacteria that affect plant growth. *Appl. Environ. Microbiol.* 51, 1089-1098 (1986).
- Bashan, Y., de-Bashan, L. E., Prabhu, S. R. & Hernandez, J.-P. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998-2013). *Plant*

*Soil* 378, 1-33 (2014).

- Bashan, Y., Hernandez, J.-P., Leyva, L. A. and Bacilio, M. Alginate microbeads as inoculant carrier for plant growth-promoting bacteria. *Biol. Fertil. Soils* 35, 359-368 (2002).
- Calvo, P., Nelson, L. & Kloepper, J. W. Agricultural uses of plant biostimulants. *Plant Soil* 383, 3-41 (2014).
- de-Bashan, L.E., Hernandez, J.-P., and Bashan, Y. The potential contribution of plant growth-promoting bacteria to reduce environmental degradation - A comprehensive evaluation. *App. Soil Ecol.* 61, 171-189 (2012).
- Nussinovitch, A. Polymer macro- and micro-gel beads: Fundamentals and applications. (Springer, 2010).



**Yoav Bashan**

The Bashan Institute of Science, Alabama, USA & CIBNOR, Mexico  
[ybb0001@auburn.edu](mailto:ybb0001@auburn.edu)

Prof. Yoav Bashan is the President of The Bashan Foundation, the President of The Bashan Institute of Science (USA), the Group Leader of Environmental Microbiology at the Northwestern Center for Biological Research (Mexico) and is also affiliated with Auburn University (USA). He obtained his doctoral degree from the Hebrew University of Jerusalem in Israel. His main field of research is the interactions among plant growth-promoting bacteria and plants in agricultural and environmental settings, mostly using immobilization techniques. Prof. Bashan has published over 360 scientific works, 211 of which have been published in peer-reviewed journals with impact factors. He has obtained over 14,700 citations of his studies; H-index: 62; i-index: 182.