100% Plant-Based Solutions for the Formulation of Microbial Products

INTRODUCTION

With the rising demand from growers for greater sustainability, microbial-based products bring a strong alternative to conventional products in agriculture for plant protection.

Developing a successful commercial biocontrol product from a promising microbial strain remains a substantial challenge. The need is for formulations that are stable, cost-effective, high quality, and easy to apply, and that deliver consistent and marked improvement in the field.

HARNESSING THE POWER OF BIOCONTROL FOR SUSTAINABLE AND EFFECTIVE PEST MANAGEMENT

Biocontrol is the use of living organisms to control pests and diseases that affect plants, and it offers several advantages as a sustainable and environmentally friendly approach to pest management.

Biocontrol products can be highly specific to the target pest or disease, and they can be used in combination with other pest management techniques. Additionally, they have a shorter residual period than conventional pesticides, reducing the risk of pesticide buildup in soil and water.

A DEDICATED RANGE FOR MICROBIAL STABILIZATION

Roquette, a global leader in plant-based ingredients, has developed a range of plant-derived products that can be used to formulate and manufacture stable biocontrol products.

In the development and manufacture of stabilized microbial formulations, Roquette products can act as the following:

- High-performance, biodegradable, readily soluble drying supports
- Water absorbent fillers that promote granule disintegration
- Binders, flow and extrusion aids, and compression agents to improve the efficiency of wet and dry granulation processes
- Stable fillers/bulk excipients with excellent biocompatibility
- Cryoprotectants for use in freeze drying applications
- Functional ingredients for the reduction of water activity GLUCIDEX® maltodextrin

Table 1: Roquette Microbial Stabilization product range

Product	Type	Grade	Viscosity	Solubility	pH
GLUCIDEX®	Maltodextrin	17,19 or 21	Low	Fully (600g/L)	~5
NEOSORB®	Sorbitol	P60 or 70/20	Low	Fully	Low (3-4)
	Mannitol	60	Low	Low to High (by conc.)	~9.5

STABILIZATION OF *BACILLUS AMYLOLIQUEFACIENS* SPORES FOR SEED COATING APPLICATION

Studies were conducted among our portfolio of plant-based ingredients to test the stabilization efficacy of *Bacillus amyloliquefaciens* spores using two specific Roquette ingredients. The goal of this experiment was to ensure the inherent stability of *B. amyloliquefaciens* spores is maintained when treated and stabilized with a mixture of GLUCIDEX® IT 19 (20%) and NEOSORB® 70/20 (5%) over an 8-month storage period at room temperature in sealed containers.

The method involved inducing sporulation in *B. amyloliquefaciens*, followed by treating the resultant spores with lyophilization and the specified Roquette ingredients. The stability of the treated spores was then assessed by plate counting to measure viable spores. The experiment aimed to monitor the viability of the spores throughout the 8-month storage period, ensuring that the formulation maintained spore stability and effectiveness in long-term storage conditions.

B. amyloliquefaciens Viability Spore Viability in Stabilization Formualtion

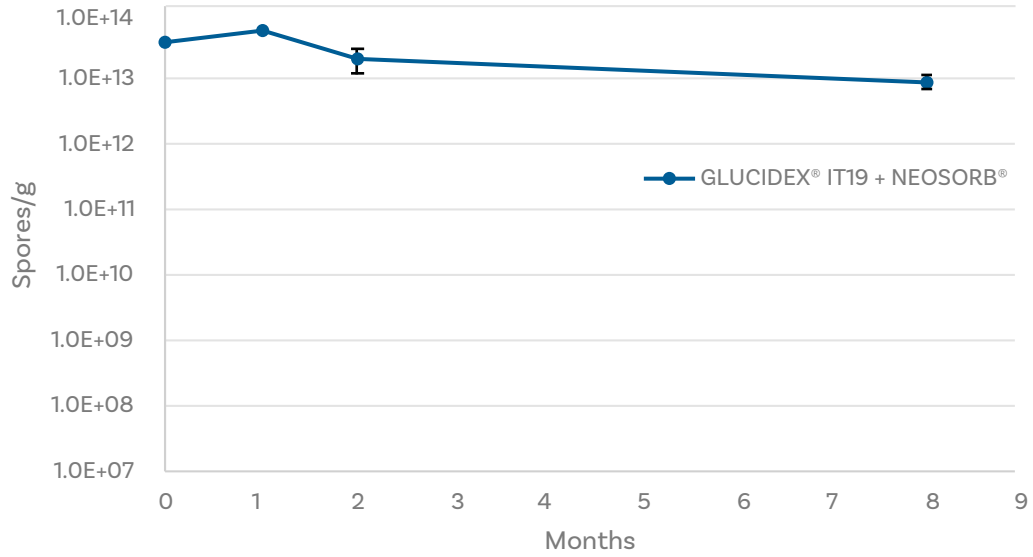

Figure 1: Stabilized *B. amyloliquefaciens* Viability

A small-scale seed coating protocol was developed to evaluate the impact to long-term spore viability when the GLUCIDEX® IT 19 + NEOSORB® 70/20 spore preparation is added to an established AGROBIND™ S780 seed coating formulation. Corn seeds (10g) were coated with the AGROBIND™ S780 formulation containing a range of dosages of the stabilized spore preparation.

Table 2: Coating Formulation Composition

Coating Type	Film
Seed Type	Corn
Mass of Seeds (g)	10
Dosage (g/kg)	12
Formulation Mass (g)	0.12
Spore Lyo. Prep (g/ml)	0.5, 0.2, 0.1, 0.05

Components	Ratio
AGROBIND™ S780	7.0%
Dye	5.0%
Microbe culture	12.5%
Water	75.5%

To assess on-seed spore enumeration, a sampling of coated seeds were washed with sterile PBS buffer to remove the coating formulation. The resulting wash was serially diluted for standard plate counting on Nutrient Agar to quantify viable spores present on the seed after coating. This procedure was repeated at each subsequent time point of the stability test.

B. amyloliquefaciens Viability Spore on Seed - Post Seed Coating

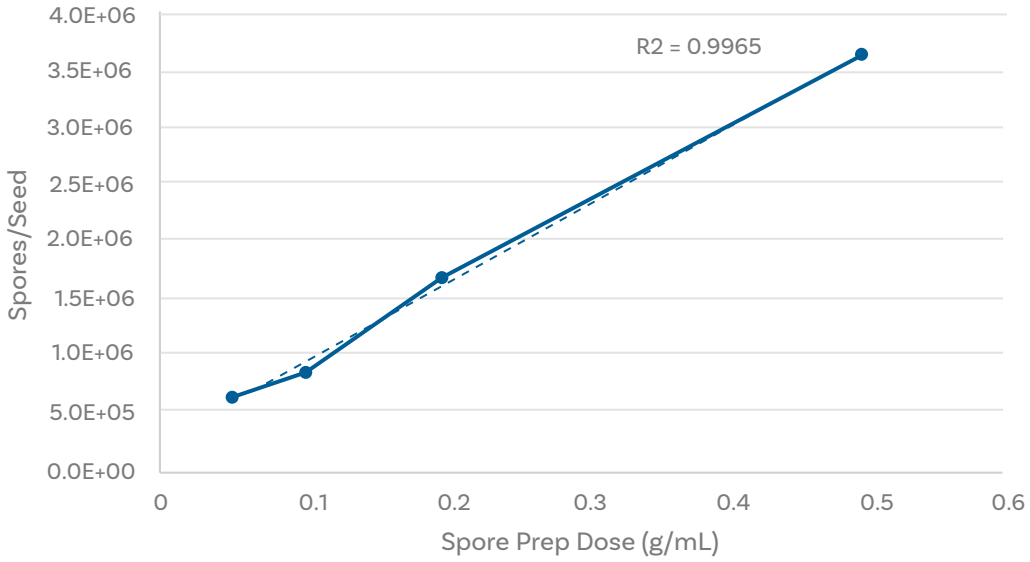

Figure 2: Spore Counts – Post Seed Coating

RESULTS

The coated seeds were stored at room temperature without humidity control, and the viability of *B. amyloliquefaciens* spores was monitored over an 8-month period. The results demonstrated that the spores retained their viability effectively, with less than a 1 log reduction in CFU counts observed. This indicates that the combination of GLUCIDEX® IT 19 and AGROBIND™ S780 in the coating formulation successfully maintained the stability of the spores on the seeds over an extended storage period.

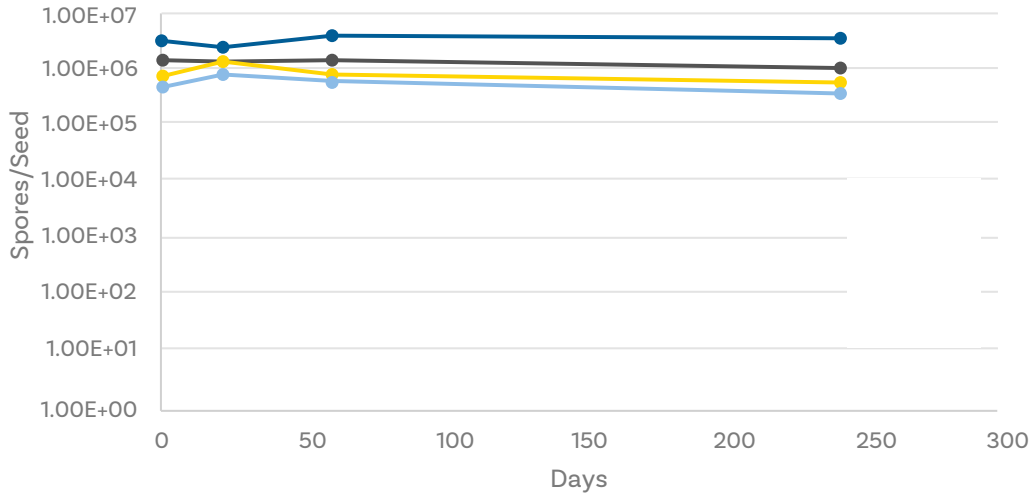

Figure 3: *B. amyloliquefaciens* Viability on Seed

PLANT-BASED CARRIERS FOR MICROBIAL DELIVERY

Other studies were conducted to identify the most consistent and effective live microbial formulation for spray application. In one specific study, the formulation was required to be water soluble, or able to pass through sprayer nozzles, with a final microbial cell count of 108 CFU/g (Colony Forming Units/g).

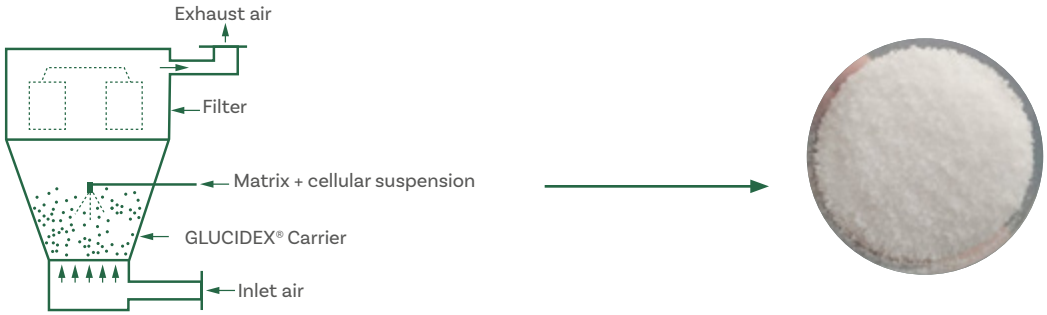

Figure 4: Schematic of a fluidized bed dryer set-up for microbial stabilization studies (left); an example product from the study (right).

The live culture was mixed with different stabilization matrices at concentrations of 10, 20 or 30% to produce candidate formulations and subsequently applied to an inert carrier, GLUCIDEX® IT 19 maltodextrin, using a table-top fluidized bed dryer (see figure 5). Figure 5 shows data from stability/viability studies of the resulting products, involving measurements of cell count over a period of 30 days.

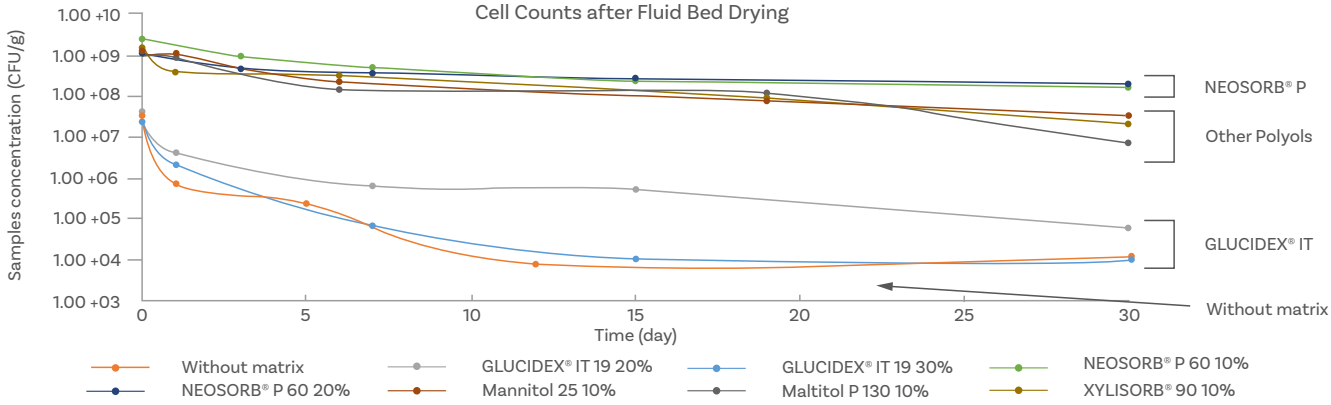

Figure 5: NEOSORB® P 60 sorbitol-based formulations exhibit optimal stabilization for this bacterial strain but other polyols also perform well and almost all matrices improve performance relative to the baseline “no matrix” case.

These results show that for this species and fluidized bed process, NEOSORB® P 60 sorbitol offers optimal performance, though the other polyols tested also perform well. NEOSORB® and the other polyols act as stabilization matrices that protect the bacteria during the drying process, as evident from higher Day 0 cell counts, and enhance stability.

Cell counts with NEOSORB® P 60 sorbitol are in excess of the 108 minimum at 30 days and relatively stable suggesting good shelf life, especially when compared with the formulation containing no matrix. No significant concentration effect is observed.

In summary, in this example, a formulation containing 20% NEOSORB® P 60 sorbitol, in combination with a GLUCIDEX® IT 19 maltodextrin as a carrier, produced a stable microbial product that maintained the required cell count throughout the 30-day test period. With a >93% dry matter content and good water solubility, this product meets all initial requirements.

To find out more about our plant-derived products for plant nutrition and protection products and how they can help you formulate an optimized plant care solution for your needs, check our website and contact us

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