

# How to prepare a liquid microbe formulation with extended shelf life and excellent biological efficacy





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Regulatory pressure on chemical pesticides and fertilizers, public demand for more healthy food and rising resistances against pests drive the agricultural market towards new sustainable biological solutions for crop protection and growth enhancement. Agricultural solutions based on microbial actives could become attractive alternatives to chemical products with new modes of action. They have a high potential as biofertilizing, biostimulating, as well as biopesticidal products.

However, microbial agrosolutions are often not effective in the field and are therefore still not widely established. There are several root causes for this performance gap. First and very important is the limited shelf life. For products based on sensitive microbes such as fungal spores, this is often only of a few weeks. Many of these actives are sensitive to moisture and pH changes, need cold storage and are

difficult to formulate. Therefore, most products are solid with low dispersing properties in water. All these reasons complicate the transport, storage, application, and handling of these products.

A second reason for the performance gap is an insufficient field delivery. Some of the product is lost during spray application due to drift and/or low adhesion and retention on the leaves. If soil applied, the distribution in the soil is limited. These delivery issues lead to low and/or inconsistent field efficacy. The price/performance ratio is considered as too high, and this prevents the acceptance for such products.

There are important levers for an increased shelf life of microbial agrosolutions. The viability of microorganisms depends on several criteria. Besides suitable growth conditions during production and appropriate downstream processing, the right formulation and suitable additives help to reduce the loss of viability of microbial active ingredients. One essential lever for a long viability of microorganism is the optimal water activity within the formulation. At specific low water activities fungal spores reduce their metabolism, remain viable in the dormant phase and survive during storage and transport. The water activity describes the availability of water in a solid or liquid formulation rather than the amount of water present. It defines to what extent water acts as a plasticizer for cell membranes. Values range from 0.0 – so to say “no free water” - to 1.0 as it is in distilled water. If the water activity is very high, the cell membranes are

very mobile leading to cell proliferation and ending the dormant state of microbial spores. If the water activity is too low, cell membranes are too brittle, and the cells die. Microbial spores usually need water activity values of about 0.2 to stay dormant. Manufacturers try to reach this water activity by creating solid products. However this method is not always sufficient so many commercial solid products require cooling during storage and yet have a limited shelf life of not more than 12 months. Examples include the commercially relevant species *Trichoderma harzianum*, *Coniothyrium minitans*, *Clonostachys rosea* and *Metarhizium anisopliae*.

In addition to an improved shelf life, microbial based agrosolutions need to be improved with respect to an enhanced and consistent field performance and a more convenient handling to achieve more acceptance in the market.

Evonik has developed a biocompatible carrier fluid and biocompatible additives to overcome the mentioned issues with microbe based agrosolutions. BREAK-THRU® BP 787 is a water-free carrier fluid with the ability to coordinate water and to lower the water activity if mixed with microorganisms. It secures optimal conditions for living microbial actives like *Trichoderma* spp., *Bacillus* spp., *Clonostachys rosea*, and *Beauveria bassiana* and thereby enhances their shelf life, even without cooling. Due to the stability of the microbes in the carrier fluid hybrid solutions – the combination of microbial and chemical actives - in one formulation are possible.

With BREAK-THRU® BP 787 the formulations can be customized as it is mixable with Evonik BREAK-THRU® additives and Silica products to achieve properties needed in the different applications. The biocompatibility of the BREAK-THRU® additives has been published already in 2019. BREAK-THRU® additives do not negatively affect the shelf life of microbes but enable even longer ones. In addition, they are compatible with the new carrier fluid and therefore enable customized solutions. If the microbes are immobilized on a solid carrier, a dispersant should be added to the carrier liquid to

disperse the actives. Wetting agents reduce the surface tension of water and thereby enhance the deposition, adhesion, and retention of spray solutions. Additional effects are a reduction of small droplets which are prone to drift, an increased rain fastness of foliar applied solutions and a better soil distribution of soil applied solutions. These effects lead to a better field delivery and enable an improved biological efficacy of microbial based agrosolutions. These are crucial prerequisites for an effective and economical use of these products.

Trichoderma harzianum spores were formulated into a low-viscous dispersible concentrate based on BREAK-THRU® BP 787 (table 1). Spores can easily be stirred into the blend of the multifunctional carrier liquid and the biocompatible additives.

BREAK-THRU® BP 787 also enables convenient handling as it is a cold stable liquid with good rheology behaviour. It is water soluble with no need for an emulsifier and thereby reduces complexity in the formulation and eases the handling in foliar, soil or seed applications.

**Table 1**  
Water free Guideline Formulation for microbial spores (water activity (aw) = 0.2): Dispersible Concentrate (DC)

|   | FUNCTION                      | SHARE |
|---|-------------------------------|-------|
| Trichoderma harzianum strain A 2781 (25% on silica) | Biostimulant                  | 20%   |
| BREAK-THRU® DA 646                                  | Dispersant                    | 5%    |
| BREAK-THRU® S 301                                   | Super spreader                | 3%    |
| BREAK-THRU® BP 787                                  | Multifunctional carrier fluid | 72%   |

The formulation is easily dispersible in water and the added readily biodegradable BREAK-THRU® S 301 provides excellent soil distribution. The water activity value of this formulation is 0.2, which is optimal for Trichoderma harzianum spores and therefore the formulation is storage stable for several months at room temperature. This was proven in an accelerated storage stability test at 40°C for four weeks in comparison to a commercial WP formulation. **Figure 1** shows longer viability of the spores within the DC formulation in comparison to the WP product. Furthermore, the formulation is also physically stable as no syneresis could be detected after four weeks at 40°C.

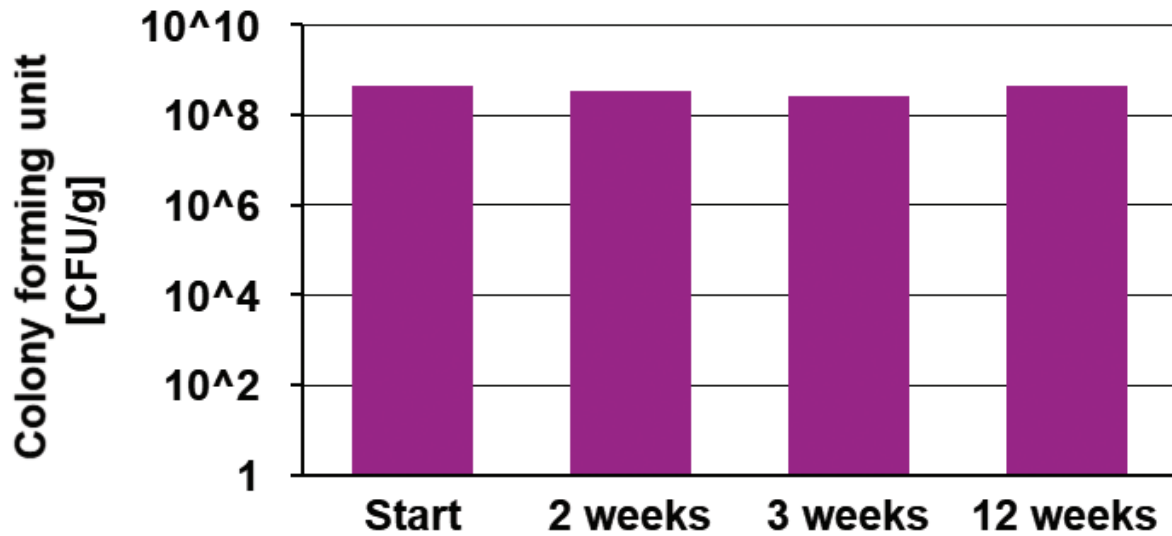
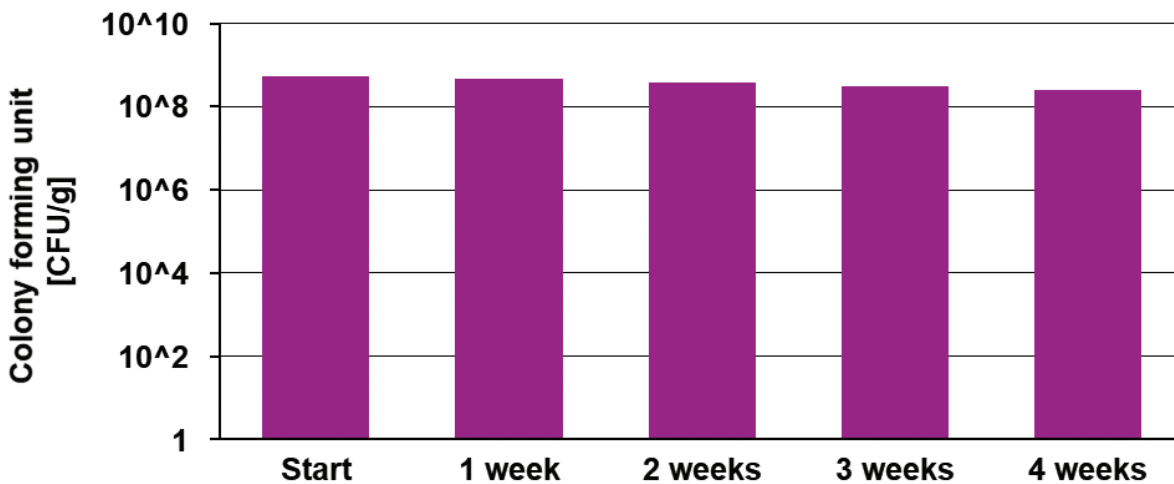
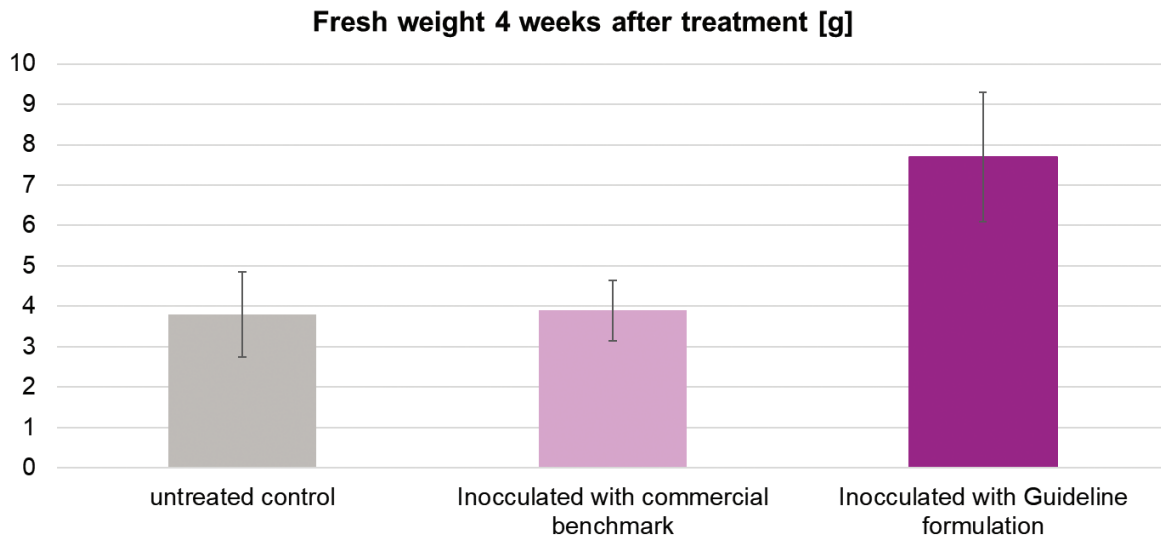


Figure 1: Viability of *Trichoderma harzianum* in formulation a) at 25°C



b) at 40°C

The DC formulation of *T. harzianum* based on BREAK-THRU® BP 787 was tested in a greenhouse trial for nutrient stress tolerance in maize in infertile soil. Oxisol soil has extremely low availability of nutrients, particularly of phosphate. Soil was sampled from the Vogelsberg area, Lich in Hessen, Germany (0.32 mg CAL-P / kg soil; 6.67 mg CAL-K / kg soil; 3300 mg Total C / kg soil; pH 5.5 (0.01 mol / L CaCl<sub>2</sub>)). The soil was mixed with expanded clay (v/v 1:1) and supplemented with NK fertilizer and 50 ml of a 0.5% tricalcium phosphate suspension (slowly soluble). Two maize seeds per plot were inoculated after germination and two weeks after sowing with the different *T. harzianum* formulations. The spore concentration was adjusted to 105 cfu/ml and 10 ml were applied per plant. Each treatment had five replicated pots. **Figure 2** shows the significantly higher amount of fresh corn weight under nutrient stress when maize seedlings were treated with the guideline formulation of *T. harzianum* based on BREAK-THRU® BP 787 in comparison with the commercial *T. harzianum* WP product.



**Figure 2:** Maize fresh weight 4 weeks after treatment

BREAK-THRU® BP 787 is readily biodegradable (OECD 301F), has no tox labelling and is a patented Evonik technology. The multifunctional carrier fluid enables physically stable dispersible concentrates based on living microorganism with extended shelf life. Due to its compatibility with high performing, biocompatible BREAK-THRU® additives it enables customized solutions with excellent dispersing of microbial actives and a targeted field delivery. Depending on the application drift control, adhesion & retention of spray droplets, spray coverage, uptake, penetration, and soil distribution can be adjusted and thereby the biological efficacy can be improved. BREAK-THRU® BP 787 is a convenient solution enabling liquid formulation of microbes.

The reason for the above mentioned multifunctionality of BREAK-THRU® BP 787 is based on additional effects we will publish in detail in the AgroPages Seed treatment special issue in June 2022 (<https://news.agropages.com/News/NewsDetail---42791.htm>). We will show results that the shelf-life enhancement of microbials in the formulations can also be achieved for the microbes once applied on the seeds. In addition, together with Silica BREAK-THRU® BP 787 provides binder functionality with excellent dust reduction. As it is readily biodegradable it is a microplastic free binder solution. As BREAK-THRU® BP 787 is mixable with Evonik BREAK-THRU® additives and silica products other seed treatment parameters like low rub-off, good flowability and coverage can be achieved. BREAK-THRU® BP 787 is compatible with water-based products and thereby enables easy handling and low complexity. We will present a significant acceleration of seed germination showing an improved biological efficacy also in this application.

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