Jungbunzlauer

Potential of citrate esters as bio-based, non-aqueous liquid carriers for microbial agents in agriculture:

Background

As an alternative to synthetic agrochemicals, microbial biocontrol and biostimulant agents are increasingly becoming popular. A range of fungal and bacterial strains are known to improve plant vigor and resistance to pests and diseases. To ensure effective application, suitable formulations to maintain shelf life and viability during application of these microbial agents is crucial. Most common formulation types include wettable powders (WP) and wettable granules (WG), as well as dispersion concentrates (DC).

Offering straight-forward application for example by spraying, soil drenching or seed coating, liquid formulations have certain advantages. However, when water-based, they are more susceptible to spoilage and require elaborate preservation which does not compromise on the live microbial agents.

Non-aqueous, liquid carriers are therefore of interest in formulation of microbials. For example, the use of blends of polyethylene glycol and glycerol has been patented as liquid non-oil, non-aqueous carrier of *Bacillus amyloliquefaciens* (Huang, Zhengyu, et al. "Non-aqueous, non-oil bacillus amyloliquefaciens compositions." U.S. Patent No. 10,251,401. 9 Apr. 2019). Citrate esters are already used in standard agrochemical formulations as solvents for synthetic pesticides, where they are appreciated for their non-volatility and good plant compatibility.

In this context, we evaluated the potential of citrate esters as bio-based, non-aqueous carriers for microbials. Under the brand name CITROFOL®, Jungbunzlauer offers triethyl citrate, tributyl citrate and acetyl-tributyl citrate with differing physicochemical properties. In an initial test, the compatibility of these carriers with living Gram negative and Gram positive bacterial biocontrol and biostimulant strains was investigated.

Test set-up

Bacillus amyloliquefaciens was cultivated in liquid medium under continuous shaking at 27°C. Bacteria were harvested by centrifugation at 4500 rpm for 10 min after 24 h. The supernatant was discarded, the cell pellet re-suspended in 0.9% NaCl for washing, centrifuged again at the same settings and re-suspended in filter-sterilised CITROFOL® AI (triethyl citrate), BI (tributyl citrate) or BII (acetyl-tributyl citrate).

Bradyrhizobium japonicum was cultivated in liquid medium under continuous shaking at 27°C. Bacteria were harvested by centrifugation at 4500 rpm for 10 min after 48 h and suspensions in CITROFOL® prepared as described previously.

The amount of CFU/ml in all bacterial suspensions was determined by plating a serial dilution of the original suspension in 0.9% NaCl on plate count agar and incubating for 24 or 48 h at 27°C respectively.

All suspensions were stored at 4°C for 24 h. Subsequently, a serial dilution was prepared to determine the cell viability by plate counting and thus check for the effect of the carrier.

Observations

As confirmed by plate counting, the original bacterial suspension of B. amyloliquefaciens contained 7 x 108 CFU/ml. The re-suspension of the cell pellet in CITROFOL® resulted in the formation of cell agglomerates (Fig. 1). Possibly, a surfactant may have to be included to homogeneously suspend the cells in the hydrophobic carrier. Due to the agglomeration, a precise quantification of viable cells by plating a serial dilution was not possible. However, a dense bacterial lawn was observed on agar plates streaked with a 1000-fold dilution of the suspension of B. amyloliquefaciens in all CITROFOL® types (Fig. 2). This indicates a very good maintenance of cell viability of B. amyloliquefaciens in these carriers and suggests their use as liquid carriers. Similarly, for B. japonicum, a quantification was not possible due to inhomogeneous dispersion, but dense growth was observed in all CITROFOL® types in 1000-fold dilution.

Figure 1: *Re-suspension of B. amyloliquefaciens in CITROFOL® AI leads to formation of cell agglomerates.*

Figure 2: *Dense bacterial lawn of B. amyloliquefaciens in in 1000-fold dilution of suspension in CITROFOL® AI.*

Conclusion

Preliminary results encourage further investigations on the use of citrate esters as liquid carriers for microbial formulations in agriculture. A general compatibility of these materials with a Gram negative and Gram positive model organism was confirmed.

As previous trials have shown, a combination of CITROFOL® AI and xanthan gum lowers the evaporation rate of water from droplets (L. Hutson, New AG Int. 2019, 18–29; + FACT sheet). This could be a further benefit especially in foliar application of microbial agents, protecting them from desiccation and thus maintaining their viability. Furthermore, it was previously shown that CITROFOL® AI in a concentration as low as 0.5% increases the spreading of droplets on a hydrophobic surface. This effect could support a better distribution of microbial cells on the target surface.

Citrate esters are therefore promising liquid carriers in the formulation of microbial biocontrol and biostimulant agents in agriculture, which are in line with the sustainability claim of such preparations.