Formulating with microbes : 3 key focus areas

1. **Physical stability of formulation – concentrate and in dilution**
   - Selecting the correct formulation
   - Maintaining good distribution of the biological
   - Formulation aid suitability
2. **Enhancing bioactivity**
   - Improving efficacy of biologicals
   - Ensuring the microbe reaches the target
   - Systemic or contact mode of action
3. **Maintaining viability of microbe**
   - Understand the properties of your biological (your specific strain of microbe)
     - Water sensitivity
     - Impact of shear
     - Shelf life of the biological

Testing for compatibility & viability

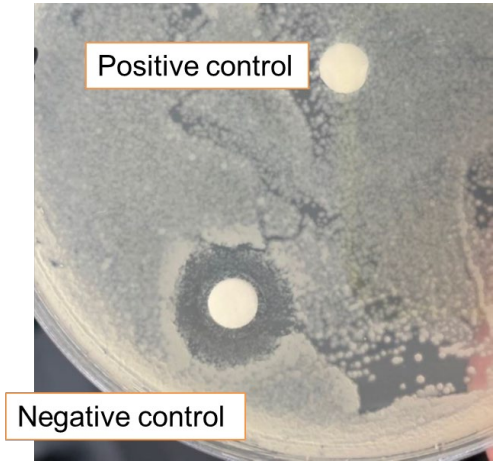
Microbe	Type	Pesticide type	Mode of action
Trichoderma	Fungi, spore forming	Fungicide	Systemic
Bacillus	Bacteria, Gram positive, spore forming	Fungicide	Systemic

- Two model microbes were used to screen Croda products covering a range of sensitivities
- Several different methods were used to assess compatibility and viability depending on the microbe product to be evaluated:
  - **Zone of inhibition (Kirby-Bauer test)**
  - **Colony forming unit count (CFU)**
  - **Conidia germination test (CGT) (direct viability)**
  - **Croda's PrecisionBio™ method**

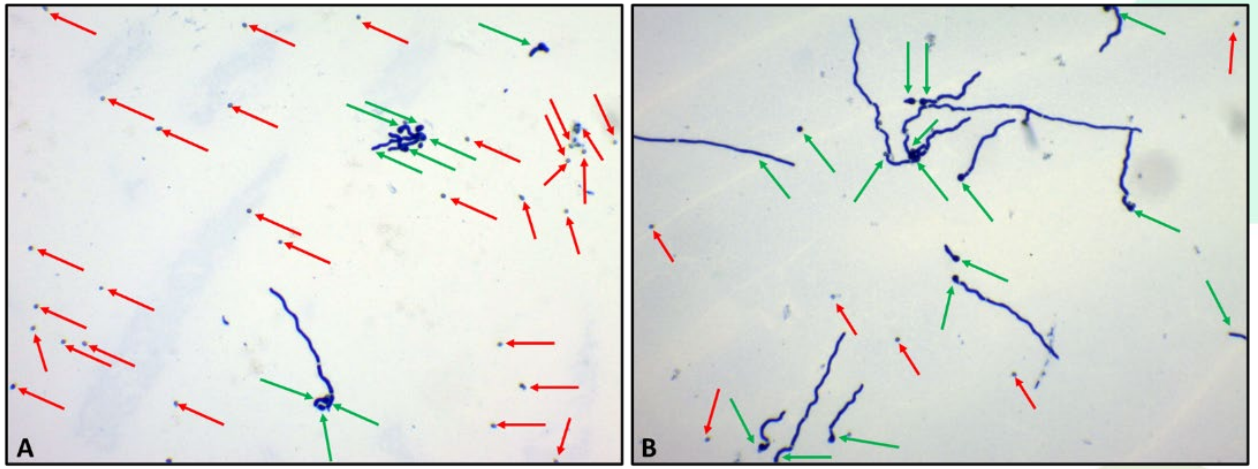
Increasing depth of understanding of a microbe

Testing for compatibility & viability

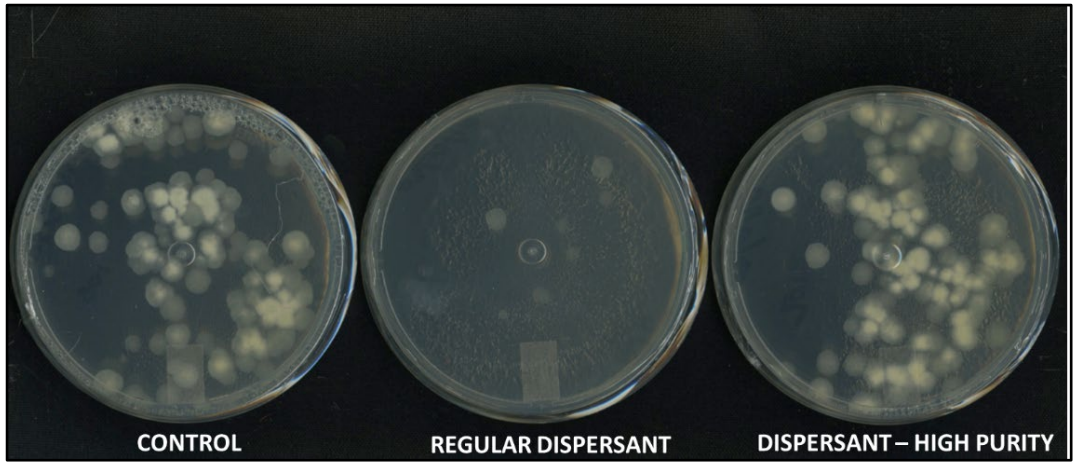
Zone of inhibition



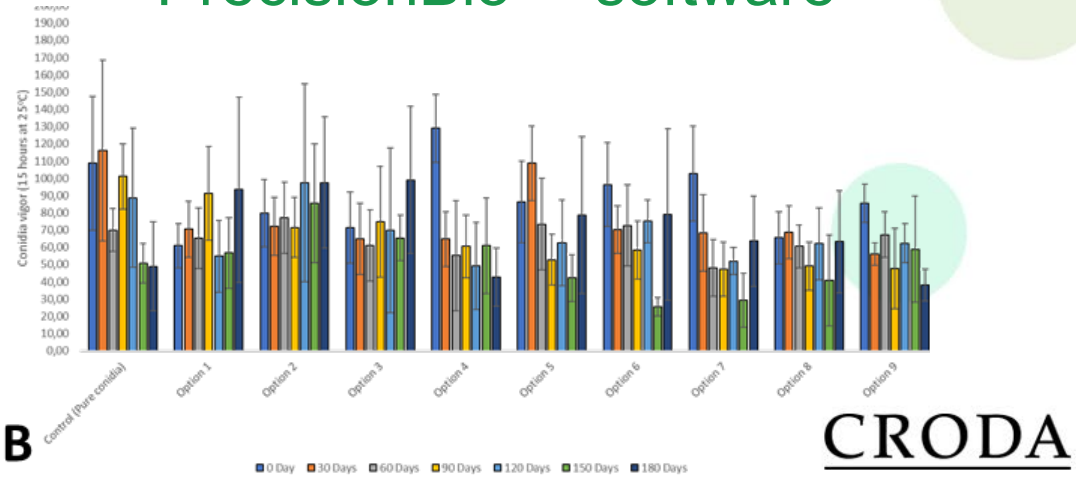
Conidia germination test (CGT)



CFU testing with *Trichoderma sp.*



PrecisionBio™ software



CRODA

Methods for testing compatibility of different formulation aids with microorganisms

– the importance of formulation design and optimisation

Importance of formulation

Formulation is integral to commercialising an agriculturally beneficial microorganism. An optimised formulation allows a microbe to be successfully applied in the field, whether that be within a coating on a seed, in soil for root uptake or foliar applied for contact or systemic modes of action.

Each microbe presents its own unique properties meaning formulation design should be tailored to each specific microbe. Some common challenges with microbe formulations and potentially solutions are:

- **UV sensitivity** – formulation aids can be incorporated to act as UV blockers
- **Shelf life/ storage requirements** – formulations without water or solid formulations can often extend product lifetime
- **Efficacy** – microbe formulations are often lower efficacy than chemical pesticides, adding adjuvants into a formulation can improve performance in the field

Formulation can be useful but can also impact the viability of a microbe. Figure 1 displays three different film coatings with the same *Trichoderma* strain after 24 hours.

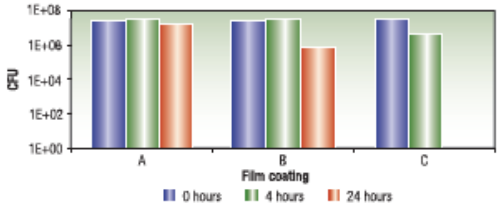


Figure 1: CFU count of *Trichoderma sp.* spores after 0 hours, 4 hours and 24 hours in the presence of three different film coat formulations for seeds

Film coat A is the only suitable formulation for this strain of *Trichoderma sp.* as there was a significant drop in CFU in the presence of film coat B and C after 24 hours. After 24 hours, film coat C had no colonies present.

Different strains of the same species of microbe can behave differently with the same formulation. Figure 2 shows film coat A with three different *Trichoderma sp.* strains after 24 hours.

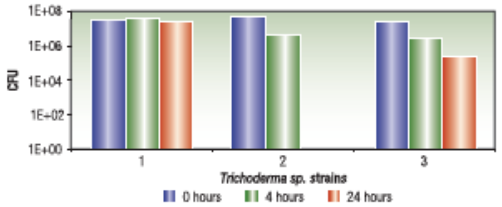
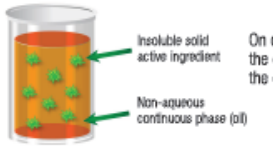


Figure 2: CFU count of three different *Trichoderma sp.* strains in the presence of one film coat after 0 hours, 4 hours and 24 hours

OD formulations



On dilution in the spray tank the oil phase is emulsified and the other solids are dispersed

Benefits of OD formulations:

- Excellent delivery system for water sensitive microbes
- Oil continuous phase acts as a built-in adjuvant
- Enhanced spray retention and penetration
- No preservative required – preservatives can negatively impact microbes

Limitations:

- Complex to produce
- Often require several surfactants to stabilise

Testing for compatibility and viability

Several different methods can be used to assess compatibility and viability depending on the microbe being evaluated. Croda focus on the four methods listed when assessing microbes.

- **Zone of inhibition (Kirby-Bauer test)**
- **Colony forming unit count (CFU)**
- **Conidia germination test (CGT)**
- **Croda's PrecisionBio™ method**

Zone of inhibition – test for compatibility

The zone of inhibition test allows for high throughput screening of formulation aids to understand the compatibility of different chemistries with a microbe.

Discs of filter paper are saturated with a product and placed on an agar plate. The plate is inoculated with microbe and the area around the filter paper is observed – if there is suppression of growth of the microbe, the formulation aid is not compatible with the microbe and should not be used within a formulation.

Formulation aid	Microbe		
	Bacillus sp.	*Trichoderma sp.*	*Pseudomonas sp.*
Atlox™ 4916			
Atlas™ G-1086			
Tween™			

CGT method – confirming germination capacity

The CGT gives a visual indication as to whether a microbe can form conidia and if those conidia are able to germinate in the presence of different surfactants. The method uses optical microscopy images and conidia are identified by small dots whereas germinated conidia have long tails. Figures 4 and 5 show the images of two CGTs with *Trichoderma sp.* and different purity surfactants.

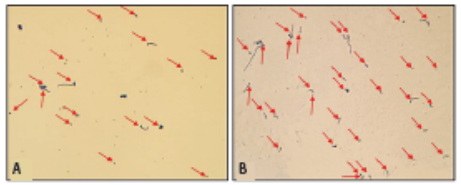


Figure 4: Effect of surfactant A and surfactant B on a *Trichoderma sp.* formulation after 135 days with conidia identified by red arrows and germinated conidia identified with green arrows

More conidia have formed with surfactant B but there is no improvement on germinated conidia compared to surfactant A (no green arrows present in Figure 4). Therefore neither surfactant is suitable for use with this particular microbe.

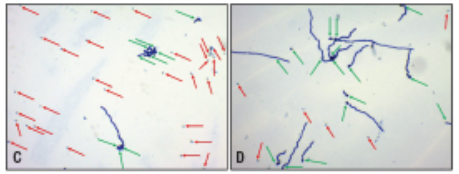


Figure 5: Effect of surfactant C and a high purity surfactant D on *Trichoderma sp.* after 135 days with conidia identified by red arrows and germinated conidia identified with green arrows

Surfactant C produces few conidia able to germinate (indicated by green arrows). Surfactant D, a higher purity surfactant, shows increased level of germination from the conidia formed. The surfactants in Figure 5 are the most suitable for use with this microbe, more than those in Figure 4, specifically, surfactant D.

Higher purity surfactants are a good option when formulating with highly sensitive microbes such as gram-negative bacteria.

Added	Oxidation products	Unreacted
■ Residual catalyst ■ Water ■ Bleach	■ Peroxides ■ Aldehydes, e.g. formaldehyde and acetaldehyde ■ Organic acids, e.g. formic acid and acetic acid	■ Free fatty acid ■ Fatty acid soaps (sodium and/or potassium salt)

Table 2: Common surfactant impurities which can impact microbe viability

Smart science to improv

CRODA

Resources

Developing microbial oil dispersion (OD) formulations datasheet

Chassis systems recipes

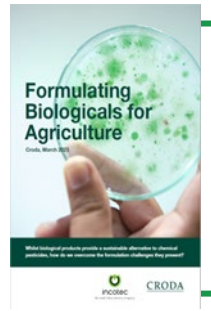
Full formulation example recipes (including a microbe)

Formulating oil dispersions toolbox chapter

- Covers the principles of formulating ODs

Formulating with microbes toolbox chapter

- Covers the principles of formulating microbes



Biologicals Whitepaper