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Battelle UK Ltd / Battelle Memorial Institute

# Encapsulation of biologics for agricultural applications

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# Battelle

- One of the world's largest Contract Research Organisations
- Manages several US government National Labs
- Battelle Memorial Institute HQ in Columbus, Ohio
  - Registered charity
- Battelle UK Ltd leads Agribusiness / Crop Protection sector
  - Chelmsford, UK : e-fate, residues, reg affairs, modelling
  - Havant, UK : Formulation Development & Testing - experienced team, senior staff have 25+ years experience

# Encapsulation of biologics for agricultural applications

- Collaborative effort : Havant Formulations team plus Columbus Microbiologists & Advanced Materials Chemists
  - Internal, Battelle-funded research
  - Client-funded development projects (confidential)
- Main focus : prolonging in-pack shelf life & longevity on treated seed of microbial biopesticides & biostimulants
- Also : ‘matrix’ formulations for in-furrow application

# Living microbes e.g. bacteria & fungi

- Usually produced by fermentation
- Single species / mixture of selected species / complex consortia
- Robustness of microbes varies hugely between species / strains
- Conventional Formulation criteria modified, but still apply, e.g. :
  - Chemical stability = microbial viability
  - Physical stability : microbes can behave similarly to solid particles

# Main Formulation Challenges

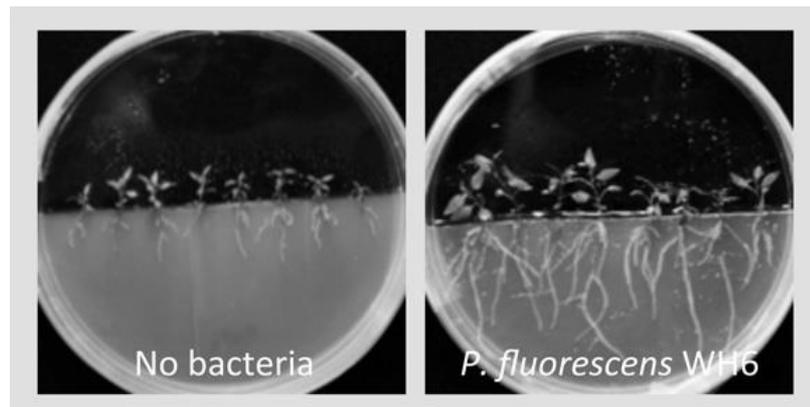
- Finding balance between keeping the microbes alive (viable) & avoiding rapid growth
- Complex consortia of microbes
  - Beware 'selecting' which survive through formulation process & storage
- Formulation processes plus inerts that do not harm microbes
- Shelf life
  - Many products currently on the market limited to e.g. a few months stored at low temperature

# Delivery mechanisms

- Foliar application
  - limited : hostile environment for microbes – dry surfaces & light can cause mortality
- Soil incorporation / in-furrow
  - Natural environment for many microbes
  - Gets microbes close to the roots
- Seed treatment
  - Similar to soil incorporation – more targeted (?) but....
  - Need to keep microbes alive on seed surface prior to sowing = hostile (dry), plus other materials present...

# Beneficial Microorganisms

- ***Pseudomonas fluorescens***
  - aerobic, gram-negative, present in agricultural soils and well adapted to grow in the rhizosphere
  - many traits to act as a biocontrol agent and to promote the plant growth ability
- ***Penicillium bilaiae***
  - plant growth promoting fungus
  - enhance P availability to *plants*
  - promotes P mobilization from organic P sources
- ***Bradyrhizobium japonicum***
  - nitrogen-fixing, forms root nodules specifically on soybean
  - added to legume seed to improve crop yields in areas where the bacterium is not native



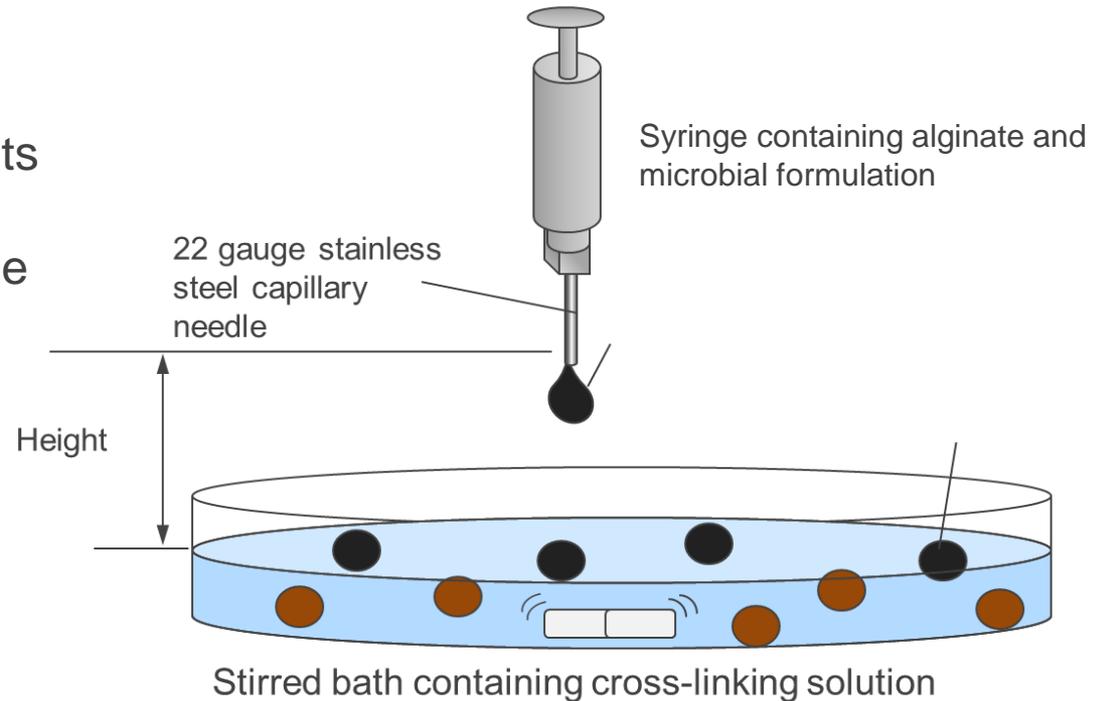
*Populus tremuloides* on nutrient agar containing 25  $\mu\text{M}$  Pi. Plants were not inoculated (left) or inoculated with *Pseudomonas fluorescens* strain WH6



*B. japonicum* nodules on soybean plant

# Encapsulation Process

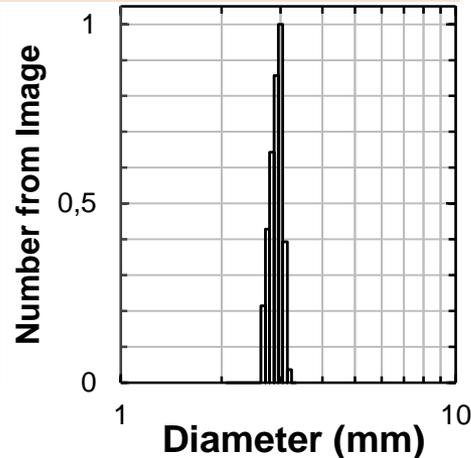
- Room temperature process
- Bio-based & biodegradable matrix
- Absorbs water
- Fast synthesis
- VOC free / No solvents
- Variable particle size
- Stable pH 4 to 6 range



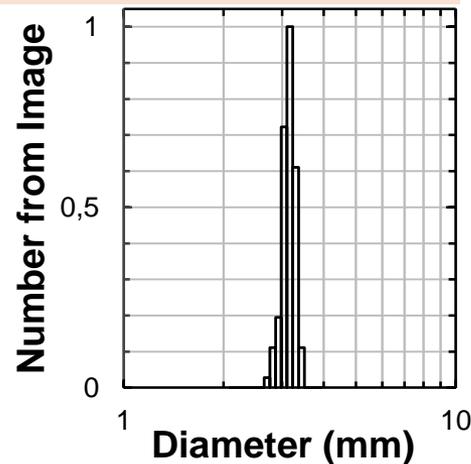
Battelle IP

# Encapsulation Process

## Alginate+ Cross linker #1

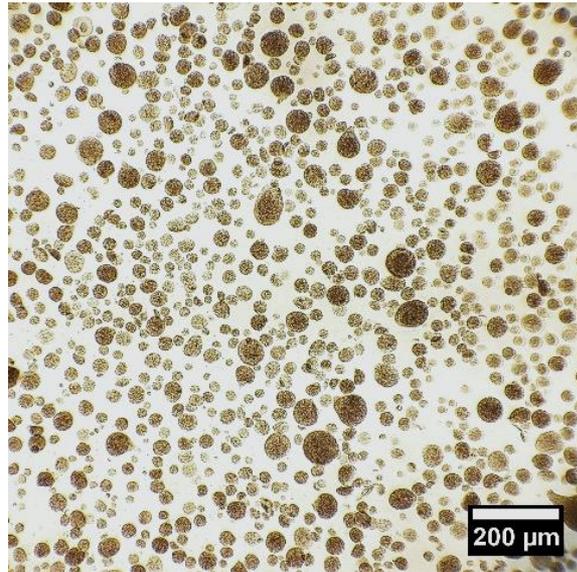
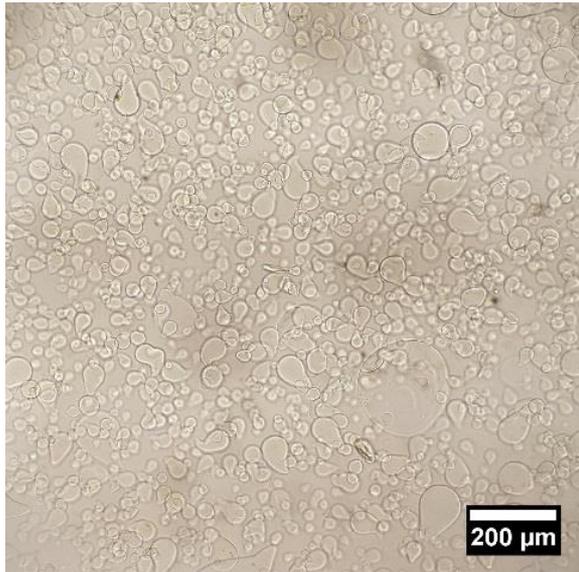


## Alginate + Cross linker #2

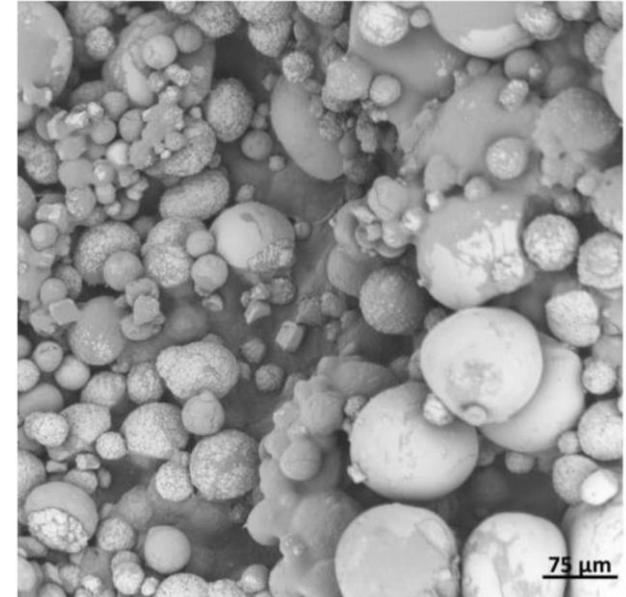


Sample (#)	Bead Diameter (mm)	Bead Volume (mL) $(4/3)*\pi*(D/2)^3$
#1	2.8	0.0115
#2	3.0	0.0141

# Encapsulation of Selected Microorganisms



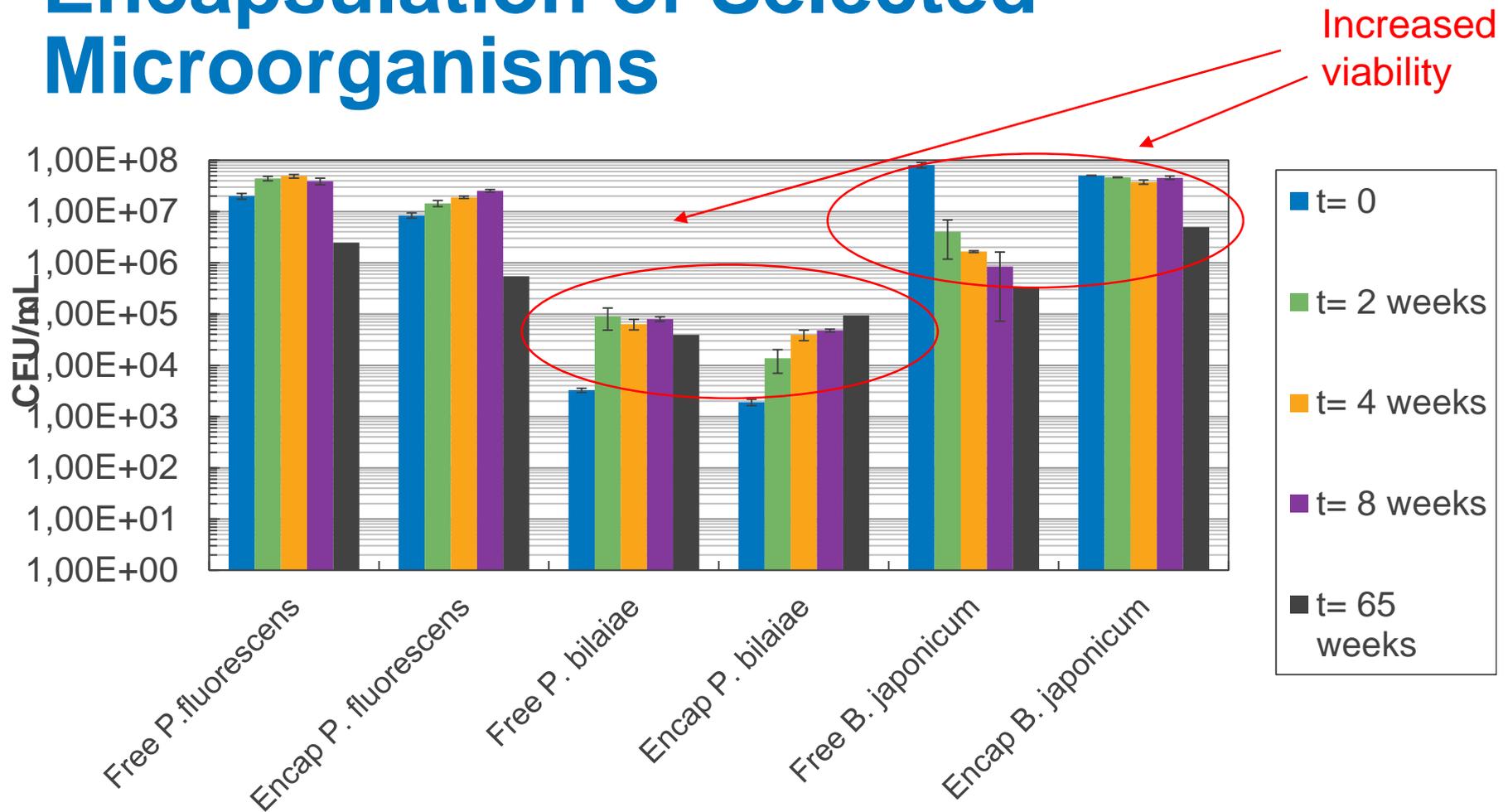
Microscopic image of empty capsules and encapsulated *P. fluorescens*



SEM image of encapsulated *P. fluorescens*

**Goal, no more than 1 log drop in viability after 60 days of encapsulation (~ 8 weeks)**

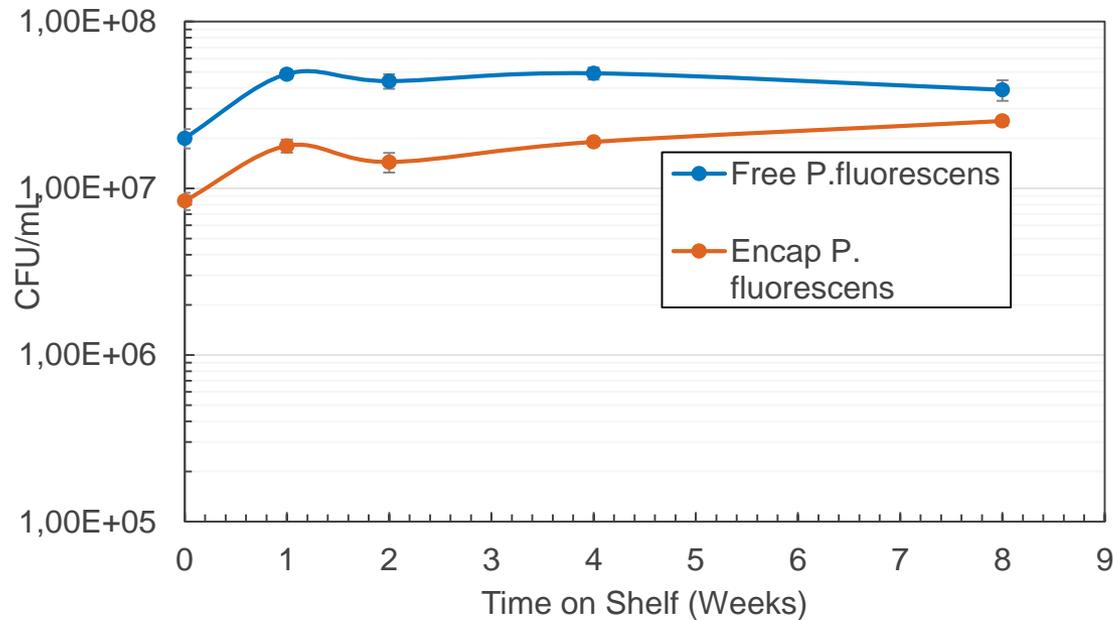
# Encapsulation of Selected Microorganisms



On-shelf stability data for alginate encapsulated microorganisms. For each microbe (both free and encapsulated), the cluster bars show stability over the course of 65 weeks (15 months)

# Encapsulation of Selected Microorganisms

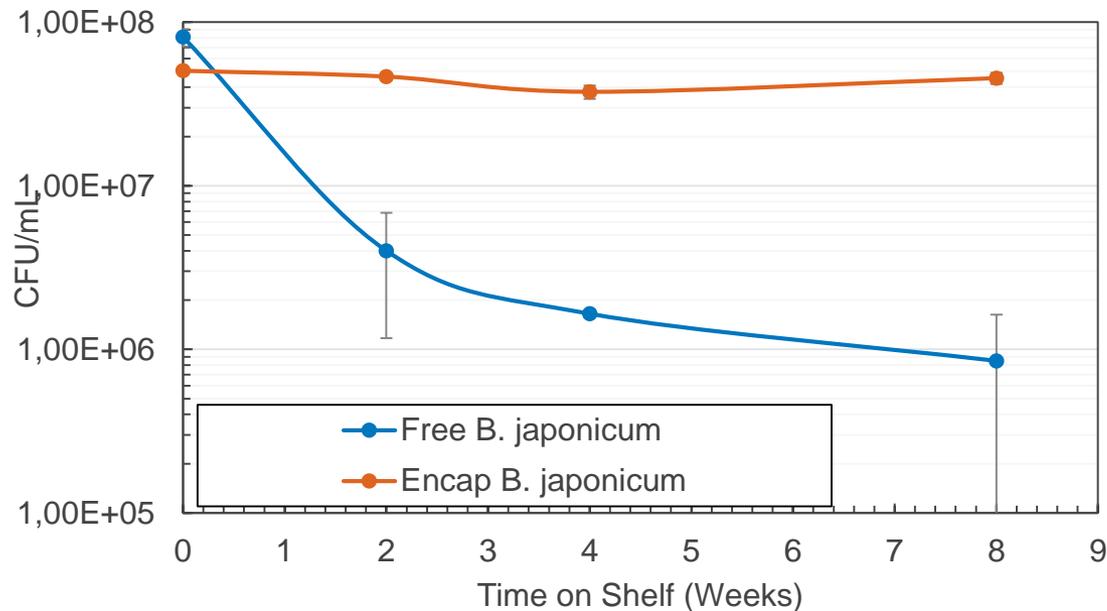
## Stability of *Pseudomonas fluorescens*



- *P. fluorescens* stable both as a free suspension, and thus, there was no room for improvement via encapsulation.
- However, presence of protective capsules may improve stability on seed, or protect the microbes from other active ingredients when dispersed in a formulation with more than just DI water.

# Encapsulation of Selected Microorganisms

## Stability of *B. japonicum*



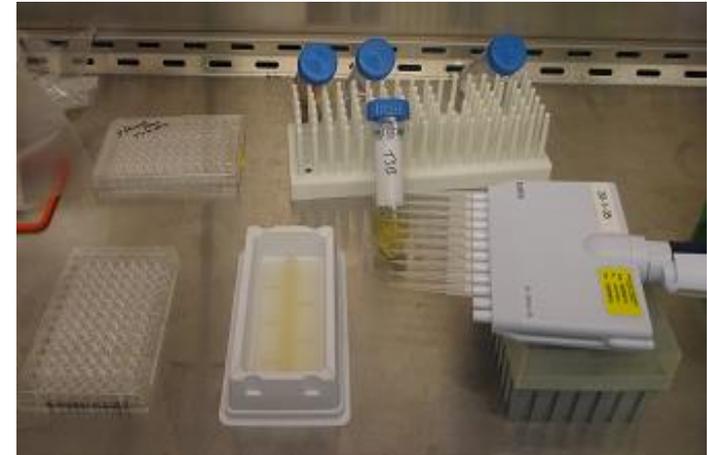
- The free *B. japonicum* shows ~ 2 log loss in viability after only 2 weeks
- Encapsulated counterparts have shown no loss in viability after 8 weeks (~60 days). CFU's fluctuated within 10% of the initial value
- Stability testing over 15 months showed drop in stability

# Many 'amendments' reported to provide benefit

Amendments	Description	References
Carriers	Liquid (oils, water, etc) or powder (talc, kaolin)	Schisler et al.
Sugars	Trehalose, lactose, etc. can stabilize proteins and whole organisms during drying and storage	Schisler et al.; Bieganski et al; other journal articles; Battelle reports
Polysaccharides	Polysaccharides (e.g., corn starch) can stabilize proteins and microorganisms	Battelle patent applications 2008/0219951; and US 7,141,542;
Gums	Gums (e.g., xanthan gum and gum arabic) stabilize proteins and used as thickening agents for biopesticides	Battelle patents; Agarwal et al.; Schisler et al.
Other gel forming agents	Gels (e.g., calcium alginate and chitosan) have encapsulation and stabilization properties for microorganisms (probiotics)	Schisler et al; Battelle experience; Prakash and Martoni review
Germination inhibitors	Long chain alcohols inhibit spore germination;	Srivatsan and Wang 2008; Cortezzo et al 2004; Yasaki et al 1978
Other	Polymers, proteins, detergents, desiccants, binders, stickers, and UV protects all have shown to impact microbial behaviour	Schisler et al; Ravensberg 2011, Jackson et al 2006; Deaker et al; others; Battelle experience

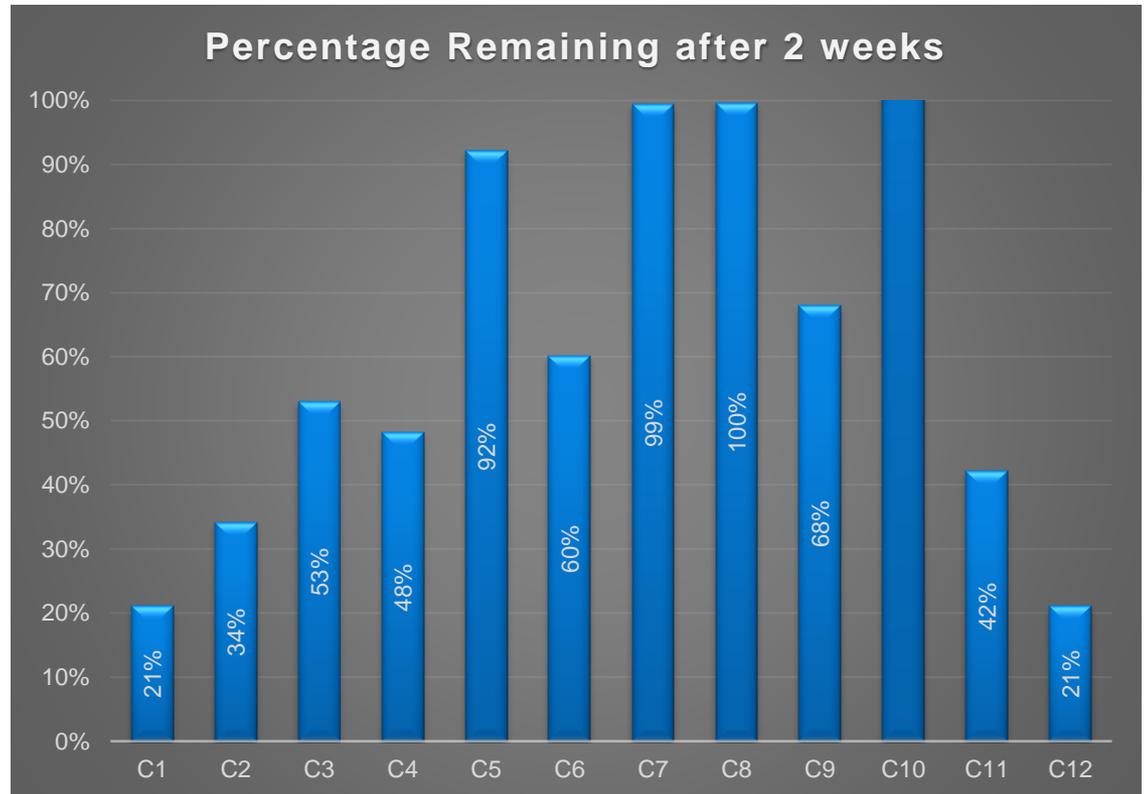
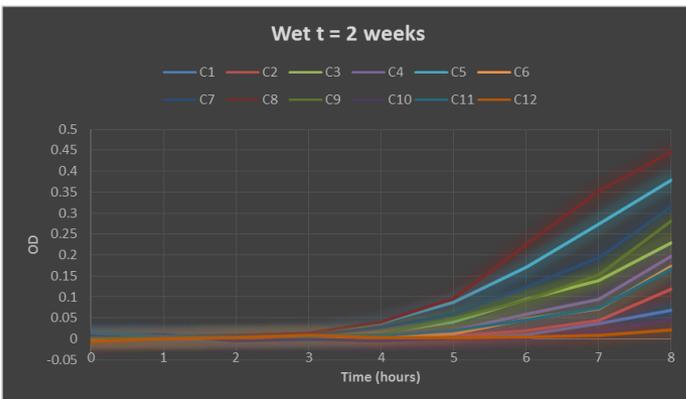
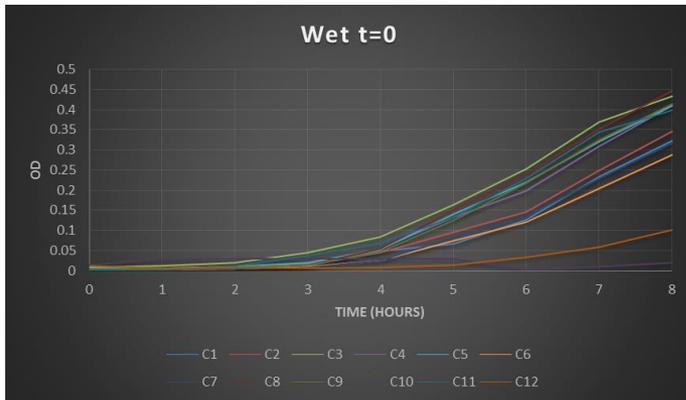
# Semi-high throughput screening

- Similar to strategy proposed by Schisler, et al. 2004. *Phytopathology* 94:1267-1271.
- Organism grown to desired titer, aliquotted, suspended in carriers and amendments added → 96 microbe / amendment combinations
- Formulations (dry and wet) stored at room temperature or other desired temp/ humidity
- Viability measured over time using optical density (OD) absorbance readings at 620 nm



# Rapid Screening of Stability Over Time

- WET : several amendments show enhanced viability



Example Data Set (12 of 96 formulations shown)

# Matrix formulations for in-furrow application

- Suspension of microbes absorbed onto carrier powder
- Powder agglomerated, dried, screened to various sizes (if required)
- Powder coating onto fertiliser granules also possible

# Microbial delivery - Pellets



# Summary

- Microbial shelf-life can be increased through encapsulation
- Matrix formulations (pellets) provide a useful alternate approach in some cases
- Selected 'amendments' (additives) can provide additional microbial longevity in combination with the above
- Battelle internal research is continuing – to provide further improvements
- We can apply what we already know, to help clients get their microbes to market - now

# ***BATTELLE***

**It can be done**

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