Characterization of encapsulated agrobiologicals

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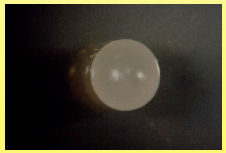
- Introduction
- overview of important characteristics
- shelf life
- enhanced efficacy



Encapsulation of active ingredients
to solve storage and application problems

A suitable capsule improves the characteristics of an active ingredient:

- **improved handling, protection of workers and clients**
- **protection from biotic and abiotic stress factors**
  - **heat, dryness, UV light, contamination,...**
- **enhanced shelf life**
- **slow/controlled release into a matrix from a „depot“ or retain a.i.**
  - **controlled by environmental conditions and capsule material properties**
- **enhanced efficacy**
- **application cost reduced by decreased number of applications**
- **construction of bait formulations**

Frost & Sullivan (2002). European Microencapsulation Technologies (Report B059)
Burges HD (1998). Formulation of microbial pesticides. Dordrecht: Kluwer Academic Publishers
Pflanzenschutz-Kurier 2/93. Die hohe Kunst des Formulierens

Encapsulation of agrobiologicals
Overview

Agrobiologicals

- **biological control agents**
- **plant-growth promoting cells**
- **N-fixing microorganisms**
- **mykorrhiza**
- **plant cells, esp. somatic embryos**

Encapsulation materials and methods
Characteristics I

Materials

- **Molecular weight, - distribution, degree of substitution, counter ions, gelation mechanism**
- **Rheology**
- **Primary, secondary, tertiary structures in solution**
- **Source, batch no.**
- **Toxicity, FDA approval**
- **Cost about 5 €/kg**

Capsules

- **Mechanical stability**
- **Particle size, -distribution**
- **Physico-chemical parameters**
  - **diffusional characteristics: cut-off, internal and external diff. limitations**
  - **redissolvability by pH, temperature, ion exchange, enzymatic, …**
- **Biological degradability**
- **Form: capsule, foil, fibre, blocks, …**

Encapsulation materials and methods
Characteristics II

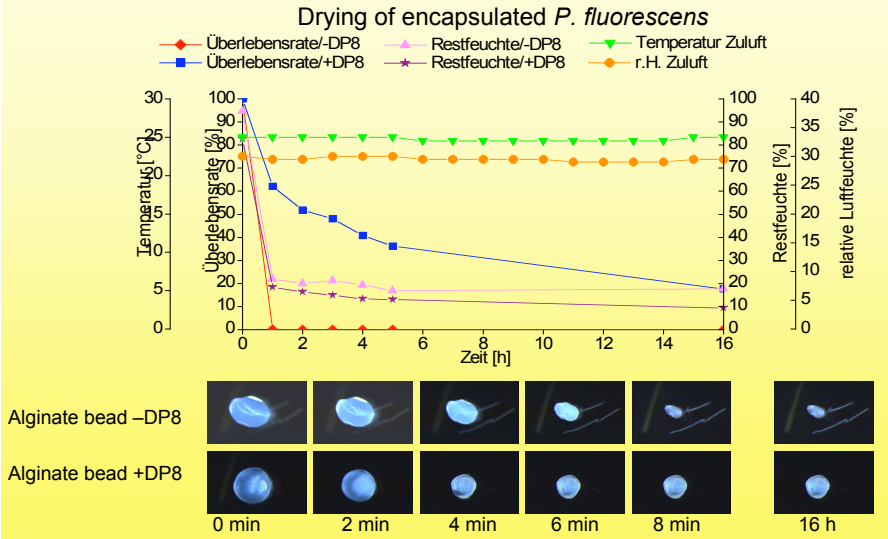
Moist and dried encapsulated cells

- **Preconditioning**
- **Flowability**
- **Formulation additives**
  - **Fillers, humectants, drying protectants,…**
- **Reswelling**

Key characteristics of commercial encapsulated agrobiologicals

- **Shelf life**
- **Enhanced efficacy**

Characterization of reswelling
Reswelling and cell growth



Characterization of reswelling
Reswelling and cell growth

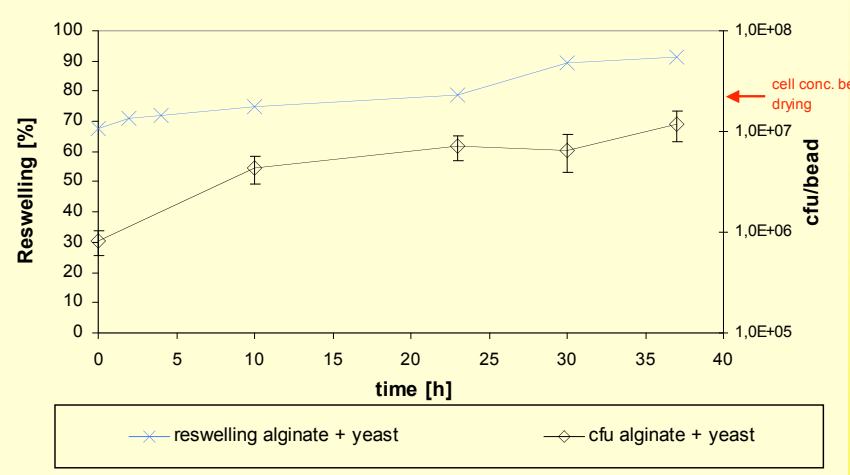
Test method	can be standardized
incubate in tap water	no
incubate in deionized water	yes
incubate in 0.9 % NaCl	yes
place on wet filter paper	yes
place on water agar	yes
place on quartz sand	yes
place on sterile or unsterile field soil	no

Characterization of reswelling
Reswelling and cell growth

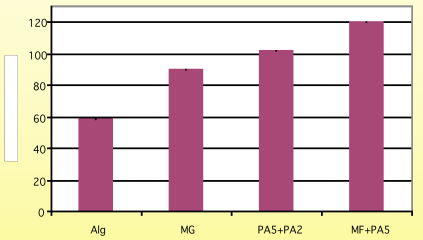
on water agar

on soil

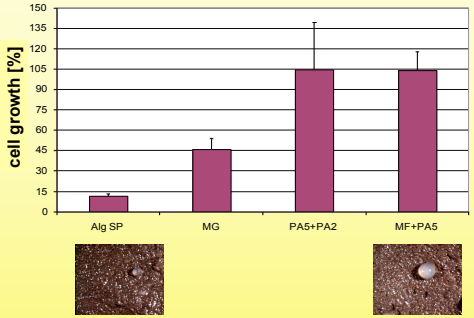
Characterization of reswelling
Reswelling and cell growth



Characterization of reswelling
Reswelling and cell growth

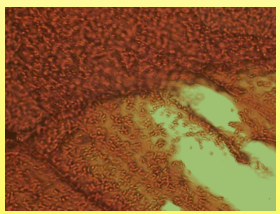


bead diameter measured,
volume calculated



Characterization of shelf life
Determination of viability

- **determination of cell viability**
  - **oxygen consumption**
  - **viability stains**
  - **growth of cells out of capsule**
  - **cfu**
- **viable but but not culturable („vnbc“)**



vitality stain with acridin orange:

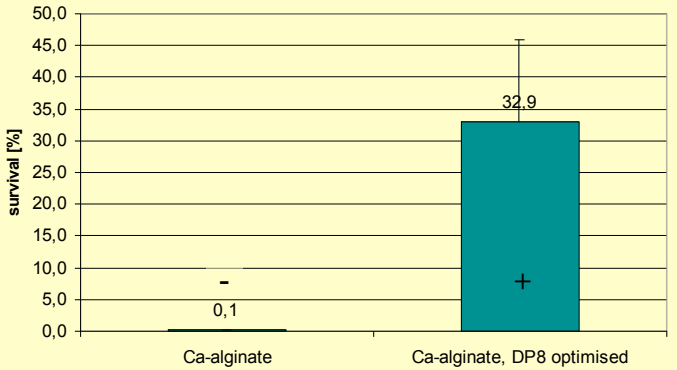
bead diameter: 1.0-1.2 mm

biomass content: ca. 2 % wet biomass

10^7 cfu/bead

Characterization of shelf life
Accelerated storage test

Influence of an optimised treatment with drying protectant DP8 on survival of dried *P. fluorescens* encapsulated in Ca-alginate beads



Characterization of efficacy
Accelerated storage test

overview

microorganism	reference
archaebacteria	Sakane, T et al. (1992)
Lactobaccillus brevis	Desmons, S. (1998)
plant viruses	Yordanova, A. et al (2000)
Lactococcus sp.	Achour, M. et al (2001)

Characterization of shelf life
Accelerated storage test

Basic idea:

loss of cells during storage follows

$\log N = \log N_0 - k*t$

k_i : specific rate of degradation, t : time

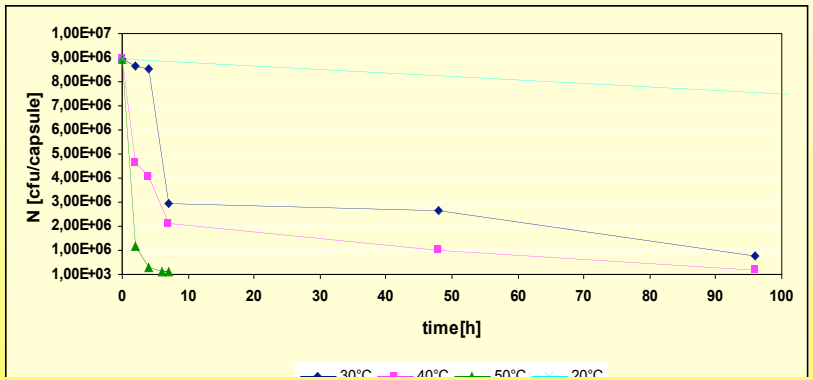
where k = f(1/T)

according to Arrhenius equation:

$\log k = -(\Delta Ha/2303*R)*1/T$

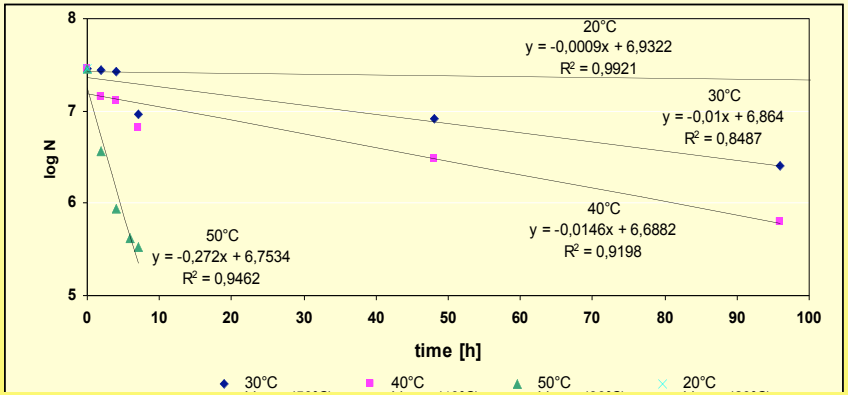
Characterization of shelf life
Accelerated storage test

cfu as a function of time for different temperatures



Characterization of shelf life
Accelerated storage test

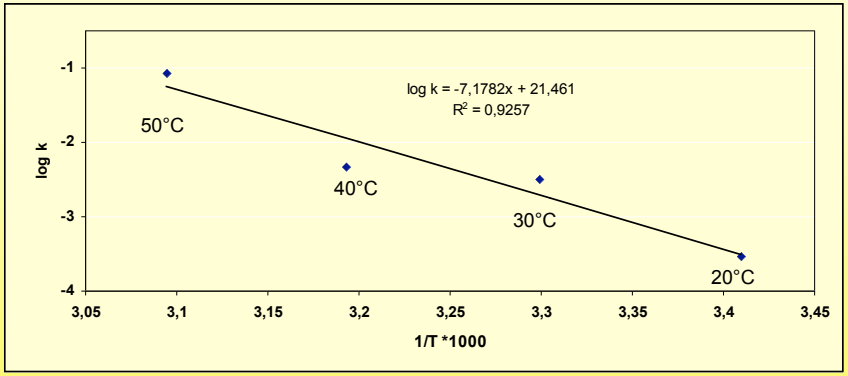
Plot of logN as function of time for different temperature



slope of the curves = k values

Characterization of shelf life
Accelerated storage test

Plot of logk as function of different temperatures



$\log N = \log N_0 - k*t$

k_i : specific rate of degradation, t : time

Characterization of shelf life
Accelerated storage test

Model for prognostication of cells alive after storage of formulation MF+PA5 at defined temperatures *T*

$$\log N = \log N_0 - 10^{-7,1782\,(1/T \times 1000) + 21,461} xt$$

k_i : specific rate of degradation, T : Temperature

Prognosticated and real cfu in formulation stored at 20°C)

MF+PA5 capsules	cfu / capsule	cfu / g capsules
N_0 (Cfu at t=0 h)	$8,96*10^6$	$4,19*10^{10}$
2 weeks storage	$3,66*10^6$	$1,71*10^{10}$
2 weeks (prognost icated)	$4,28*10^6$	$1,97*10^{10}$
4 weeks storage	$1,88*10^6$	$8,78*10^9$
4 weeks storage (prognost icated)	$1,78*10^6$	$8,22*10^9$

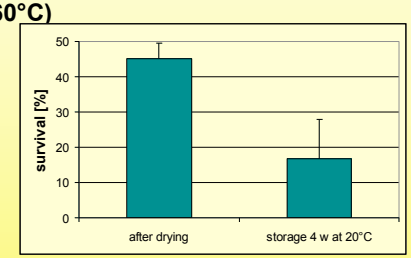
Storage time	temper ature	cfu/ capsule	cfu/g capsules
6 months	20°C	$5,52*10^2$	$2,58*10^6$
12 months	20°C	$3,00*10^{-2}$	$1,40*10^2$
6 months	4°C	$6,16*10^6$	$2,87*10^{10}$
12 months	4°C	$4,24*10^6$	$1,98*10^{10}$

storage of cells suspended in NaCl resulted in 75 % cells less.

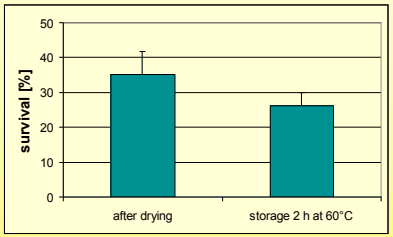
Characterization of shelf life
Accelerated storage test

Shelf life of encapsulated and dried *P. fluorescens* BA2002

after 4 weeks of storage at 20°C



accelerated storage test (2 h at 60°C)



For a fast estimation of shelf life, incubate formulation 2 h at 60°C.

Characterization of efficacy
Method overview

Lab, greenhouse, field

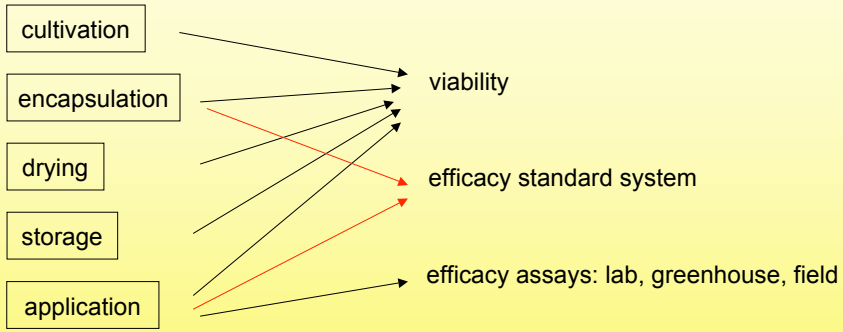
Test system

- **No standard systems available!**
- **Complex plant-pest-soil-capsule system**
- **What works in the lab does often not work in the field**

Data

- **Dose-response curves**
- **Effect of soil type, soil humidity, temperature, time of application,… on efficacy**
- **Effect of capsule material, biomass concentration, drying, storage,… on efficacy**

Characterization of efficacy
Method overview



Characterization of efficacy
Establishment in soil

Establishment of *H. rhossiliensis* by encapsulation in hollow beads

Characterization of efficacy
Field trials

Field trials 2005 with encapsulated bacterial antagonists: application of granules

Characterization of efficacy
Field trials

Field trials 2004

- **Encapsulation of bacterial antagonists**
  - three bacterial strains (BA2002, F50, F54) raised in a bioreactor on ½ TSB medium.
  - 60 g bacterial biomass + 540 g autoclaved baker's yeast + 3000 g of a biopolymer solution
  - Jet Cutter into a 2 % $CaCl_2$ solution, 20 min crosslinking time
  - Drying of beads
- **Application of bacterial antagonists**
  - incorporated of encapsulated and free cells, respectively, in the pellet surrounding commercial sugar beet seeds.
  - Field trials
    - plot trial, six replications,
    - at six locations in Europe
      - Germany, France, The Netherlands
    - Efficacy: early and final emergence of seedlings in the field (most important parameters)

Characterization of efficacy
Field trials

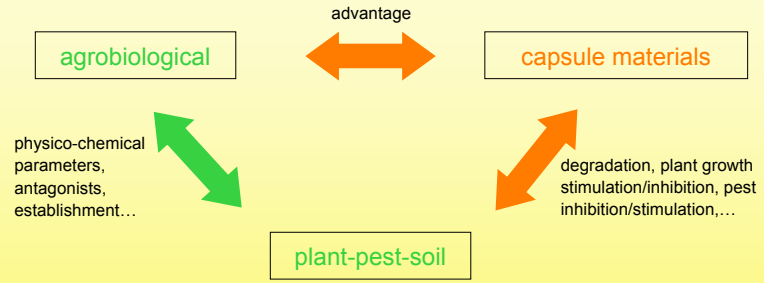
Field trials 2004 with encapsulated bacterial antagonists at Seligenstadt, Germany

		Early field emergence	
Formulation	**Antagonist**	**[%]**	**% of standard**
bead	F30	71,1	112,1
bead	F54	70,0	110,3
bead	BA2002	68,0	107,3
culture broth	BA2002	67,7	106,8
culture broth	F54	66,1	104,2
culture broth	F30	64,9	102,3
standard (no pesticides)	-	63,4	100,0

Efficacy of bead formulation tended to be higher than the liquid formulation at the other five locations, too.

Experiment carried out by Dr. R. Tilcher, KWS Saat AG, Einbeck, Germany

Characterization of efficacy
Need for research



Characterization of encapsulated agrobiologicals
Summary

- **Overview of important characteristics**
  - **mechanical stability, particle size, -distribution, physico-chemical parameters, biological degradability, flowability, reswelling, formulation additives, preconditioning**
- **Reswelling**
  - **several test methods in or on media**
  - **few data on reswelling properties and cell growth**
- **Shelf life**
  - **viability**
  - **accelerated storage tests**
- **Efficacy**
  - **lab, greenhouse, field**
  - **no standard test system**
  - **complex systems**