**Synergistic interaction of co-encapsulated *Saccharomyces cerevisiae* and *Metarhizium brunneum* used for biological pest control**

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***Highlights***

* Calcium alginate-based formulation acts as microfermenter for the entomopathogenic fungus *M. brunneum*
* Co-encapsulation of *S. cerevisiae* improves sporulation of *M. brunneum*
* Co-encapsulation of *S. cerevisiae* and *M. brunneum* leads to steep oxygen gradients in alginate beads as revealed by microelectrode measurement

**1. Introduction**

In the last few years wireworm damage has become an increasing problem in both conventional and organic potato cultivation. Wireworms are polyphagous soil dwelling larvae of click beetles (*Agriotes spp*.) and even low populations lead to severe economic losses since they can live up to five years in soil. Wireworms use CO2 gradients established in soil by plant roots to locate potential hosts. However, effective plant protection products are currently not available since chemical insecticides have recently been restricted or abandoned. Consequently there is a tremendous need for alternative biological control options. In previous work, a biological bead formulation for wireworm control was developed, based on an attract-and-kill approach that exploits the insect’s behavior [1-4]. The calcium alginate beads contain both *Saccharomyces cerevisiae* that produce CO2 as an attractant and the entomopathogenic fungus *Metarhizium brunneum Cb15 III* acting as the kill component. Additionally, a nutrient supply is added. When the beads are placed in soil, they absorb its moisture, thereby initiating the CO2 production process as well as the growth of fungus out of the beads. Virulent aerial conidia are formed on the bead’s surface. Therefore the co-formulation represents a “microfermenter” [5] that needs to be investigated in more detail. Co-cultivation can alter cell growth [6] and, moreover, lead to morphological and physiological changes possibly resulting in attenuated virulence induced by contact-dependent interaction [7]. Hence, the main objective was to investigate the synergistic interaction of *Saccharomyces cerevisiae* and *Metarhizium brunneum* inside the bead.

**2. Methods**

*M. brunneum* was grown in submerged culture for 48 h at 25 °C and 150 rpm in shaking flasks with baffles. Mycelial biomass and blastospores were separated by vacuum filtration.

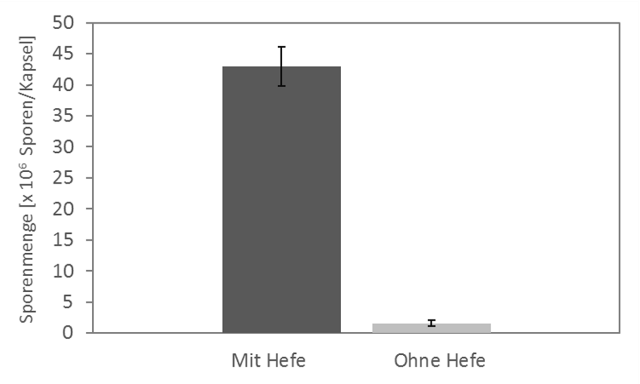
The encapsulation suspension was prepared by mixing sodium alginate and sterile native corn starch. Then, *S. cerevisiae* and *M. brunneum* biomass were added. For bead formation, the suspension was dripped into a sterile CaCl2 solution. Diameter of beads was 4-5 mm. Subsequently, beads were dried, resulting in a bead diameter of 3 mm.

Formed aerial conidia were rinsed off from the bead surface with a 0,1% Tween 80 solution and conidial concentrations were determined by counting conidia with a Thoma cell counting chamber

Microelectrodes (Unisense, Denmark) with a diameter of 50 µm were used measuring oxygen-, pH- and temperature gradients. For this, beads were fixed, and microelectrodes inserted stepwise (100 µm) into the beads with a manual micromanipulator.

**3. Results and discussion**

We found that co-encapsulation of *S. cerevisiae* enhanced the sporulation of *M. brunneum* from the beadssignificantly (Figure 1), probably serving as nitrogen source, since they consist of 80% nitrogen.



Aerial conidia [x106 / bead]

b

a

without S. cerevisiae

with S. cerevisiae

Figure 1: Sporulation of M. brunneum on alginate beads with and without S. cerevisiae. Different letters above bars indicate significant differences based on t-test at p < 0.05 (means ± SD, n = 4).

Further analysis revealed steep oxygen gradients when *S. cerevisiae* is added to the formulation (Figure 2), indicating oxygen limitations in the center. Diffusion limitation in alginate beads can limit cell growth and lead to metabolic changes [8, 9]

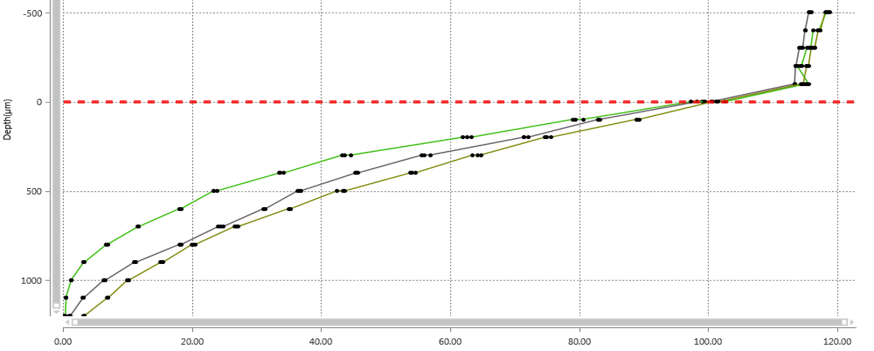
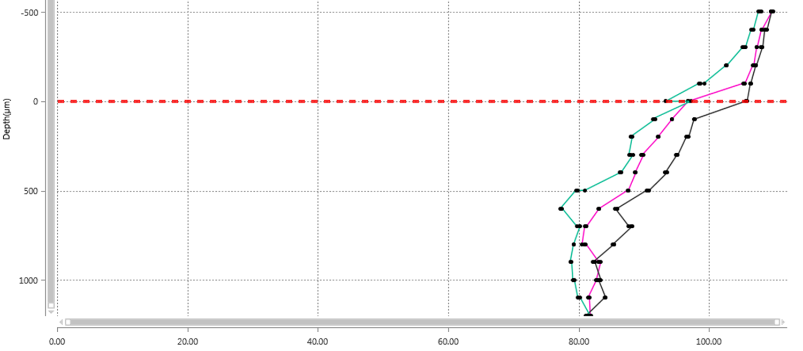


Figure 2: Oxygen gradients in alginate beads loaded with starch and M. brunneum (top) and with starch, M. brunneum and S. cerevisiae (bottom). Abscissa in mV. Red dotted line indicates bead surface.

**4. Conclusions**

Preliminary results indicate a biological impact of co-encapsulation on fungal development, especially sporulation. A novel method for bead analysis was established in order to examine the physicochemical and biochemical processes. Future work will focus on small-scale submerged co-cultivation (BioLector Pro).

The influence of oxygen limitations will be examined and circumvented by reducing both bead diameter and cell concentration in order to improve efficacy.

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