Low cost and efficient formulation of microencapsulated Sinorhizobium meliloti formulation cultivated in starch industry waste water

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Abstract

Sinorhizobium meliloti grown in starch industry wastewater was microencapsulated for agricultural application using low viscous sodium alginate as an encapsulation matrix. Sodium alginate mixed with cell concentrate was developed in canola oil and emulsion technique was used for the production of microbeads. CaCl2 (1 M) was used as a cross-linking agent. This Microencapsulated formulation was coated on alfalfa seeds and ~5.0x10^4 CFU/seed survived after 3 months of storage which was close to the highest level of the prescribed requirement (10^3- 10^5 CFU/seed) The nodulation efficiency and plant yield were clearly improved when icroencapsulated formulation was applied to seed (dry matter was 238 mg ;while in control, it was 50 mg).

Introduction

Commercial rhizobial bio-inoculants are available as powder, granules and liquid, while encapsulation formulation is an interesting approach. Alginate is a promising polymer having versatile properties in encapsulation of microorganisms (Wang et al. 2006) and is totally an environmentally friendly material. Encapsulation has the same advantages than any other microbial formulation as it can be applied along with the seeds during sowing by simple mixing. Encapsulated microorganism can survive in beads due to the protective effect of matrix and there will be controlled release of microorganism by virtue of slow release of bacteria (Bashan, 1986).

Methods

- S. meliloti strainA2 (Agriculture and Agrifood Canada, Sainte-Foy, QC, Canada) was used in the current study
- Fermentation in flask ,30 °C ,200 rpm, 48 h.
- Steps in development of microencapsulated formulations:

➲ (a)-production of rhizobia,
➲(b)-centrifugal recovery,
➲(c)-cell concentrate,
➲(d)-cell suspension in alginate,
➲(e)-mixing of oil and microglobule formation,
➲(f)-addition of CaCl2 and hardening of microbeads
➲(g)-separation of oil and water phase.

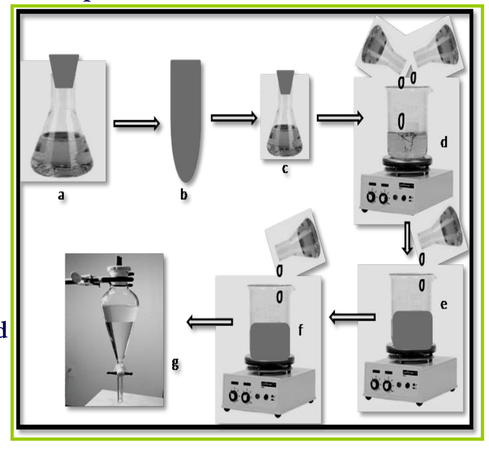

Fig. 1 Microencapsulation steps

Seed coating

- One gram of surface sterilized alfalfa seeds (Medicago sativa) were mixed for 5 min. using a vortex mixer with 1 mL of formulation and dried under laminar airflow for 2h.
- Emulsion formulation used for seed coating was prepared by mixing canola oil with cell concentrate (50% v/v). Seeds were stored at at 4 °C for 3 months.
- For testing he Survival of cells: 10 seeds were mixed vigorously with 10 mL of 0.001 g/L Tween-80 solution and diluted with phosphate buffer (pH 7) followed by plating on YMA.

Results

- The scanning electron micrograph (Fig.2) shows the morphology of microbeads:
  - ➲ beads were generally irregular in shape and size and there was also rare occurrence of aggregated beads (one with other) which might be due the additives used.
  - ➲ there was no large aggregated bead formation during cross-linking with calcium ions
  - ➲The beads were generally smaller than 100 µm size and can be utilized for seed coating of even smaller seeds, such as alfalfa.

- Fig. 3 showed the survival of S*inorhizobium meliloti* on alfalfa seeds from one day after coating:
  - ➲It was observed that 5.6 times more cells survived on encapsulated formulation coated seeds than emulsion coated seeds.
  - ➲ A decline was observed in the survival of cells in concentrated broth coated seeds.

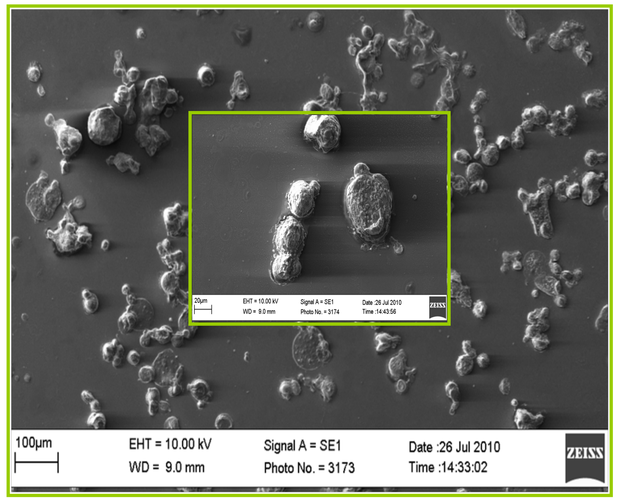

Fig.2:: Electron micrograph of microencapsulated beads.

Nodulation efficiency

➲The nodulation efficiency of microencapsulated formulation was tested on alfalfa plants.
➲clear indication on the effect of nodulation by encapsulated bacteria:
➲maximum nodulation index (18):the nodules were found both in tap roots and secondary roots (Table 1).
➲The plants grown from seeds treated with microencapsulated formulation attained height as high as 74-82 mm and significantly higher dry biomass up to ~238 mg. than the control plant without inoculation (dry biomass up to ~50 mg),
➲ plants inoculated with SIW grown culture (dry biomass up to ~155 mg) and YMB grown culture (dry biomass up to ~153 mg) (Table 2).
➲The control plant without any inoculation had no nodules in root system and plants were yellowish due to the lack of nitrogen, a major element in chlorophyll synthesis (Fig. 4).

Seed coating

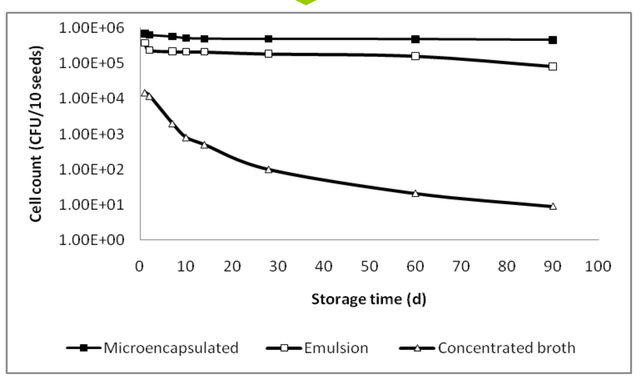

Fig. 3 Survival of S*inorhizobium meliloti* on alfalfa seeds

Fig.4: Plant growth, A: Plants treated with microencapsulated formulation and B: Plants treated with YMB grown Sinorhizobium. Nodulated roots, C: Plants treated with microencapsulated formulation and D: Plants treated with YMB grown Sinorhizobium. Solid black arrows show nodule on tap root and white arrows show nodules on secondary roots.

Table 1: Effect of formulation on nodulation and growth after 28 days of growth

Inoculant (*Sinorhizobium*)	Nodulation index (10 pouches)	Stem dry weight (mg/pouch)	Root dry weight (mg/pouch)	Plant height (mm)
Uninoculated (control)	0 (a)	30±6.3 (a)	20±3.4 (a)	25-32 (a)
YMB grown (10^6 CFU)	12-18 (b)	120±17.9 (b)	35±4.5 (b)	50-57 (b)
Starch industry wastewater grown (10^6 CFU)	12-18 (b)	113±19.7 (b)	40±8.9 (b)	51-56 (b)
Microencapsulated (10^6 CFU)	18 (b)	183±8.2 (c)	55±10.5 (c)	74-82 (c)

Conclusions

- The results of the present study on microencapsulation of nodulating bacterium emphasized the advantages of development of stable formulation.
- The stability of the seed coated formulation was increased by encapsulation and emulsification together.
- During agricultural application the nodulation capacity increased due to the controlled release of cells from microbeads and there was a corresponding enhancement in the growth of plants.

References

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