Microencapsulation of microbial cells for improving shelf life through Spray drying technique

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Microencapsulation, a technique of coating active particles or molecules to overcome the problems in spray drying is the latest development. Microencapsulation is defined as a process in which tiny particles or droplets are surrounded by a coating or embedded in a homogeneous or heterogeneous matrix to give small capsules with many useful properties (Gharsallaoui *et al*., 2007) and is used by several researchers to provide living cells with a physical barrier against the external environment (O'Riordan *et al*., 2001). The microcapsule may consist of a core surrounded by a wall or barrier of uniform or non-uniform thickness, which can be formed by one or more polymers (Krishnan *et al*., 2005). A biological control product to compete commercially with chemical products must have a minimum shelf life of 1–2 years at room temperature. Dehydrated fungal formulations are attractive because of their long stability, easy handling and storage at room temperature. Three kinds of propagules that can be used in formulations are hyphae, chlamydospores and conidia. The use of hyphae is avoided due to its lack of resistance to dehydration. Conidia and chlamydospores withstand adverse environmental conditions and hence it is the preferred choice as propagules in formulations. There are several products in the market using microbial spores or conidia as active ingredient, obtained either by two-phase solid fermentation or liquid fermentation. However, in both cases the biomass must be dried to obtain a stable product with prolonged shelf-life. For large scale production of powdered formulations of microbial agents different drying techniques are used, among them spray drying is widely preferred due to its low cost. However, spray drying can produce cellular damage as a result from the elevated temperature, dehydration and oxidation of macromolecules during storage. microencapsulation with biopolymers can help to overcome some of these problems. First hand information about the success of use of humic acid as encapsulant for *B. bassiana* through spray drying technique will be discussed step wise.

Basic terminology

Definition of microencapsulation: it is the process by which individual particles or droplets of solid or liquid material (the core) are surrounded or coated with continuous film of polymeric material (shell) to produce capsules in micrometer to millimetre range, known as microencapsulation.

Microcapsule: Generally microcapsule consist of two main components

i) Core material or active ingredient or material to be coated

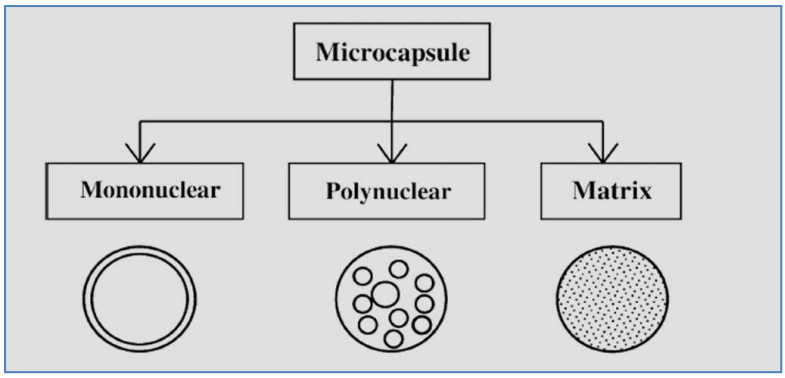
ii) Coat or wall or shell material, which is used to provide encapsulation for surface protection of core material.

Types of microcapsules: The type and morphology of microcapsules depends mainly on the core material and the deposition process of the shell.

1- Mononuclear (core-shell) microcapsules contain the shell around the core.

2- Polynuclear capsules have many cores enclosed within the shell.

3- Matrix encapsulation in which the core material is distributed homogeneously into the shell material. - In addition to these three basic morphologies, microcapsules can also be mononuclear with multiple shells, or they may form clusters of microcapsules.



Properties of coating material:

- Stabilization of core material
- Inert toward active ingredients
- Controlled release under specific conditions
- Film-forming, pliable, tasteless, stable
- Non-hygroscopic, no high viscosity, economical
- Soluble in an aqueous media or solvent, or meltin
- The coating can be flexible, brittle, hard, thin etc

Coating materials:

- **Gums:** Gum arabica, sodium alginate, carrageenan
- **Carbohydrates:** Starch, dextran, sucrose
- **Celluloses:** Carboxymethylcellulose, methylcellulose
- **Lipids:** Bees wax, stearic acid, phospholipids
- **Proteins:** Gelatin, albumin

Spray dryer: Line diagram and components

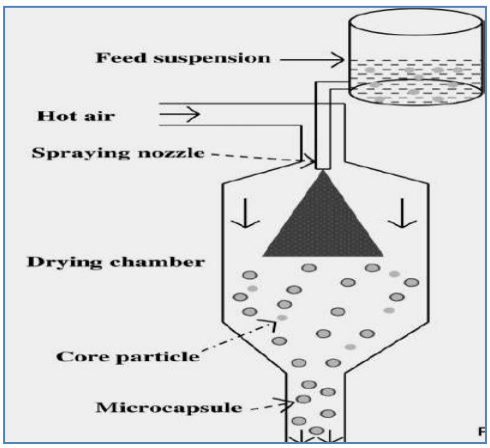


Fig 1: Line diagram of Spray dryer

Fig 2: Spray dryer LSD-48

The line diagram showed in fig1 shows the working principle of co-current flow method adopted in the spray dryer model LSD-48 (fig 2) which is being used at DOR, Hyderabad for microencapsulation of *B bassiana* spores with bio-polymers under institute research programme.

The main components of standard spray dryer

- An air heater
- Atomizer
- Main spray chamber
- Blower or fan
- Cyclone
- Product collector

Air flow: There are three modes of contact

1. Co-current
2. Counter-current
3. Mixed -flow

Sample preparation and spray drying for microencapsulation

Material required

1. Microbial agent (*B. bassiana* spore in powder form)
2. Coating material (Humic acid, Sucrose, Gum arabica, Chitin)
3. Sterile water

4. Conical flask 500ml, 100ml
5. Tween solution (80%)
6. Magnetic stirrer
7. Vortex shaker
8. Mini spray dryer

Instrument Parameters:

1. Start the instrument, ensure the motor starts
2. Set the aspirator to between 40-50 to ensure vacuum build up of 80-100
3. Set the inlet temperature: 170^{o}C
4. Wait for 5 minute to ensure drying of glass chamber and cyclone
5. Set feed pump speed to20
6. Set aspirator to 40

Procedure

- Add 0.5g of *B. bassiana* spore little by little to 50ml sterile water in conical flask by vortexing to form homogenous solution
- Add a drop of tween (0.02%) solution for proper mixing of spore powder in water
- Add 0.20g humic acid in another 40ml sterile water and mix properly to form homogenous solution
- Add humic acid solution to spore solution slowly by vortexing
- Make up the final volume to 100ml
- Feed water for one minute when the desired inlet temperature was ready
- Run the sample (100ml) for microencapsulation
- After sample, run the instrument with water for cleaning the tubings and nozzle
- Stop the feed pump, zero the temperature and switch off the aspirator.
- Switch off the control panel only after the inlet temperature reaches room temperature preferably or at least falls below 60^{o}C.
- After cooling, remove cyclone and collect the product
- Dismantle the assembly and clean all the glassware and the nozzle, dry and again assemble the equipment for next run.

Evaluation of humic acid as encapsulant through spray drying technique

Spray drying of *B. bassiana* (Bb) conidia was standardized with varying inlet temperatures of 110, 130, 150, 170, 175 and 180^{0}C. Recovery of viable conidia increased with the temperature only till 175^{0}C with corresponding $\log_{10}$ CFUs (*L*CFU) of 16.69, 16.54, 17.30, 19.87, 19.98 and 19.18 per recovered product although lower than the value of 21.19 (*L*CFU) before spray drying. Based on the results, best combinations of two inlet temperature and three concentration of sodium humate (SH) was tried. Spray drying of Bb conidia was undertaken at 170 and 175^{0}C with 0.1, 0.15 and 0.2% SH. Product recovered from spray drying with humic acid was dark brown coloured smooth, dry and free flowing powder containing Bb-SH microcapsules.

Bb powder before spray drying contained 21.19 *L*CFU. Spray drying at 170 and 175^{0}C with 0.1% SH resulted in a *L*CFU of 19.60 and 19.72 respectively per recovered product while, spray drying with 0.15% SH resulted in a value of 20.39 and 20.45 *L*CFUs per recovered product for above two temperatures respectively. However, spray drying of the conidia along with 0.2% SH only at 175^{0}C resulted in complete retention of conidial viability of 21.18 *L*CFU per recovered product in the resulting powder containing Bb-SH microcapsules. The outlet air temperature was 86.5$\pm$1.3^{0}C for the inlet temperature of 175^{0}C. In our study, microencapsulation of pure conidial powder of *B. bassiana* with 0.2% SH through spray drying at 175^{0}C with an outlet air temperature of 86.5$\pm$1.3^{0}C resulted in complete recovery of viable Bb conidia. Thus use of SH as an encapsulant has enabled effective spray drying at a high temperature of 175^{0}C.

Shelf-life studies: Shelf-life studies of the samples stored at room temperature (30^{0}C) revealed a significant lowering to 14.34 *L*CFU by the end of six months from the initial value of 21.59 *L*CFU for Bb conidial powder. *L*CFU of powder containing Bb-SH 0.15% microcapsules was lowered to 16.64 from the initial value of 20.74 *L*CFU. While, a minimal lowering counts i.e. 21.11 *L*CFU from the initial value of 21.54 *L*CFU was observed in case of powders containing Bb-SH 0.2% microcapsules.

Bb-SH 0.2% microcapsules did not lose viability while Bb conidia that were not spray dried lost their viability rapidly after 6 months storage at room temperature, thus, showing that humic acid is a good candidate for microencapsulation of Bb conidia facilitating extended storage at room temperature.

References

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