Chapter 6
Encapsulation of Biofertilizers, Biopesticides and Biocontrol Agents

Geeta Singh and Ishani Paithankar

Abstract Increasing the yield of crop plants is possible by alleviating biotic and abiotic stresses and by improving fertilization. Classical agrochemicals are gradually being replaced by biological inputs such as biofertilizers, biopesticides and biological plant growth enhancers. Biofertilizers and biopesticides are, for instance, soil microorganisms that contribute to plant growth and protect plants from diseases. Here, the targeted delivery of these microbes at their site of action is important. In this chapter we review the encapsulation process for targeted delivery of biofertilizers, biopesticides and biocontrol agents. Strategies include microbial encapsulation, and encapsulation in natural and artificial polymers. Spray drying, freeze drying, extrusion, and emulsion are used to prepare capsules or beads or formulations. We present materials for microbial encapsulation, preparation of encapsulated microbial formulations, and applications.

Keywords Biofertilizers · Biopesticides · Plant growth promoting microorganisms · Encapsulation · Microcapsules · Beads

Abbreviations

ACC	Aminocyclopropane-1-Carboxylate
CFU	Colony forming units
IAA	Indole 3-acetic acid
OSAN	Octenyl succinic acid anhydride

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6.1 Introduction

Biofertilizers, biopesticides and biocontrol agents together encompass groups of microorganisms that contribute to the growth and development of plants in an environment friendly manner. Biofertilizers are microbial formulations which help in availability of nutrients using their metabolic activities and thus, improve soil health and fertility (Noumavo et al. 2016). The availability of macronutrients nitrogen, phosphorous, potash as well as secondary and micronutrients to the crop plants are significantly regulated by the diverse group of soil microorganisms. Some bacteria and fungi are able to reduce molecular nitrogen (N_2) to ammonia (NH_3) and make it available for plants through the action of nitrogenase enzyme (Newton 2000; Franche et al. 2008; Dixon and Kahn 2004). These microbes exist as symbiotic or asymbiotic associations with plants. Some well-known examples include *Rhizobium*, *Bradyrhizobium*, *Klebsiella, Azospirillum*, and *Burkholderia*. *Rhizobium* is known to fix N_2 in association with leguminous plants of Fabaceae family (Willems 2007).

High reactivity of phosphate renders it into insoluble forms including inorganic phosphate or mineral phosphate (e.g., apatite) and organic phosphate (Ionositol phosphate, phosphomonoesters, phosphodiesters) (Khan et al. 2009). The soluble forms of P ($H_2PO_4^-$ and HPO_4^{2-}) are available for assimilation by plants. The conversion of these insoluble inorganic and organic phosphate compounds into soluble forms is primarily mediated by soil microorganism. This is accomplished by production of organic acids carboxylic and gluconic acids resulting in lowering of pH leading to dissolution of phosphates (Rodriguez and Fraga 1999). Organic phosphates are solubilised by production of phosphatases enzymes hydrolysing phosphate mono- and diesters (Rodriguez and Fraga 1999; Tao et al. 2008).

Besides, enhancing the plant nutrient availability microbial biofertilizers also stimulates the plant growth and development by production of some phytohormones including auxins, gibberellins, and cytokinins. Plants often are unable to produce optimal levels of auxin required for root growth (Pilet and Saugy 1987). However, there are some soil bacteria that are able to synthesize indole 3-acetic acid (IAA), precursor of auxin hormone, from L-tryptophan released from root exudates. Most common IAA producing bacteria include *Rhizobia* (rice), *Azospirillum* (wheat), *and Pseudomonas* (radish) (Badenoch-Jones et al. 1984). Another method by which IAA producing bacteria affect plant growth is by reducing ethylene levels in plants. The IAA secreted by bacteria works with endogenous IAA to activate synthesis of ethylene synthesis pathway enzyme 1-Aminocyclopropane-1-Carboxylate (ACC) synthase (Penrose and Glick 2001). ACC synthase synthesizes ACC from S-adenosyl-methionine. This ACC synthesized by plants is assimilated by bacteria and degraded to ammonia and α-ketoglutarate using enzyme ACC deaminase. Thus, these microbes act to regulate the levels of ethylene in plants and prevent it from inhibiting plant growth.

Biocontrol agents are the microbial organisms that protect plants against biotic stresses. Their mechanism of action against plant pathogens includes production of antibiotics (Compant et al. 2005; Haas and Keel 2003; Mazurier et al. 2009),

synthesis of lytic enzymes (Frankowski et al. 2001) or production of siderophores (Dowling et al. 1996; Kloepper et al. 1980), competition for plant nutrients. Soil borne microorganisms often synthesise lytic enzymes including glucanases, cellulases, chitinases proteases, lipases that hydrolyse cell wall components of pathogens and thus inhibiting them from colonising or infecting plant parts. The siderophores take up/deplete iron from rhizosphere thereby limiting the colonization of pathogenic fungi (Dowling et al. 1996; Kloepper et al. 1980). The well-known examples of the biocontrol organisms include fungi of *Trichoderma sp, Pseudomonas*. Currently bio-formulations having biofertilizers as well as biocontrol agents are primarily available as powder form (solid) or as liquid formulations. The major constraint encountered in these is loss of viability of the active organisms over the period of storage, transportation as well as at the time of application. In addition, the problem with contamination with undesirable organisms is also a major limitation. After their application at the target site, the sustained and gradual release is also not possible in these formulations. Therefore, by resorting to bioencapsulation process these limitations can be successfully overcome.

6.2 Encapsulation

Encapsulation is defined as confinement of any solid, liquid or gaseous material within a semi-permeable wall of polymeric material resulting in formation of small microcapsules (da Silva et al. 2014; Martinis et al. 2013; Nedovic et al. 2011). The capsular wall serves as a protective shield against external conditions including pH, temperature, humidity etc. that may adversely affect the activity of the core / enclosed material. In this manner, the capsule facilitates regulated release of encapsulated material only in the presence of conditions favouring its activity at the desired place (Suave et al. 2006). Encapsulation is classified as one of the immobilization techniques along with entrapment and covalent bonding/cross linking. Entrapment is the irreversible immobilization technique, in which the immobilized material is entrapped in a matrix or fibres for support (Górecka and Jastrzębska 2011). Encapsulation differs from immobilization. In immobilization, the material in entrapped entirely within the matrix, while in encapsulation a coating material is used to enclose the matrix, which is contained within capsule forming core of entrapped material.

Immobilization allows exposure of small portion of material surface, while encapsulated material is totally enclosed within capsule (King 1995). Encapsulation harbours a number of advantages over immobilization. Encapsulation involves enclosure of material within a semi-permeable membrane, facilitating diffusion of nutrients and also high strength of the wall material enables retention of the material. Encapsulation is categorised on the basis of bead size as microspheres (10–100 μm) and macrospheres (>100 μm) (John et al. 2011; Rathore et al. 2013). It can also be classified on the basis of bead structure or morphology (John et al. 2011). Solid spheres are known as beads while hollow spheres made of a liquid core

are referred as capsules. Capsules are further classified as microcapsules (1-1000 μm) and macrocapsules (mm to cm) (Rathore et al. 2013). Thus, encapsulation process is divided into two different types- Microencapsulation (bead size 1–1000 μm) and Macroencapsulation (bead size mm to cm).

This technique is employed for immobilization of diverse substances including enzymes, pharmaceuticals, flavours, cell organelles, plant and animal cells (Rathore et al. 2013). Recently, this technology has captured the imagination of biologists for entrapment of microorganisms. The encapsulated microorganisms have found applications in food industry, pharmaceutical, environment, agriculture etc. It has also been widely used for treatment of industrial waste water (Martinis et al. 2013), formulation of probiotics for yoghurt preparation (Krasaekoopt et al. 2003). In agricultural sector, it is being exploited for producing formulations of biofertilizers, biopesticides or biocontrol agents.

6.2.1 Advantages of Encapsulation

The most commonly used inoculants include liquid inoculants, that are cultures of broth in water, organic or mineral oils, or peat carrier formulations. The liquid formulations are applied as dips or sprays for seeds. Peat formulations are directly inoculated into the seeds. However, both of these formulations decrease microbial survival as they are unable to provide protection to the material from external conditions and also the products are rendered to higher chances of contamination during storage, transport or application in soil, which reduces the shelf life of product (Bashan et al. 2002). The encapsulated formulations harbour a number of advantages over conventional inoculants in terms of preserving microbial viability, shelf life, protection against unfavourable external conditions and regulation of release in target environment.

6.2.2 Microcapsule Structure

Microcapsules are made of natural or synthetic polymers. These are formulated as gel beads or as dried powder form. Due to presence of pores in their smooth or irregular walls, they lack encapsulation efficiency and stability (Mortazavian et al. 2007; Favaro-Trindade et al. 2008). Thus, these capsules are coated with suitable wall materials (Mortazavian et al. 2007). Structurally, a microcapsule consists of an inner, centrally located core enclosed by a polymer layer forming wall or membrane of the capsule.

6.2.2.1 Coating Material

The essential features deciding the suitability of a given material for it usage for making capsule membrane are non-reactive response towards core material or active agent, provision of protection to the core against external, adverse conditions, ensure proper sealing of the material inside the capsule and economic viability. It should also facilitate the efficient release of the material under suitable, favourable conditions at the target place (Gharsallaoui et al. 2007; Nazzaro et al. 2012).

A number of materials can be employed for coating microcapsules. Most commonly used materials include both natural and synthetic polymers. These include carbohydrates such as starch, modified starch, dextrins, sucrose, chitosan; gums, Arabic gums, alginate, carrageenan; lipids, wax, paraffin, hydrogenated oils and fats; proteins, gelatine, casein, albumin; and inorganic compounds: Calcium sulphate, silicates (Favaro-Trindade et al. 2008). Synthetic polymers used for encapsulation include polyethylene glycol, polyvinyl alcohol, polyurethane, polypropylene, sodium polystyrene sulphate and polyacrylate (acrylonitrile-sodium methallylsulfonate). Khorramvatan et al. (2013) used three different natural polymers starch, gelatine and sodium alginate as coating material of encapsulated formulation of *Bacillus thuringiensis*. It was found that sodium alginate was most effective coating material against UVB (385 nm) and UVC (254 nm).

6.2.2.2 Common Natural and Synthetic Polymers

Various natural and artificial polymers used for preparation of microcapsules and their properties are listed in Table 6.1 (Gasperini et al. 2014; Wandrey et al. 2010; Olabisi 2015).

6.3 Techniques for Formulation of Microbial Inoculants

The entire process of production of encapsulated particles is completed in two phases: encapsulation and drying. This section describes the microbial encapsulation process for selected organisms. Mainly two types of microcapsulation methods have been described and used by various researchers.

6.3.1 Microencapsulation Phase

6.3.1.1 Extrusion Method or Droplet Method

It involves dripping of encapsulation matrix containing cell suspension through an orifice into a hardening bath. The mixture dripped in the form of droplets is converted into gelled spherical capsules upon contact with hardening solution

Table 6.1 Properties of commonly used polymers for microbial encapsulation

Name	Chemical/structure	Origin	Commercial modifications	Nature	Properties
Agarose	β-D-galactopyranose, 3,6-anhydro-α-L-galactopyranose	Red algae-*Gelidium* and *Gracilaria*	–	Carbohydrate	Cost effective Readily available in number of pore sizes Mechanical resistant Modulation of particle size
Carrageenan	βD-galactose, αD-galactose (anionic polymer)	Red algae-Rhodophyceae	*Kappa, iota, lambda* forms	Carbohydrate	Water soluble at 60°–80 °C Forms gels at room temperature Form viscous solutions Thermally reversible gels upon cooling in presence of cations Stability at neutral-alkaline pH
Alginate	β-1,4 linked β-D-mannuroic acid, α-L-guluronic acid (anionic polymer)	Brown sea weed, *Pseudomonas* and *Azotobacter*	Sodium alginate	Carbohydrate	Large pore size Form gels in presence of cations Water soluble at 60°–80 °C Insoluble in acid media
Chitosan	N-acetyl β-D-glucosamine (cationic polysaccharide)	Crabs and shrimp shells	–	Carbohydrate	Soluble at pH less than 5.4
Cellulose	β-1,4-D-glucose	Cell wall of plants, algae, oomycetes	Sodium carboxy-methyl cellulose, Ethylmethyl cellulose, Ethylcellulose, Hydroxypropyl cellulose, Hydroxypropyl methylcellulose	Carbohydrate	Thermogelation Soluble in cold water High strength Flexible films and transparent Low cost Requires treatments before use for entrapment

Starch	α-1,4 D- glucose units linear (amylose) and α-1,6 branched (amylopectin)	Plant parts including leaves, roots, tubers, stems	Corn starch, maize starch, rice starch OSAN (octenyl succinic acid anhydride) modified starch	Polysaccharide	Low cost Readily available Lower gel strength
Dextrin (starch gums)		Degradation of starch		Polysaccharide	Formulations dried easily Lower tensile strength
Collagen	Triple helix with every third amino acid glycine, proline and hydroxyproline	Extracellular matrix, fibroblast and osteoblasts		Protein	High strength Biocompatible and non-toxic
Fibrin	Glycoprotein, 3 polypeptides linked by disulphide bonds	Blood clots		Protein	
Polydextrose	Branched polymer of D-glucose	Heating dextrose with acid catalyst		Synthetic polysaccharide	Soluble Low calorific value Non-digestable High degree of polymerisation
Poly (ethylene glycol)	Ethylene glycol units	Reaction of ethylene oxide and water, ethylene glycol or ethylene glycol oligomers	Polyethylene glycol-vinyl sulfone, dimethacrylated polyethylene glycol	Synthetic	High strength
Polyvinyl alcohol	Vinyl alcohol units	Polyvinyl acetate		Thermoplastic	Non-toxic
Polyurethane		Reaction of polyol and di-isocyanate		Elastomer	
Polypropylene	Propylene units	Polymerisation of propylene with Ziegler-Natta catalyst		Thermoplastic	

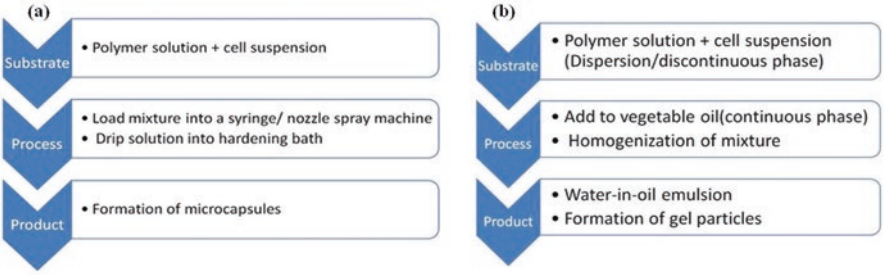

Fig. 6.1 Steps involved in bio-encapsulation (**a**) extrusion or droplet method and (**b**) emulsion technique

(Fig. 6.1a). The size of microcapsules formed is determined by diameter of orifice, viscosity of matrix, distance from hardening solution, and the concentration and temperature of hardening material. Based on the gelling method, this technique is further divided as Thermal gellation, ionic gellation and complex coacervation (Vemmer and Patel 2013).

Ionic gelation is used mainly for hydrocolloid biopolymers alginates, carrageenan and pectin. In case of alginate for encapsulation, the method involves following steps: Hydrocolloid solution preparation in water, adding cells to the hydrocolloid to form suspension, dripping droplets of cell suspension via a syringe into a hardening solution ($CaCl_2$) (Chen and Chen 2007). This hardening solution is made of divalent cations including Ca^{2+}, Mg^{2+} etc. In the $CaCl_2$ solution, the Ca^{2+} ions enable alginate polymers to form 3-D lattice around the cells by forming cross-linkages. The mechanism behind gel formation involves a bond formation between carboxylic free radicles of polymers and the positively charged cations in the solution (Champagne and Fustier 2007). This result in gel formation and the droplets formed are called beads (Gbassi and Vandamme 2012). The main advantage of this method lies in its easy procedure, gentle operations with minimal injury to cells high viability and low cost. Due to slow formation of microcapsules, the method cannot be employed for large-scale productions. It produces relatively larger beads of size 2–5 mm. Also, it often lacks compatability with high viscosity matrices.

6.3.1.2 Emulsion Technique

It involves two different phases the dispersion phase and continuous phase. Here, slurry of cells and polymer serve as dispersion phase and vegetable oils including sunflower, corn or paraffin oils act as continuous phase. The dispersive phase is added to continuous phase resulting in formation of water in oil emulsion. The resulting capsules are collected using centrifugation or filtration (Sheu and Marshall 1993; Gbassi and Vandamme 2012). For alginate beads, the process includes mixing of encapsulation solution with fat soluble acetic acid to lower the pH. This is followed by addition to water to separate oil phase. Figure 6.1b briefly gives steps

involved in the process. Overall, this technique is better than extrusion in that it can be used for large scale productions and it produces relatively small-sized beads (25-2 μm). However, requirement of additional purification steps for removal of oil phase and lack of control over size of microcapsules produced, create roadblocks in the use of this technique (Ding and Shah 2009; Rathore et al. 2013).

The above techniques have been used for encapsulation of microorganisms employed for number of purposes. In case of probiotics, extrusion method is employed for formation of alginate beads. Alginate is often used with a number of different polymers acting as coating materials. Jankowski et al. (1997) encapsulated probiotic bacteria *Lactobacillus acidophilus* using a formulation of alginate and starch. Krasaekoopt et al. (2006) used alginate alongwith chitosan coating material for formulation of alginate beads of *Lactobacillus acidophilus*, *Bifidobacterium bifidium*, *L. casei*. Another well known coating polymer for alginate beads is poly L-lysine. Champagne et al. (1992) used a alginate beads coated with poly L-lysine for encapsulating *Lactococcus lactis* for probiotics production. Other most widely used materials for formulation of probiotics include gellan gum and xanthan gum, K-carrageenan and Cellulose acetate phthalate. Gellan and Xanthan gums were used in combination for encapsulation of *Bifidobacterium lactis* (McMaster et al. 2005). K-carrageenan was used for encapsulation of *Bifidobacterium bifidium* by Dinakar and Mistry (1994). Rao et al. (1989); Favaro-Trindade and Grosso (2002) encapsulated *Bifidobacterium pseudolongum* using cellulose acetate phthalate.

Alginate is also used in agricultures for producing formulations of biofertilizers, biocontrol agents. Farhat et al. (2014) used alginate for encapsulation of two plant growth bacteria *Serratia marcescens, Enterobacter sp*. Santos et al. (2018) used alginate and clay for encapsulation of plant growth promoting microbes including *Azospirillum brasilense, Burkholderia cepacia, B. thuringiensis, B. megaterium, B. cereus, B. subtilis, Trichoderma spp*. Ivanova et al. (2005) encapsulated *Azospirillum brasilense* using Na-Alginate, standard and modified cornstarch. Bashan (1986) encapsulated *Azospirillum brasilense* using Na-Alginate with skim-milk. Young et al. (2006) used alginate and humic acid for encapsulation of bacteria *Bacillus subtilis*. Van Elsas et al. (1992) tested three combinations of Na-alginate for encapsulation of *Pseudomonas fluorescens*. These combinations included: Na-alginate, Na-alginate and skim-milk and Na-alginate, skim-milk and bentonite. Other plant growth microorganisms encapsulated were *Bradyrhizobium japonicum* with carboxymethyl cellulose with starch coating (Júnior et al. 2009) and *Rhizobium japonicum* with synthetic polymer polyacrylamide (Dommergues et al. 1979). Alginate has also been employed for formulation of biocontrol agents in agriculture. Fravel et al. (1985) used alginate, pyrax (clay) for encapsulation of *Talaromyces flavus, Gliocladium virens, Penicillium oxalicum*. Shah et al. (1998) used only Na-alginate for formulation of biocontrol agent *Erynia aphidis*.

Synthetic polymers including polyvinyl alcohol, polyurethane and polysulfone have been used for bioremediation purposes. Cunningham et al. (2004) encapsulated hydrocarbon degrading bacteria with the help of polyvinyl alcohol. Briglia et al. (1990) used Polyurethane foam for encapsulation of Pentachlorophenol

degrading microorganisms *Rhodococcus chlorophenolicus, Flavobacterium sp*. Ben-Dov et al. (2009) encapsulated waste-water bacteria using agar and polysulfone.

6.3.2 *Drying of Encapsulated Cultures*

The microcapsules produced are dried in order to convert them into minute particles (granules) or powder form. This is required to improve the shelf life and stability of the cultures during storage. Here, a few commonly used methods are presented.

6.3.2.1 Spray Drying

Spray drying method involves conversion of a fluid product into a solid in the form of powder (Fig. 6.2). This is accomplished by dispersion of the droplets of the fluid by using hot air within a hot chamber (Rodríguez-Hernández et al. 2005). The energy from hot air acts to disintegrate the liquid, dividing it into small particles, which results in mist or spray of droplets (Finney et al. 2002). It is one of the most widely used methods for microencapsulation of biological materials and food products. The reason behind its wide applicability is lower exposure time of the product

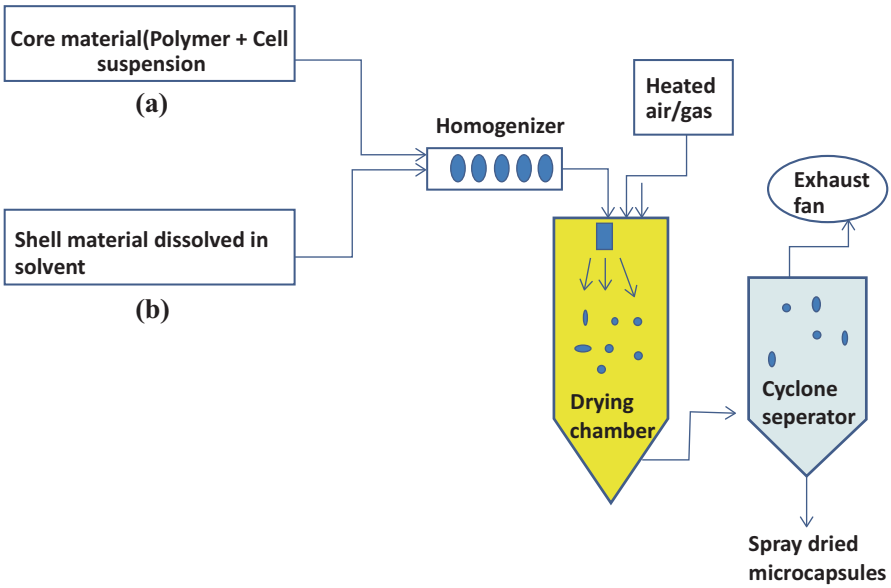

Fig. 6.2 Spray drying (**a**) The core material and coating solutions are homogenized. (**b**) The shell material is dissolved in solvent. The solution is passed through drying chamber where hot air acts to disintegrate it into small particles to form mist or spray of droplets. The spray dried particles are recovered in cyclone separator

to high temperatures, minimal thermal damage and higher yields. However, this method results in increased losses in viability. The origin of this technology dates back to nineteenth century, when it was used for drying eggs. However, the industrial application of this method began only in 1920s.

Milk and washing powder were the first industrial products to be produced by this method. O'Riordan et al. (2002) used spray drying method to encapsulate *Bifidobacterium* using gelatinised modified starch. Amiet-Charpentier et al. (2008) encapsulated rhizobacteria *Pseudomonas fluorescens-putida* using methacrylic copolymer from Eudragitrange. Jin and Custis (2011) used spray drying for producing conidia of *Trichoderma harzianum* using three different sugars, sucrose, molasses or glycerol for encapsulation. Paul et al. (1993) used dry air for encapsulation of *Azospirillum lipoferum* using alginate. It was observed that very high rate of drying of beads, adversely affected the cell viability than lower drying rate. Sinkiewicz-Enggren et al. (2015) encapsulated *Lactobacillus reuteri* using spray drying with following parameters of spray dryer: inlet temperature (120 °C), outlet temperature (73–74 °C), aspirator: 100%, pump: 20%, nozzle cleaner: 6–8. Spray drying device used was BUCHI, mini-spray dryer B290, Essen, Germany. Behboudi-Jobbehdar et al. (2013) found that an inlet temperature of 133.34 °C and feed rate of 7.14 ml/min were optimum for production of highly viable encapsulated *L. acidophilus*.

6.3.2.2 Spray Chilling

In this method, a dry core material is sprayed with a lipid-based material to serve as coating. The lipid-based material is sprayed in form of mist on the core material, which is kept in motion. This is followed by solidification of coating by using cold air with temperatures between 10–50 °C. This technique has been used for encapsulation of various food materials including vitamins, minerals, and other heat sensitive materials (Gibbs et al. 1999).

6.3.2.3 Coacervation

This method involves separation of a hydrocolloid/polymer from the solution followed by deposition over the emulsified core material. The principle behind the method is that after the phase separation, the polymer coating material forms a coacervate, which coalease to decrease the surface area and total interfacial free energy of the system and this favours its adsorption over the core material surface and form a uniform coating on core particle. This coating material is solidified by crosslinking reaction using thermal, chemical or enzymatic methods (Desai and Park 2005). The main advantages of the process include proper control of release of encapsulated material, a high pay load of 99% and its operation at room temperature, making it suitable for heat-sensitive bacteria. However, the materials used in techniques, result in a higher costs and complexity of the process make it relatively disadvantageous over commonly used techniques like spray drying.

6.3.2.4 Freeze Drying

It involves freezing of solution of carrier matrix and biological agent at low temperature, which is followed by removal of solvent by sublimation by applying low pressure or vaccum. This method is also termed as lyophilisation. Since, the process does not involve melting, it is considered as mild and hence enables preservation of characteristics of microcapsule. However, high cost of the method makes its use disadvantageous (Santivarangkna et al. 2007).

6.3.2.5 Vacuum Drying

It involves sublimation of frozen sample by applying low pressure similar to freeze drying. However, in this method sample solution of matrix and biological agent is not frozen but is converted from liquid to solid by phase transition. The application of this method for microbial encapsulation is however limited (Broeckx et al. 2016).

6.3.2.6 Fluid-Bed Agglomeration and Coating or Fluidized Bed Drying

This technique was first developed by pharmaceutical industry with purpose of making dry, enteric coatings for targeted and controlled release of drug in gastrointestinal tract (Dewettinck and Huyghebaert 1999). Today, it is being widely utilised by other industries like food, feed, agrochemicals, cosmetics for formulation and preservation of various products (Boerefijn and Hounslow 2005; Guignon et al. 2003; Saleh et al. 2003). A dehumified and filtered air is used to fluidise particle bed of the product. The technique is divided into three processes, the dehydration, heating and cooling. It finds applications, primarily in food industry, where it is used for commercial production of baker's yeast *Saccharomyces cerevisiae.* In agriculture, it is used for drying process of microencapsulated biocontrol formulations of fungi, bacteria, yeast or protein toxins of *Bacillus thuringiensis* (Brar et al. 2006).

6.3.2.7 Co-crystallization

This method involves dispersal of core material in a supersaturated sucrose solution, which is maintained at a high temperature. This is followed by a gradual dissipation of the heat resulting in crystallization of solution and core material. The crystals formed are dried and sieved (Bhandari et al. 1998). Table 6.2 briefly describe the microbial encapsulation process for selected organisms with their advantages and limitations. It shows bioencapsulation of beneficial microorganisms used as biofertilizers, biocontrol agents or biopesticides using spray drying and freeze drying and their advantages or disadvantages.

Table 6.2 Bioencapsulation of biofertilizers, biocontrol agents or biopesticides using spray drying and freeze drying

Microorganisms	Encapsulation material/ parameters	Technique	Advantage/disadvantage	References
Trichoderma harzianum	MaltodextrinDE10, MaltodextrinDE20, gum arabic, 1:1 MaltodextrinDE10 – gum arabic	Spray drying	Survival (storage and drying): MaltodextrinDE10 – gum arabic formulation 86%	Muñoz-Celaya et al. (2012)
Trichoderma harzianum	Sucrose (0.5, 1, 2, 4, 6 and 8%), molasses, glycerol BUCHI mini spray dryer B-290 Inlet temperature: 40–140 °C Outlet temperature: 20–81 °C	Spray drying	Survival (storage and drying): Increase survival CFU 7.5×10^{10}/g of dried conidia Optimum sucrose concentration: 2% Inlet temperature: 60 °C Outlet temperature: 31 °C	Jin and Custis (2011)
Bacillus cereus C1L	Maltodextrin (0–25%) and gum arabic (0–25%) Outlet temperature: 60–100 °C Inlet temperature: 150–250 °C	Spray drying	Viability: 42% at outlet temperature of 73.5 °C Best yield at maltodextrin (18.3) and gum arabic (12.5%)	Chen et al. (2013)
Beauveria brongniartii	Skim milk and polyvinylpyrrolidone (PVPK90) Outlet temperatures: 53+/−2 °C	Spray drying	Yield: 25% Viability: 92%	Horaczek and Viernstein (2004)
Beauveria brongniartii and *Metarhizium anisopliae*	Skim milk and polyvinylpyrrolidone (PVPK90) Inlet temperature: 60 °C Outlet temperature: 40 ± 2 °C	Spray drying	Loss of fungal material Lower germination rates	Horaczek and Viernstein (2004)
Beauveria bassiana	Dextrin (10%), skimmed milk (10%), polyvinylpyrrolidoneK90 (5%)	Spray drying	Viability (after 6 months storage at 4 °C): 80%	Liu and Liu (2009)

(continued)

Table 6.2 (continued)

Microorganisms	Encapsulation material/ parameters	Technique	Advantage/disadvantage	References
Beauveria bassiana	Sodium humate Inlet temperature:175 °C Outlet temperature: 86.5+/−1.3 °C	Spray drying	Good viability after 6 months storage at room temperature	Qureshi et al. (2014)
Tsukamurella paurometabola C-924	Starch cream (10% w/w) Mobile Minor™ (Niro atomizer, Denmark) atomizer Inlet temperature: 130 °C Outlet temperature: 55° or 65 °C Feed flow rates: 1.9 or 1.7 L/h	Spray drying	Stability (at room temperature and 4 °C): More for encapsulated Shelf life: longer than 1 year	Hernández et al. (2007)
Nitrogen-fixing bacteria associated with lupin nodules	Sodium-alginate: Maltodextrin (0:15,1:1, 2:13) LabPlant SD-05 dryer Nozzle diameter:1.5 mm Inlet temperature: 100 °C Outlet temperature: 65 °C Air-flow rate: $73 m^3 h^{-1}$ Feed rate: $5.3 g min^{-1}$	Spray drying	Highest survival at Na-alginate: maltodextrin ratio 1:14: 79% Microcapsule yield: 27%	Campos et al. (2014)
Bradyrhizobium japonicum	Gum-acacia (1,3,6%) and maltodextrin (13,15,30%) Buchi mini spray dryer(B-290) Inlet temperature: 65°–70 °C Outlet temperature: 30–32 °C Air flow rate: 40 units Feed pump rate: 20–23%	Spray drying	Viable cell count: 1×10^8 CFU/g with gum-acacia (1%) and maltodextrin (15%) Survival: 90% Increased root of soyabean biomass by 2%	Dey et al. (2018)
Pantoea agglomerans, Trichoderma harzianum	Alginate-glycerol-kaolin	Freeze drying	Shelf life: Increased Protection from UV-C radiation	Nussinovitch (2016)

(continued)

Streptomyces sp. Di944	Alginate beads Durum flour (starch) granules, Talcum powder	Freeze drying	Stability (4 °C): Talcum powder (14 week)-stable and 100% viable Granular forms (10 week)- stable and 100% viable Alginate beads (12 and 24 week)- less stable Disease suppression (tomato damping-off): Talcum formulation-90% Alginate beads-30% Starch granules-22%	Sabaratnam and Traquair (2002)
Beauveria brongniartii, Metarhizium anisopliae	Skim milk and polyvinylpyrrolidone (PVPK90)	Freeze drying	Viability: *B. brongniartii*: 68% *M. anisopliae*: 4%	Horaczek and Viernstein (2004)
Azospirillum brasilense Cd	Na-alginate (wet and dry beads) Na-alginate and skim milk (wet and dry beads)	Freeze drying	Microbead diameter 10–20 m Some bacteria killed during microbead formation Enhanced growth of wheat and tomato seedlings in unfertile soil	Bashan et al. (2002)

6.4 Encapsulation of Plant Growth Promoting Microorganisms

There have been several studies for encapsulation of plant growth promoting microorganisms and many of them have resulted favourable outcomes (Table 6.3). A method for encapsulation of potential biocontrol agents like – ascospores or conidia of *Talaromyces flavus* (Tf1/Tf-I), *Gliocladium virens* (GL3), *Penicillium oxalicum* or *Trichoderma viridae* (T-1-R9) or cells of *Pseudomonas cepacia* (POP-SI) by mixing with a solution containing sodium alginate (1%) and Pyrax (1%) followed by dripping into a solution of $CaCl_2$(0.25 M) or Ca-gluconate (0.1 M) was attempted by Fravel et al. (1985). It was observed that all strains of fungi but not *Pseudomonas cepacia* (POP-SI) remained viable after forming pellet in $CaCl_2$ and after drying. However, all fungal and bacterial strains were able to retain their viability in Ca-gluconate for a longer time period after pellet formation.

In another study, sodium alginate along with wheat bran, a food carrier base was used for encapsulation of 11 isolates of *Trichoderma spp.* and *Gliocladium virens* to check their biocontrol efficacy against *Rhizoctonia solani* infected seeds of beet in soil (Lewis and Papavizas 1987). The biocontrol activity of isolates was tested in 6 different soils. All the isolates were effective against the pathogen in natural soil. It was found that eight isolates of *Trichoderma spp* were effective in reducing the survival of *R. solani* by 34–78%. Most effective strains were *T. harzianum* (Th-58) and *T. hamatum* (TRI-4). *Trichoderma* isolate TRI-4 was highly effective against the pathogen in all 6 soils (>70%) and against 6 *R. solani* isolates in loamy sand. A minute amount of biomass of isolates showed efficacy comparable to very large biomass. However, the effectivity of all the formulations was reduced after 6 weeks of storage at 5° or 25 °C.

Sodium alginate was used for formulation of *Erynia neoaphidis*, a pathogenic fungus of aphid pests. It was observed that the optimal concentration of sodium alginate for effective encapsulation of fungal mycelium was 1.5%. 0.1 M and 0.25 M $CaCl_2$ were found to be equally efficient as gelling agents. Freshly produced alginate beads with fungal conidia showed an infectivity of 27–32% in aphids of pea. However, the performance did not differ significantly from fresh mats of mycelia or plugs from petri dish cultures. A reduction in survival (63–97%) of conidia was observed after drying and storage of beads in comparison to freshly prepared beads (Shah et al. 1998). In further studied the factors involved in production of alginate granules of *Erynia neoaphidis*. Granules were formed by entrapment of fungal mycelia in alginate polymer. It was found that addition of sucrose, potato starch or chitin to alginate significantly improved conidia production from granules (Shah et al. 2010). The performance of alginate pellets of entomopathogenic fungus *Beauveria bassiana* was evaluated for biocontrol of *Solenopsis invicta* (Red Imported Fire Ant) under field conditions (Bextine and Thorvilson 2002).

Many commercial formulations of biocontrol, biopesticide and biofertilizer agents have been prescribed by several researchers are in different plants (Table 6.4). A comparison of the performance of sodium alginate beads of mycoherbicide

Table 6.3 Encapsulation of microbes used in agriculture

Formulation	Microbe used	Results	Reference
Alginate-glycerol-kaolin	*Pantoea agglomerans, Trichoderma harzianum*	Increased shelf life. Protection from UV-C radiation.	Nussinovitch (2016)
Alginate-humic acid	*Bacillus subtilis* CC-pg104	Increased cell viability. Storage till 5 months without much cell loss. Successful growth promotion of lettuce.	Young et al. (2006)
CM-cellulose/xanthan	*B. subtilis*	Bacterial release efficiency: Xanthan: 90.2% CM-cellulose: 76.6% Xanthan formulation showed better biocontrol activity against *Meloidogyne* incognita, Xanthan beads inoculated tomato plants showed decreased galls	Pacheco-Anguirre et al. (2016)
Na-alginate-bentonite	*Pseudomonas putida* Rs-198	Better survival than non-encapsulated cells.	Li et al. (2017)
Na-alginate (2–3% w/w)	*Bacillus thuringiensis* sub sp. *kurstaki* (Bt-KD2)	70% spore viability.	Khorramvatan et al. (2017)
Na-bentonite and alginate	*Raoultella planticola* Rs-2	100% encapsulation efficiency. Survival rate of 81% at 4 °C and 88.9% at room temperature. Increased survival during drying. Increased stability during storage.	He et al. (2015)
Na-alginate	*Klebsiella oxytoca* Rs-5	High degree of root colonization. Increased survival rate. Increased retention time. Relieves salt stress of cotton seeds.	Wu et al. (2013)
Na-alginate and starch	*Azospirillum brasilense*	76% viability after one year storage.	Schoebitz et al. (2012)
CM-cellulose, corn, starch, potato starch, autoclaved baker's yeast	*Metarhizium brunneum*	Max. Survival 82%.	Przyklenk et al. (2017)
Chitosan	*Rhizobium, Azotobacter, Azospirillum*	Increased plant growth.	Namasivayam et al. (2014)

(continued)

Table 6.3 (continued)

Formulation	Microbe used	Results	Reference
Alginate, bentonite, skim milk	*Pseudomonas fluorescens*	Increased colonization in soils. Better survival. Less sensitivity to dry/wet fluctuations in soils. Drying beads resulted in reduced survival than moist beads. Moist beads colonized wheat roots after 63 days.	Trevors et al. (1993)
Na-alginate (wet and dry beads) Na-alginate and skim milk (wet and dry beads)	*Azospirillum brasilense* Cd	Microbead diameter 10–20 m. Some bacteria killed during micro-bead formation. Enhanced growth of wheat and tomato seedlings in unfertile soil.	Bashan et al. (2002)
Na-alginate (2–4%) Agarose Polyurethane	*Flavobacterium sp.* (ATCC 39723)	All three formulations showed capacity of Pentachlorophenol degradation. All encapsulated cells showed stability upon storage at 4 °C and retained biodegradable activity.	Stormo and Crawford (1992)
Na-alginate	*Glomus versiforme*	Encapsulated spores able to germinate and retained ability to infect plant roots.	Declerck et al. (1996)
Na-Alginate prills (0.2%)	*Trichoderma koningii* (biocontrol to phytopathogens)	*T. koningii* alginate prills+wheat bran (2 g) remained activity on 2-year storage at 5 °C.	Mónaco and Rollán (1999)

Alternaria cassia with kaolin or corn cob as filler material and fermentation medium with or without Potato dextrose broth was attempted. It was observed that in case of un-supplemented fermentation medium alginate beads with Corn cob grits filler materials performed better in terms number of spores than kaolin alginate beads. Using fermentation media added with Potato dextrose broth enhanced spore production in both the cases. Potato dextrose broth and corn cob grits act as nutrient source for encapsulated mycelia, accelerating spore production. Therefore, a higher spore yield was observed when corn cob grits were used as fillers for alginate beads and the yields improved when corn cob grits were supplemented with Potato dextrose broth (Daigle and Cotty 1992).

Studies were undertaken to evaluate appropriate concentration of chitin with Na alginate to be used for effective encapsulation of *Beauveria bassiana*. Among the different concentrations of chitin used with or without wheat bran, three times increase in conidia production was observed with 2% chitin and 2% wheat bran upon 21 days storage. It was observed that increasing chitin content of alginate

Table 6.4 Commercially-used biocontrol, biopesticide and biofertilizer agents

Name	Microbe	Form	Application	Use	Plant	Reference
Mycostop®	*Streptomyces griseoviridis*	Wettable powder	Seed dressing	Biocontrol of *Fusarium* and *Alternaria*	Crucifers, carnations	Sabaratnam and Traquair (2002) and White et al. (1990)
Galtrol®	*Agrobacterium radiobacter*	Agar plate culture of bacteria	Plant dip treatment as water suspension: spray, root dip, root drench	Control of crown gall disease caused by *Agrobacterium tumefaciens*	Woody plants	Kerr (1989)
Quantum 4000®	*Bacillus subtilis*	Wettable powder	Seed dressing			Connick Jr. et al. (1990)
Dagger-G®	*Pseudomonas fluorescens*	Wettable powder	Seed dressing			Currier et al. (1988)
Blue Circle®	*Pseudomonas cepacia*	Wettable powder	Seed dressing			McLoughlin et al. (1992)
BINAB-TW®	*Trichoderma harzianum*	Granular/solid (alginate beads)		Control of soil-borne pathogenic fungi	Ornamentals and vegetables	Lumsden et al. (1995)
GlioGard®	*G. virens*	Granular/solid (alginate beads)		Control of soil-borne pathogenic fungi	Ornamentals and vegetables	Lumsden et al. (1995)
Lipel ™	*Bacillus thuringiensis* var. kurstaki	Wettable powder (nutrient medium, residues, sodium chloride, dextrose, spores, endotoxin)	Slurry in water: Foliar spray Dry powder: Foliar dusting	Biopesticide		Agri Life, India
Bionemagon ™	*Bacillus firmus* strain NCIM 2673	Wettable powder (Kaolin spores)	Dry: Soil amendment Mixture of water+powder (filtered and decanted): Drip irrigation	Biopesticide		Agri Life, India

(continued)

Table 6.4 (continued)

Name	Microbe	Form	Application	Use	Plant	Reference
Sheathguard™	*Pseudomonas fluorescens* strain IIHR-PF2	Wettable powder (Carboxymethyl cellulose, talc, cells)	Slurry in water+sugar: seed coating Dry: nursery bed treatment, soil application and compost enrichment	Biopesticide		Agri Life, India
Serenade® Opti	*Bacillus subtilis* QST713	Wettable powder	Dilution with water: Foliar spray, soil drench	Biopesticide		Bayer Crop Science LP, USA
FZB24® TB	*Bacillus amyloliquefaciens ssp. plantarum*	Dry powder (talcum, corn starch, skim-milk powder, glycerol, spores (lyophilized)	Dry treatment of seeds and tubers, soil amendment	Biofertilizer		ABiTEP GmbH, Germany
FZB24® WG	*Bacillus amyloliquefaciens ssp. plantarum*	Wettable powder (corn starch, skim-milk powder, glycerol, spores (lyophilized)	Slurry with water: tuber treatment, seed treatment, soil drench	Biofertilizer		ABiTEP GmbH, Germany
NITROFIX™ – AC	*Azotobacter chroococcum* strain MTCC 3853	Wettable powder (kaolin, dextrose, lignite, spores)	Slurry with water+ sugar: seed coating Slurry with water+manure: Seedling root dip Mix with compost: soil amendment Add to irrigation stream: soil drench	Biofertilizer		Agri Life, India

pellet decreased conidial numbers. However, using wheat bran with any concentration of chitin resulted in increased number of conidia. Also, chitin incorporation in alginate pellet reduced saprophytic fungal contamination (González et al. 2007).

Testing of Sunflower oil, Groundnut oil and talc for encapsulation of entomopathogenic fungus *Lecanicillium lecanii*. The three basic carriers (Sunflower oil, Groundnut oil), talc was evaluated independently as well as in composition with chitin and chitosan. The most suitable proportion of carrier material: technical ingredient and viability were evaluated by using CFU (Colony forming units) count of the formulations during 3 months storage period. It was observed that enrichments of both Groundnut Oil and Sunflower Oil with chitin exhibited best viability and thus, were found to be best carriers for fungal encapsulation (Nithya and Rani 2017).

Encapsulation of Na-alginate granules *Trichoderma hamatum* for biocontrol of *R. solani* colonization along with wheat bran, rice straw, oat bran, eucalyptus leaves and corn meal were attempted. Their addition was found to reduce the *R. solani* saprophytic activity and maintained 100% viability after 3 months storage. Wheat bran was found to be the most effective (Mafia et al. 2003). Alginate encapsulated chlamydospores of *Trichoderma spp.* and *Gliocladium virens* with bran as bulking agent showed a higher survival and viability than alginate encapsulations of conidia and kaolin bulking agent in soil (Lewis and Papavizas 1985).

Application of the encapsulated *Trichoderma hamatum* (TRI-4) and *Gliocladium virens* (GI-3, GI-21, GI-32) for biocontrol of *R. solani* damping-off of eggplant led to a decrease in saprophytic growth of pathogen. It was effective in a reduction in post-emergence damping-off in other plants including cucumber and pepper seedlings (Lewis et al. 1998). However, A biocontrol formulation comprising of *Cladorrhinum foecundissimumto* consisting of bran, alginate prills and flour/clay was found effective for damping off pathogen control in eggplant and pepper (Lewis and Larkin 1998).

Material like wheat bran, rice straw, oat bran, eucalyptus leaves and corn meal were employed to encapsulate Na-alginate granules. These formulations showed 100% viability and it was observed that addition of food sources to Na-alginate reduced the saprophytic activity of *R. solani* (Mafia et al. 2003). Higher survival was observed in the alginate encapsulated chlamydospores of *Trichoderma* spp. and *Gliocladium* (Lewis and Papavizas 1985). Application of formulations having encapsulated *Trichoderma hamatum* (TR 4) and *Gliocladium virens* (GI 3, GI 21, GI 32) using Na alginate and Biodac (cellulose) on soilless mix showed reduction in the disease of eggplant and decreased saprophytic growth of *R. solani* (Lewis et al. 1998).

Further, formulations of *Cladorrhinum foecundissimumto* developed using bran could successfully reduce the disease and plant stands produced were comparable to those in non-infected control plants after 4 weeks (Lewis and Larkin 1998). Entrapment of the wet biomass of *Trichoderma viridae* in gluten matrix yielded 10^6–10^7 CFU/g soil and was more effective at lower dose as compared to non-encapsulated counterparts (Chen-Fu and Wen-Chien 1999). In the second week after inoculation, the formulations produced a biomass of 10^6–10^7 CFU/g soil.

Different food bases additives wheat bran, corn cobs, peanut hulls, soy fiber, castor pomace, cocoa hulls and chitin for encapsulation of *Gliocladium virens* and *Trichoderma spp*. were evaluated and found to be effective against damping-off. All combinations of alginate prills could perform well in soil against damping-off of cotton plants (Lewis et al. 1996). They also believed that the biocontrol effect of the formulation depended on the intrinsic activity of the isolate used.

In order to use alginate prills, organic carriers were used for encapsulation of *Talaromyces flavus* for biocontrol of *Verticillium dahlia* causing wilt in eggplant (Fravel et al. 1995). The results of green house experimentation using three different soils (two loamy sands and silt clay) revealed their suitability as an efficient carrier for an effective delivery of the bioformulation.

Encapsulated biocontrol agents *Bacillus subtilis* and *Pseudomonas putida* against the root rot pathogen *Pythium aphanidermatum* and *Fusarium oxysporum* f.sp. *cucurbitacearum* were able to survive over a range of temperature up to 45 days. Immobilization using materials like kaolin clay, vermiculite, bacterial broth carriers showed more population than other carriers. Vermiculite, peat moss, wheat bran, bacterial broth carriers were found to be best for survival and population growth of *P. putida* (Amer and Utkhede 2000). Use of alginate with or without wheat bran for encapsulation of *Beauveria bassiana* for biocontrol of green bug (*Schizaphis graminum*) infecting wheat crop was undertaken and superiority of alginate-wheat bran combination was observed in terms of improved shelf life (Knudsen et al. 1990).

Jain et al. (2010) studied the efficacy of phosphate solubilising activity of fungus *Aspergillus awamori* using two different polymers for encapsulation- Ca-alginate and agar. Two types of insoluble phosphates were used namely, Udaipur Rock Phosphate and Tri-Calcium Phosphate. When the three formulations were compared, Agar encapsulation showed maximum solubilisation of Udaipur Rock Phosphate followed by Ca-alginate and un-encapsulated (free) cells, while Ca-alginate encapsulation showed maximum solubilisation of Tri-Calcium Phosphate as compared to agar and free cell formulations.

In addition to biocontrol agents the technique of encapsulation is also widely applied to biofertilizers like phosphate solubilising and nitrogen fixing bacteria and fungi. Bioencapsulation of nitrogen fixing *Azospirillum* by formulation of alginate (3%) standard starch (44.6%) and modified starch (2.4%) to yield biodegradable formulations which were characterized with high viability (Ivanova et al. 2005). Similar advantage of encapsulation of P-solubilizing bacterium *Enterobacter* using alginate combined with skim milk (Vassileva et al. 1999) was observed. It was recorded that encapsulated bacteria could induce better growth in plants than non-encapsulated bacteria. The alginate-skim milk beads also enhanced plant growth. Similar observations pertaining to the use of alginate along with skim milk as the matrix for the encapsulation of phosphate solubilising rhizobacterial strains *Pseudomonas fluorescens* BAM-4 and *Burkholderia cepacia* has been found successful (Minaxi and Saxena 2011). Hence, it is concluded that alginate is best polymer with easy application and low cost for development of N-fixing biofertilizers.

6.5 Conclusion

In this article, various techniques available for encapsulation of microorganisms for various purposes have been discussed. The variety of naturally obtained biodegradable materials as well as artificially synthesised polymers is available. These can be used individually or in combinations optimized in proportions for making of biological formulations providing best possible yields and their viability and activity. The methods available for production of microbeads or capsules containing biological agents are used keeping in mind the suitability of the organism to be immobilized. Much research has been done in encapsulation of biofertilizers and biocontrols for agricultural delivery. Alginate is the most widely used biomaterial in combination with various other natural or artificial materials for coating. Another most widely used polymer matrix for microbial encapsulation is Carboxy-methyl cellulose.

These two polymers are used with a number of coating materials including starch, wheat bran, talc, bentonite, skim milk etc. It has been observed that supplementing of main carrier matrix with wheat bran, corn starch, talc or peat significantly enhance the performance of the formulations. Spray drying and freeze drying are the primary methods for drying of the capsules. More biodegradable and cheaper materials need to be explored for encapsulation of biofertilizers and biocontrols. A major challenge is the loss of viability of most formulations during drying phase and storage. However, research conducted over the years has shown that encapsulated microbes are superior to their non-encapsulated counterparts in terms of all the parameters like viability, shelf life, survival, activity and efficiency.

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