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Activity and Survival of Spray-Dried *Beijerinckia* sp. Microencapsulated in Different Carbohydrates

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Abstract

This study examined the possibility of preserving *Beijerinckia* cultures by encapsulation using a spray drier, for use in biotechnological processes in the production of biopolymers. An adequate choice of the wall (coating) material is one of the factors that will determine the degree of cell survival and the maintenance of fermentative activity in the encapsulated inoculum. Malt dextrin, dehydrated glucose syrups, modified starch, and acacia (gum arabic) were used as wall materials. The results showed that spray-dried *Beijerinckia* encapsulated in malt dextrin, stored for 2 mo, and inoculated into sterile must after rehydration presented the greatest stability with respect to fermentative activity, although the glucose-encapsulated cells showed the highest percentage of viability during spray drying and during the storage period.

Index Entries: Fermentation; spray drying; *Beijerinckia*; encapsulation; biopolymer.

Introduction

Microbial polysaccharides have been compared with traditional plant polysaccharides. The advantages of microbial polysaccharides are that they are new, that they possess constant functionality and reproducible chemical and physical properties, and that their cost and supply are stable. They offer a potentially new source of functional biopolymers for food, industrial, and medical applications. Bacterial polysaccharides are incorporated

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into food as thickeners or suspending or gelling agents, in order to improve food quality and texture. They are also used in metal recovery, water clarification, and oil well drilling *(1)*.

Although exopolysaccharides are produced by a large number of microorganisms, reports on the *Beijerinckia* species are very scarce. Pure culture inoculation of broth is not always a convenient means of inducing polysaccharide fermentation on a commercial scale because it is difficult to prepare a consistent inoculum with high fermenting activity. A new approach to this problem is the use of dried bacteria as a starter. The development of dried, concentrated cultures for inducing gum fermentations could eliminate many of the problems customarily involved in the preparation and maintenance of starter cultures in the industrial plant. According to Teixeira et al. *(2)*, culture concentrates can be evaluated and standardized for activity before shipment to the processor, making it possible to produce consistently high-quality products. Dried preparations have the advantages of long-term preservation and convenience in handling, storage, marketing, and consumption.

Bacterial cells are likely to lose their viability and activity (gum-producing capacity) during drying and the subsequent storage period. The choice of an appropriate suspending medium may be the most important factor in increasing the survival rate and activity of the microorganisms during drying and subsequent storage. The use of spray-dried cultures may be promising, having similar advantages to freeze-dried cultures, but much less costly to produce. However, special attention must be given to the operation of the spray dryer in order to provide viable cultures with rapid gum production on rehydration. This requires the use of mechanisms to minimize cellular injury during spray drying. Reports found in the literature show that the loss of cell viability during spray drying may be on the order of three logarithmic cycles *(3)*.

The amount of water remaining after drying affects not only the viability of the bacteria, as determined immediately after the process, but also the rate of loss of viability during subsequent storage *(4)*. The physiologic reactions of organisms depend on the mobility of the bound water, not of the free water. The optimum residual moisture content varies with the composition of the fluid in which the organisms are dried, with the storage atmosphere, and with the species and physiologic state of the organisms.

Spray drying can be used as an encapsulation process when it entraps "active" material within a protective matrix or carrier (wall material [WM]) formed from a polymer *(5)*. The encapsulation of viable bacterial cells has several advantages over encapsulation of isolated enzymes. The stability of enzymes in intact cells is greater than in extracts and the production achieved by cells is easily manipulated *(6)*.

Successful microencapsulation is the result of a judicious choice of WM composition for a determined core material, and the result of a well-managed process design. The carbohydrates, owing to their diversity, low cost, and widespread use in foods, have become the preferred choice

for encapsulation. Sugars, malt dextrin, modified starches, and gums are all used as WMs *(7)*.

The objectives of the present study were to determine the effect of different carbohydrates on cell survival and gum production during spray drying and the subsequent storage period.

Materials and Methods

Foods

Malt dextrin and dehydrated glucose syrups (Corn Products International, Mogi Guaçu, Brazil), modified starch (Capsul®, National Starch, São Paulo, Brazil), and acacia (Spray Gum®; Colloids Naturels Brasil Commercial Ltda., São Paulo, Brazil were used). All materials were commercial food grade.

Organism

Beijerinckia sp. was isolated from sugarcane roots, maintained at 4°C on YM agar slants, and transferred every 30 d according to Muro and Luchi *(8)*.

Preculture and Production of Cell Concentrates

Beijerinckia sp. was grown in shaker flasks containing YM medium. The medium was adjusted to pH 6.5 and sterilized at 121°C for 15 min. The preculture was inoculated with two loops of culture from a slant and incubated at 25°C and 200 rpm for 24 h in a New Brunswick model G25 shaker. This preculture was then used to inoculate a second broth (5% [w/v]). The second broth contained sugarcane molasses (1.5% [w/v]) and brewer's yeast autolysate (2.0% [w/v]) *(9)*. All fermentation broths were made using the same lot numbers of components. The cultures were incubated at 25°C and 200 rpm for 18 h. Stationary-phase cells were harvested by centrifugation (22,300*g* for 15 min at 5°C) and washed twice with sterile distilled water.

Spray-Drying Experiments

A cell pellet from 4 L of culture was resuspended in 390 mL of a sterile solution of 10% (w/v) skim milk in accordance with Mary et al. *(10)*. This medium was aseptically supplemented with 15% (w/v) nonsterilized WM. Bacterial suspensions (450 mL) were incubated at 25°C and 100 rpm for 30 min to allow for cell adaptation and then sprayed. In preliminary experiments, this ratio resulted in a suspension with a total viable count of $\geq 10^9$ cells/g. The total solids content was 25% (w/v) based on the dry weight of the bacterial cells. The proportion of the different ingredients in the formulation of the spray-dried cultures was determined from the results of the preliminary trials. Five different WMs were employed: dehydrated glucose syrups, 10 and 20 dextrose equivalent (DE) malt dextrin, Capsul,

and a blend (20 DE malt dextrin and acacia in the ratio of 3:2) according to Reineccius *(11)*. A magnetic stirrer was used for all the bacterial suspensions to maintain a homogeneous feed during drying. The viscosity of the bacterial suspensions was determined with all the WMs.

A LabPlant model SD-04 (Leeds, UK) spray dryer was used. The entrance and exit temperatures, liquid flow rate, air pressure, and nozzle diameter were, respectively, 135°C, 75°C, 10 mL/min, 5 kgf/cm^2, and 1 mm. The processing conditions were standardized from the results of the preliminary trials. Microencapsulation of the bacteria in five different WMs was performed in triplicate, maintaining the same drying conditions. The resulting spray-dried bacteria were homogenized and stored separately in 2-g quantities in sealed sterile glass bottles at 4°C. Viability and activity assays were performed on two bottles of each different powder.

Viability assays were carried out on the powders dried under the different test conditions just before drying on the feed solution, after drying, and during storage at 4°C (after 14, 30, and 60 d). Each sample of spray-dried bacteria was tested after rehydration with respect to viability and biologic activity (in terms of fermentative activity). Because the rehydrating conditions were based on the results of the preliminary trials, these gave rehydrated products with a total viable count between 10^7 and 10^8 cells/mL, a level of inoculum comparable with that obtained with the traditional loop transfer in the preculture method, according to Kidby et al. *(12)*. For all encapsulated materials, 1 g of spray-dried bacteria was rehydrated at 25°C and 100 rpm for 24 h in a shaker in 10 mL of a sterile solution of skim milk (10% [w/v]). One milliliter of the cell suspension was then subjected to serial dilutions with a sterile solution of NaCl (0.85% [w/v]) before transferring to YM agar plates and incubating at 25°C for 36 h. The total viable cell number was expressed as colony-forming units per gram of dry powder. Survival was defined as the ratio of viable cells before and after drying at each storage time, converted to the base 10 logarithm ($\log N_o/N$). Physicochemical analyses of the encapsulated samples included sugar and moisture contents and water activity.

Assay for Fermentative Activity

A 1-g sample of powder was rehydrated as described in the previous section and inoculated into sterile gum production medium in a ratio of 5% (v/v). The broth containing sucrose (1% [w/v]) and brewer's yeast autolysate (1% [w/v]), with the pH previously adjusted to 6.5 prior to sterilization (121°C, 15 min) according to Maldonade *(9)*, was inoculated and incubated in a New Brunswick model G25 shaker at 25°C and 200 rpm for 24 h. The culture broth was centrifuged at 22,300*g* at 5°C for 30 min to remove the cells. The supernatant was kept for substrate (residual sugars) and product (polysaccharide) analysis, as described next.

Analytical Procedures

Determination of Dry Cell Weights

Microbial dry weights were monitored gravimetrically. The organisms collected by centrifugation were resuspended in distilled water to remove undesired soluble material and recentrifuged. The final pellet was suspended in water again and transferred to a preweighed Pyrex dish that was dried to constant weight at 105°C. The results were expressed in biomass of concentration (grams of dry cell mass per liter of substrate).

The dry weight of spray-dried *Beijerinckia* sp., microencapsulated in different carbohydrates, was determined in the following manner. The preweighed microencapsulated cells were washed with distilled water (in a ratio of 1 g of powder/200 mL of water), to remove undesirable soluble material, and centrifuged at 22,300*g* at 5°C for 30 min. The resulting pellet was dried to constant weight at 105°C. All powders were analyzed twice. The supernatant was kept for sugar analyses as described later.

Polysaccharide Concentration

Polysaccharide concentration was estimated using gravimetric analysis. The supernatant remaining after centrifuging the bacterial cells was precipitated with 4 vol of ethanol. The precipitates were washed twice with ethanol. The precipitated gum was dried at 45°C to constant weight, and the results were expressed as grams of gum per liter. The yield of gum was reported as grams of gum per gram of sugar consumed.

Sugar Concentration

Sugar concentration in the cell-free broth and substrate was measured using the dinitrosalicylic acid method *(13)* modified according to Giordano *(14)* and expressed as grams of sugar consumed (initial sugar – residual sugar) per liter.

Moisture Content and Water Activity

Moisture content was determined by drying in a vacuum oven at 100°C and 0.6 atm, to constant weight. Water activity was determined in an Aqualab Mod CX2 (Decagon, Pullman, WA) at 25°C.

Statistical Analyses

Analysis of variance (ANOVA) by orthogonal polynomial regression was applied to the data for each variable, in order to verify if the bacteria encapsulated in different WMs behaved distinctly with respect to the following variables: viability (log N_o/N) and fermentative activity (concentration of cell and polysaccharide mass, biopolymer yield and sugar consumption) during storage at the different time intervals (periods). Subsequently a group ANOVA was carried out, combining the five individual experiments into a single analysis. The Statistics Analyses Systems *(15)* software was used for the ANOVAs. All the experiments were performed in triplicate.

Results and Discussion

Viability

The experimental results for the survival of spray-dried *Beijerinckia* sp. during encapsulation in different WMs and during storage for various times (0, 14, 30, and 60 d) at room temperature and a_w are provided in Tables 1 and 2, respectively.

The total number of viable bacteria decreased during spray drying and storage in the different WMs. From the results (Table 2) it can be seen that the WM DE 20 malt dextrin resulted in a greater retention of viability of the bacteria during storage. The decrease in viable cell counts of *Beijerinckia* sp. in the different powders varied according to the WM. Such a varied behavior of the different powders could be attributed to the effect of the original chemical composition and/or their interaction products.

ANOVA of the log reduction in viability (N_o/N) of the bacteria encapsulated in each WM for the different storage periods (days) showed that the F test was significant at the 1% level of probability for WM1, WM2, WM3, WM4, and WM5. Thus, we rejected the null hypothesis (H_o) and showed that the storage period influenced the dependant variable $\log(N_o/N)$ for all the WMs used, the percentage of variation being distinctly explained as a function of the WM used. From the coefficients of determination (R^2), it was shown that the contribution of the linear model to the explanation of the phenomenon for WM1, WM2, WM3, WM4, and WM5 was 96.32, 94.55, 67.0, 94.20, and 74.35%, respectively. The regression equations, in which Yn is $\log(N_o/N)$ of the WMn and x is the storage time, were as follows:

$$Y1 = 0.377048 + 0.04389x$$

$$Y2 = 1.4199 + 0.048273x$$

$$Y3 = 0.767015 + 0.006653x$$

$$Y4 = 0.892758 + 0.012266x$$

$$Y5 = 1.5126 + 0.011727x$$

The group ANOVA of the log reduction in viability (N_o/N) revealed that the interaction ($WM \times P$) was significant at the 1% level of probability, indicating that the storage period had an influence on the viability of the encapsulated bacteria as a function of the WM used. The solution found was to consider the analyses individually, i.e., for each WM.

Substantial differences in survival during spray drying and the shelf life were observed for the different carbohydrates. During spray drying, the maximum survival was obtained for bacteria encapsulated with 10 DE malt dextrin, followed, in decreasing order, by glucose, 20 DE malt dextrin, the blend, and Capsul. The average water activity (a_w) of the spray-dried products, in the same order, was 0.252, 0.321, 0.349, 0.231, and 0.431, respectively. However, at the end of 2 mo, the bacteria encapsulated in glucose showed a greater level of survival, which was followed, in decreasing order,

Table 1
Powder Analyses and Survival of *Beijerinckia* sp. During Spray Drying in Different WMs

WM	Moisture content (%)	Water activity (a_w)	No. of viable cells (CFU/g solids)		Log reduction of viable cells (log[N_o/N])
			Before drying	After drying	
10 DE Malt dextrin (WM1)	3.19	0.253	1.25×10^9	8.4×10^8	0.17
	3.20	0.246	1.40×10^9	8.2×10^8	0.23
	3.17	0.259	1.28×10^9	8.8×10^8	0.16
Capsul (WM2)	3.35	0.428	1.19×10^9	2.55×10^7	1.67
	3.37	0.431	1.45×10^9	4.0×10^7	1.56
	3.38	0.434	1.26×10^9	3.3×10^7	1.58
Glucose (WM3)	2.66	0.320	1.83×10^9	4.0×10^8	0.66
	2.62	0.323	1.75×10^9	4.3×10^8	0.61
	2.60	0.322	1.12×10^9	5.0×10^8	0.63
20 DE Malt dextrin (WM4)	3.25	0.349	1.58×10^9	1.96×10^8	0.91
	3.23	0.350	1.48×10^9	1.60×10^8	0.97
	3.26	0.347	1.26×10^9	1.31×10^8	0.98
Blend (gum + 20 DE malt dextrin) (WM5)	3.41	0.229	2.7×10^9	8.8×10^7	1.47
	3.46	0.232	1.3×10^9	9.5×10^7	1.14
	3.45	0.233	12.3×10^9	9.0×10^7	1.41

Table 2
Effect of WM on Survival of *Beijerinckia* sp. During Storage

	Moisture content (%)		Log reduction of viable cells after storage (log[N_o/N])			
	Storage period (d)		Storage period (d)			
WM	0	60	0	14	30	60
10 DE Malt dextrin	3.19	3.47	0.17	1.00	1.89	2.95
	3.20	3.49	0.23	1.08	1.93	2.84
	3.17	3.37	0.16	1.08	2.12	2.77
Capsul	3.35	3.75	1.67	2.18	2.41	5.40
	3.37	3.76	1.56	2.17	2.44	5.57
	3.38	3.79	1.58	2.07	2.45	5.70
Glucose	2.65	2.86	0.66	0.95	1.07	1.07
	2.62	2.81	0.61	0.95	0.99	1.13
	2.60	2.79	0.63	1.06	1.06	1.12
20 DE Malt dextrin	3.25	3.64	0.91	1.21	1.37	1.62
	3.20	3.60	0.97	1.07	1.31	1.75
	3.21	3.55	0.98	1.02	1.25	1.52
Blend (gum +	3.41	3.73	1.47	1.85	2.17	2.26
20 DE malt dextrin)	3.46	3.81	1.14	1.73	1.90	1.88
	3.45	3.89	1.41	1.73	2.13	2.14

by 20 DE malt dextrin, the blend, 10 DE malt dextrin, and Capsul. The experiments showed that the survival rates were not linearly related to a_w.

It is interesting to find ways to store microbial cultures with a high retention of viability for use as starter cultures. The "available" water for the growth of microorganisms differs considerably depending on the solute. When glucose was used as the WM, an a_w reading of 0.321 was obtained with only 2.6% moisture content, whereas with Capsul, an a_w of 0.431 was obtained with 3.3% moisture content. However, 20 DE malt dextrin and the blend produced powders with similar a_w values but different degrees of survival, in agreement with Hahn-Hägerdal *(16)*, who suggested that they probably influenced the biologic reactions in different ways, resulting in distinct levels of survival.

Amiet-Charpentier et al. *(17)* obtained similar results microencapsulating *rhizobacteria* in modified starch, but these did not survive more than a week. The maximum bacterial survival at the end of 2 mo occurred in powders with moisture contents below 3.5%, in agreement with Prajapati et al. *(18)*.

Moisture content is an important parameter in the stability of dried cultures. Microbial biomass contains 70–90% water. Biopolymers and membranes are dispersed in a water medium, and life takes place only in the presence of water. Water is a structural component of both biopolymers

and biomembranes. In addition, water as a substance is directly involved in a number of biochemical reactions. The term *bound water* is understood as that part of the intracellular water that combines directly with proteins, nucleic acids, membranes, or other substances and that is responsible for maintaining their structural organization *(19)*.

The studies reported here indicated that the loss of cellular viability appeared to be related to damage to the following cell components: cell membrane, cell wall, and DNA. In the cell membrane, it is believed that the loss of water-hydrated layers causes disorientation of the phospholipid molecules, at least in some membrane parts. Under these conditions, membrane lipids may change their configuration from lamellar to hexagonal. Such disorientation of the phospholipids in the membranes of dried organisms increases the danger of irreversible damage under these conditions. This may be owing to oxygen leakage and the presence of oxidizing reactions, with peroxidation of the lipids and with the presence of phospholipases and enzymatic degradation of the main biomembrane components. A consequence of membrane phospholipid disorientation is the destruction of the biomembrane barrier function, increasing permeability *(19)*.

A comparison of glucose and malt dextrin showed that the low DE malt dextrin and glucose showed a greater protective effect of the cells against damage during spray drying, although greater protection (survival) was observed in the higher DE malt dextrin during storage. Studies with encapsulated orange peel and trans-β-carotene in malt dextrin showed that increasing the DE extended the shelf life of the encapsulated compound, probably because a more dense, oxygen-impermeable matrix was formed *(20)*. The presence of glucose in the encapsulation system had a considerable effect owing to its antioxidant properties *(6)*. However, products encapsulated in the higher DE malt dextrin could affect the survival of cells during spray drying because of their higher hygroscopicity *(21)*. The greater the hygroscopicity of the powder, the longer it tends to stay in the drying chamber, so the drying process is relatively slower, increasing the thermal inactivation of the cells, according to Solano et al. *(3)*.

Fermentative Activity

The effects of the different types of WM on the fermentative activity of *Beijerinckia* sp. at the different storage times are provided in Table 3.

Although the average dry cell weight of the spray-dried *Beijerinckia* sp. microencapsulated in the different carbohydrates was 0.0508/g of powder and the SD was 0.007, after rehydration the initial number of viable cells in the inoculum differed according to the type of carbohydrate and storage days. The entire study was conducted after the reactivation period.

The results of the fermentations (Table 3) on a laboratory scale under similar conditions using inoculae of *Beijerinckia* sp. encapsulated in the different WMs showed that the type of WM directly influenced the bacterial fermentative activity, stored for different periods of time, with respect to sugar consumption, cell growth, and gum production.

Table 3
Fermentative Activity of *Beijerinckia* sp.
Encapsulated in Different Carbohydrates During Storage

Period	WM	Block	Dry mass (g/L)	Yield	Sugar consumed (g/L)	Concentration of polysaccharides (g/L)
0	1	1	2.02	0.42	11.25	4.70
0	1	2	2.07	0.46	11.75	5.41
0	1	3	2.14	0.50	13.70	6.85
0	2	1	1.37	0.40	7.85	3.14
0	2	2	1,57	0.45	8.25	3.71
0	2	3	1.55	0.47	8.15	3.83
0	3	1	1.95	0.17	6.15	1.04
0	3	2	1.55	0.22	7.95	1.75
0	3	3	2.34	0.26	9.30	2.42
0	4	1	3.50	0.42	12.10	5.05
0	4	2	3.86	0.45	10.80	4.86
0	4	3	3.78	0.37	11.15	4.23
0	5	1	2.63	0.37	9.10	3.37
0	5	2	2.57	0.41	8.45	3.46
0	5	3	2.60	0.44	7.65	3.37
14	1	1	1.07	0.43	7.65	3.29
14	1	2	1.15	0.45	7.95	3.58
14	1	3	1.23	0.47	8.40	3.95
14	2	1	1.30	0.26	7.40	1.92
14	2	2	1.33	0.35	8.00	2.80
14	2	3	1.42	0.41	7.85	3.22
14	3	1	0.95	0.22	9.30	2.05
14	3	2	0.85	0.29	12.25	3.55
14	3	3	0.89	0.31	13.15	4.08
14	4	1	4.46	0.44	9.25	5.07
14	4	2	3.00	0.47	10.60	4.98
14	4	3	3.80	0.47	9.75	4.61
14	5	1	2.56	0.33	9.25	3.05
14	5	2	2.54	0.34	8.60	2.92
14	5	3	2.56	0.32	8.25	2.64
30	1	1	0.95	0.40	9.60	3.84
30	1	2	1.05	0.38	9.95	3.78
30	1	3	0.85	0.46	8.45	3.89
30	2	1	1.18	0.26	7.20	1.87
30	2	2	1.08	0.31	7.45	2.31
30	2	3	1.17	0.29	6.95	2.01
30	3	1	0.79	0.39	13.65	5.32
30	3	2	0.71	0.29	12.90	3.74
30	3	3	0.84	0.34	12.20	4.15
30	4	1	2.25	0.50	12.70	6.35
30	4	2	1.95	0.39	11.60	4.52
30	4	3	1.30	0.36	11.00	3.96
30	5	1	2.47	0.33	10.77	3.55

Table 3 *(continued)*

Period	WM	Block	Dry mass (g/L)	Yield	Sugar consumed (g/L)	Concentration of polysaccharides (g/L)
30	5	2	2.50	0.29	11.60	3.36
30	5	3	2.53	0.35	11.00	3.85
60	1	1	0.45	0.40	9.80	3.92
60	1	2	0.50	0.37	9.45	3.50
60	1	3	0.70	0.41	8.90	3.65
60	2	1	0.59	0.26	7.35	1.91
60	2	2	0.55	0.30	7.20	2.16
60	2	3	0.50	0.29	6.75	1.95
60	3	1	0.76	0.43	8.80	4.35
60	3	2	0.67	0.62	11.50	7.13
60	3	3	0.81	0.58	10.35	6.00
60	4	1	1.66	0.55	12.70	6.98
60	4	2	1.45	0.52	11.60	6.03
60	4	3	1.18	0.46	11.00	5.06
60	5	1	2.50	0.30	12.50	3.75
60	5	2	2.46	0.38	11.60	4.41
60	5	3	2.52	0.31	11.30	3.50

According to Becking *(22)*, the main sources of carbon for the cellular growth of *Beijerinckia* sp. are glucose, sucrose, and fructose. Maldonade *(9)* observed that the amount of cell mass produced by *Beijerinckia* sp. during fermentation increased from 1.75 to 4.8 g/L when the glucose concentration was increased from 0.5 to 10.0% with a fixed nitrogen concentration. They also showed that sucrose was a better carbon source than glucose when used at the same concentration.

In fermentations using *Beijerinckia* sp. encapsulated in malt dextrin, there is a great variety of sugars, since, according to Dokic et al. *(23)*, industrially produced malt dextrin normally consists of a broad spectrum of saccharides. Malt dextrin can be produced by acid, acid/enzyme, or double enzyme hydrolysis, and the composition of the malt dextrin is influenced by the type of hydrolysis and conditions used. All fermentation broths were made using the same lot numbers for the components.

After drying, the fermentations carried out with bacteria encapsulated in 20 DE malt dextrin showed relatively greater cell growth, followed, in decreasing order, by the bacteria encapsulated in the blend (gum + 20 DE WM), 10 DE malt dextrin, glucose, and Capsul. According to the results in Table 2, even in the cells that survived 60 d of storage, a gradual decrease in the production of cell mass during fermentation occurred, using bacteria encapsulated in the different WMs. On the other hand, there was a distinct behavior of the encapsulated bacteria during the same period, with respect to polysaccharide yield.

ANOVAs for dry mass produced by the bacteria encapsulated in each WM after the different storage periods showed that the *F* test was significant at the 1% level of probability for WM1, WM2, WM3, WM4, and WM5. Thus, we rejected the null hypothesis (*Ho*) and showed that the storage period distinctly influenced the dependent variable dry weight for all the WMs used. From R^2, it was verified that the contribution of the linear model to the explanation of the phenomenon for WM1, WM2, WM3, WM4, and WM5 corresponded to 82.10, 96.12, 55.0, 82.0, and 80.32%, respectively. The regression equations, in which *Yn* is the weight of dry mass produced by the bacteria encapsulated in *WMn* and *x* is the storage time, were as follows:

$$Y1 = 1.772845 - 0.02274x$$

$$Y2 = 1.551228 - 0.0160x$$

$$Y3 = 1.521483 - 0.0165x$$

$$Y4 = 3.80024 - 0.04299x$$

$$Y5 = 2.581914 - 0.00174x$$

The group ANOVA for dry mass produced by the bacteria encapsulated verified that the interaction ($WM \times P$) showed a significant effect on the variable dry mass at the 1% level of probability, indicating that the storage period has a different effect on the dry mass produced by bacteria encapsulated in each WM. The WM DE 20 malt dextrin conferred relatively greater retention of cell viability and stability with respect to cell activity (production of polysaccharide) during the storage period. The cell growth in the biopolymer-producing medium showed that the encapsulated bacterial cells were capable of regenerating. With respect to the numbers of viable cells, the differences observed between the rehydrated inocula encapsulated in the different WMs after 24 h of incubation remained, owing to the kinetics of distinct growth patterns.

The carbon/nitrogen ratio in the growth medium plays an important role in polysaccharide production. The concentrations of total sugars in the dry weight of spray-dried *Beijerinckia* sp. microencapsulated in glucose, 10 DE malt dextrin, 20 DE malt dextrin, the blend (gum + 20 DE malt dextrin), and Capsul were 88.0, 94.0, 94.0, 70.0, and 94.0 (% [w/w]), respectively (SDs of 0.042, 0.021, 0.015, 0.015, and 0.02, respectively). The initial concentration of total sugars in the medium for the production of biopolymers by fermentation with spray-dried *Beijerinckia* sp. microencapsulated in the different WMs varied from 15.4 to 16.7 g/L (values lower than 0.2% [w/v]) and a fixed nitrogen concentration.

ANOVAs for the amount of sugar consumed by the bacteria encapsulated in each WM at the different storage times (days) revealed that the *F* test was significant at the 1% level of probability for WM1, WM2, and WM5, and for WM3 and WM4 at the 5% level of probability. From R^2, it was verified that the contribution of the linear model to the explanation of the phenomenon for WM2 and WM5 corresponded to 84.15 and 86.63%, respectively,

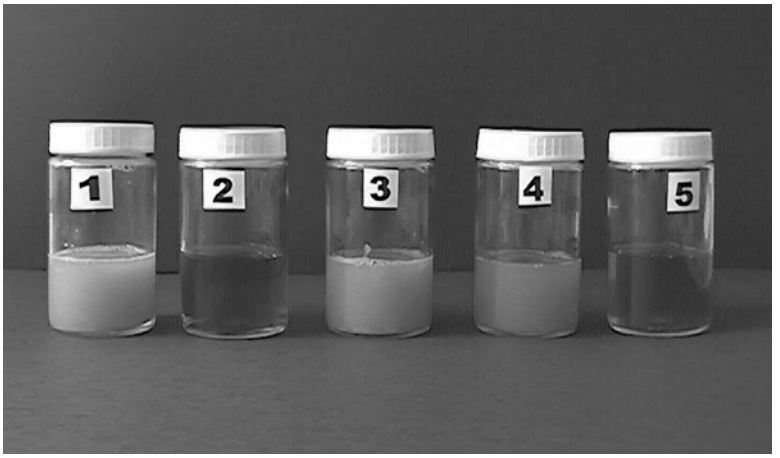

Fig. 1. Cell-free must fermented by cells encapsulated in different WMs: 1, 10 DE malt dextrin; 2, Capsul; 3, glucose; 4, 20 DE malt dextrin; 5, blend (gum arabic + 20 DE malt dextrin).

while the quadratic model contributed 87.73% in the case of WM3. The regression equations, in which Yn is the amount of sugar consumed by the bacteria encapsulated by WMn and x is the storage time, were as follows:

$$Y2 = 7.9636 - 0.001655x$$

$$Y3 = 7.897994 + 0.307863x - 0.004495x^2$$

$$Y5 = 8.401804 + 0.061693x$$

For the bacteria encapsulated in WM1 and WM4, a contribution by the cubic model to the explanation of the sugar consumption during fermentation was verified, although this has no practical justification.

For the group ANOVA, for the amount of sugar consumed by the bacteria encapsulated in each WM at the different storage times (days), it was shown that both the type of WM and the interaction ($WM \times P$) influenced consumption at a 1% level of probability.

Vendruscolo *(24)* showed that the type of sugar influenced the yield and quality of biopolymer produced by *Beijerinckia* sp., with the biopolymer yield using sucrose in the fermentation being 2% greater than that obtained with glucose. The viscosity of the 6% aqueous solution of the biopolymer, measured at 25°C, was greater when sucrose was used in the fermentation.

The cell-free fermented must (Fig. 1) was viscous and cloudy for the 10 DE and 20 DE malt dextrin WMs and for glucose, whereas for Capsul and the blend (20 DE malt dextrin + gum arabic), it was clear and transparent.

The experiments carried out using the blend (20 DE malt dextrin + gum arabic) resulted in inoculum with reasonable stability with respect to cell viability and fermentative activity, although after 24 h of incubation, the characteristics of the polymers produced were distinct, a fact also

observed with the WM Capsul. In the fermentation with *Beijerinckia* sp. encapsulated in malt dextrin, there was a great diversity of sugars, and thus the pattern of polysaccharide production can vary, since, in general, the production of biopolymers depends on the type of microorganism and the composition of the medium.

Different growth phases and alterations of the growth medium, such as by using different substrates and limiting nutrients, do not influence the primary structure but do affect the molecular mass and the yield of gum. Therefore, the biopolymer product recovered from a culture represents a mixture of the gums produced in the different growth phases *(25)*. According to Busta *(26)*, the duration of the lag phase prior to the logarithmic growth phase of the culture varies as a function of the degree of repair required, owing to reversible cellular injuries suffered by the action of the spray drier and subsequent storage. Starter cultures of bacteria prepared by spray drying show a longer lag phase before the onset of growth. Injured cells have extended lag periods before they start to grow, which means that injured cells will take longer to start their desirable activities in fermentations.

According to Casas et al. *(27)*, both xanthan gum concentrations and the average molecular weight of xanthan increase during fermentation, under any conditions, and the molecular weight of a polymer has a direct influence on its rheologic behavior, with larger molecules having higher viscosity. The biopolymer was recuperated from the fermentation liquid after 24 h of incubation by precipitation with ethanol. From the characteristics of the ethanol precipitates, it was shown that the WMs directly influenced the solubility/precipitation of the biopolymers formed after 24 h of incubation. All the fermentation liquids were precipitated using the same lot numbers of solvent and the same conditions.

The growing cultures of *Beijerinckia* entered the stationary phase after about 16–20 h. According to Fu and Etzel *(28)*, after the lag phase the production of metabolites by spray-dried inoculum, obtained under different conditions of spray drying, is equivalent. The bacterial cells encapsulated in Capsul and the blend (gum + malt dextrin) produced polysaccharides with distinct characteristics, probably owing to the relatively longer lag phase. The polysaccharides obtained from the fermentation with bacteria encapsulated in Capsul and in the blend (gum arabic + 20 DE malt dextrin) were recuperated using the following procedure: the polysaccharide was precipitated with ethanol after removing the biomass by centrifugation, dialyzed against water, reprecipitated, and freeze-dried.

ANOVAs for the amount of polysaccharide produced by the bacteria encapsulated in each WM at the different storage periods (days) showed that the *F* test was significant at the 1% level of probability for WM2 and WM3, and at the 5% level of probability for WM1 and WM5. It was not significant for WM4. Thus, the storage period influenced the dependent variable production of biopolymer for the bacteria encapsulated in WM1,

WM2, WM3, and WM5. From R^2, it was shown that the contribution of the linear model to the explanation of the phenomenon for WM2, WM3, and WM5 corresponded to 73.65, 96.35, and 51.30%, respectively, while the quadratic model corresponded to in the case of WM1. The regression equations, in which *Yn* is the production of biopolymer by the bacteria encapsulated in *WMn* and *x* is the storage time, were as follows:

$$Y1 = 5.414213 - 0.10554x + 0.0013x^2$$

$$Y2 = 3.193584 - 0.02402x$$

$$Y3 = 2.077443 - 0.066188x$$

$$Y5 = 3.127105 - 0.011874x$$

In the group ANOVA for the amount of polysaccharide produced by the bacteria encapsulated, it was shown that both the WM and the interaction ($WM \times P$) exerted an influence on the polysaccharide content at a level of 1% probability. ANOVAs for yield of polysaccharide with each WM at the different storage periods revealed that the *F* test was significant at the 1% level of probability for WM1, WM2, and WM3. The null hypothesis (*Ho*) was rejected, and it was verified that the period influenced the dependent variable, yield of biopolymer, for these three WMs. For WM4 and WM5, there was no significant storage period for the variable under study. From R^2, it was shown that the contribution of the linear model to the explanation of the phenomenon for WM1, WM2, and WM3 corresponded to 93.63, 80.10, and 98.20%, respectively. The regression equations were as follows:

$$Y1 = 0.459622 - 0.00117x$$

$$Y2 = 0.399367 - 0.00238x$$

$$Y3 = 0.200803 - 0.005482x$$

The group ANOVA with respect to the yield of biopolymer revealed that both the WM and the interaction ($WM \times$ Storage period) were significant at the 1% level of probability, although the storage period had a distinct effect for each WM.

Conclusion

An alternative method for the maintenance of *Beijerinckia* sp. inoculum is encapsulation with carbohydrates such as malt dextrin or glucose in a spray drier, which presented relatively greater viability and fermentative activity. Encapsulation technology offers new market opportunities for the use of inoculum in the fermentation biopolymer industry, but the total costs of the spray-dried encapsulated powder will vary depending on the WM chosen. Thus, this factor must be one of the determining factors in the choice of WM.

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