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Abstract: A process of microencapsulation of the strain Bacillus pseudofirmus was carried out to stabilize spores and maintain a constant viability. The spray technique was used for evaluating 12 processes in spores encapsulated in five formulations (Caolin, CMC, skimmed milk, maltodextrin, rice flour and diatomaceous earth). The initial viability (%) after the atomization process and the survival rate during storage were evaluated by counting the CFU on the plate. The percentage of viability indicated that the formulation that includes rice flour and maltodextrin obtained the best results for our strain (98.50%) compared to the rest of the formulations, using the process of atomization with the process conditions: Temperature of 110 °C, 5 mL / min of feeding and 100% of aspiration. These results showed that the microencapsulation process performed is efficient for stabilization; Rice flour improves protection in this process and adding maltodextrin generates greater stability during long-term storage.

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Research Data Related to this Submission

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There are no linked research data sets for this submission. The following reason is given:  
Data will be made available on request

Ashok Pandey  
Editor-in-Chief  
Bioresource Technology

June 25, 2019

Dear Editor,

I am writing to submit our manuscript entitled, “Microencapsulation of *Bacillus pseudofirmus* by atomization used for bio-concrete” for consideration as a *Bioresource Technology* Short Communication article.

We highlight the importance of improving the CaCO<sub>3</sub> production capacity of the bacterial strain and its storage in self-healing of cement by microorganisms. The calcium mineralization activity of the microorganism itself is crucial in evaluating the performance in self-healing. The formulation of rice flour and maltodextrin (F3), provided an improved protection in the stability of *Bacillus pseudofirmus* strain during storage. In addition, by means of the atomization method with the process conditions, high percentages of viability were obtained for the strain after the drying process, thus providing a new efficient technology for the microencapsulation of microorganisms.

Each of the authors confirms that this manuscript has not been previously published and is not currently under consideration by any other journal. Additionally, all the authors have approved the contents of this paper and have agreed to the *Bioresource Technology* 's submission policies.

Each named author has substantially contributed to conducting the underlying research and drafting this manuscript. Additionally, to the best of our knowledge, the named authors have no conflict of interest, financial or otherwise.

Sincerely,

David Tarazona

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Highlights:

- Formulation of rice flour and maltodextrin provided an improved protection in the stability of *Bacillus pseudofirmus*.
- The atomization method provided a new efficient technology for the microencapsulation of microorganisms.
- The self-healing of cement by microorganisms has proven to be a promising strategy.
- The calcium mineralization activity of the microorganism itself is crucial to improve the CaCO<sub>3</sub> production capacity of the bacterial strain and its storage.

Microencapsulation of *Bacillus pseudofirmus* by atomization used for bio-concrete

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ABSTRACT:

A process of microencapsulation of the strain *Bacillus pseudofirmus* was carried out to stabilize spores and maintain a constant viability. The spray technique was used for evaluating 12 processes in spores encapsulated in five formulations (Caolin, CMC, skimmed milk, maltodextrin, rice flour and diatomaceous earth). The initial viability (%) after the atomization process and the survival rate during storage were evaluated by counting the CFU on the plate. The percentage of viability indicated that the formulation that includes rice flour and maltodextrin obtained the best results for our strain (98.50%) compared to the rest of the formulations, using the process of atomization with the process conditions: Temperature of 110 °C, 5 mL / min of feeding and 100% of aspiration. These results showed that the microencapsulation process performed is efficient for stabilization;

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4 Rice flour improves protection in this process and adding maltodextrin generates greater  
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6 stability during long-term storage.  
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10 KEYWORDS: *Bacillus pseudofirmus*, microencapsulation, bioconcrete, atomization.  
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## 12 13 1. INTRODUCTION 14

15  
16 Concrete is one of the most used materials in construction around the world, because it is a  
17  
18 resistant and durable material; However, in severe climatic conditions, exposure to the  
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20 weather, in contact with moisture and / or mechanical forces not considered can generate  
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22 cracking; these fissures must be treated immediately to avoid concrete-associated  
23  
24 pathologies such as: water permeability (which increases the chances of corrosion of the  
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26 steel embedded in the concrete), chemical reactions between water and sulfates, chlorides,  
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28 carbonates; which are aggressive to the concrete. Being this, the only cause of the structural  
29  
30 failure, for which there is a decrease in its durability, making its maintenance cost high and  
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32 inefficient (Bashir and Tiwary, 2016; Khaliq and Ehsan, 2016; Tziviloglou et al., 2016).  
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39 The widespread application of conventional water repellents such as silane and siloxane in  
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41 cement-based materials poses a serious environmental threat due to its non-biodegradable  
42  
43 nature (Ibrahim et al., 1997). A viable solution that can be designed to address  
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45 environmental problems is to use calcite from the bacterium of the *Bacillus* genus for  
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47 microbial cementation in order to optimize the mechanical behavior of cement-based  
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49 materials. Its ability to easily grow, absorb heavy metals and biocrystallize to form calcite  
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51 has turned bacteria into promising microbes for the purpose of biomineralization in the  
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53 construction industry (Wong, 2015).  
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4 The *Bacillus pseudofirmus* is one of the microorganisms widely studied in the formulation  
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6 of the bioconcrete, due to its property of secreting CaCO<sub>3</sub> product of its cellular metabolism  
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8 (Jonkers and Schlangen, 2008).  
9

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11 The concrete constituted with bacterial spores is activated at the entrance of water and  
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13 oxygen; they multiply and germinate with the presence of calcium lactate, product of the  
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15 metabolism limestone or calcite that seals the microcracks. Oxygen consumption helps in  
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17 the bacterial conversion of calcium lactate to limestone, also reduces the oxygen content in  
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19 the concrete, due to bacterial conversion (Jonkers and Schlangen, 2008).  
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25 The objective of the present investigation was to delimit the best formulations regarding the  
26  
27 cell viability of the strain in work; to ensure its best maintenance at the time of application  
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29 in buildings and throughout the life of the work.  
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31

## 32 33 2. MATERIALS AND METHODS: 34 35

36 The research work was carried out in the Bioengineering Laboratory of the University of  
37  
38 Human Sciences and the Bioproduction Laboratory of Celera Technologies, the bacterium  
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40 *Bacillus pseudofirmus* (ATCC 700159), is cryopreserved with 10% glycerol, at a  
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42 temperature of - 80 ° C (<https://www.atcc.org/products/all/700159.aspx>). The encapsulating  
43  
44 agents used were kaolin (Merck®, CAS 1332-58-7), sodium carboxymethylcellulose  
45  
46 (CMC) (Chemical Montana Company, CAS 29384), skim milk (Laive, Peru), maltodextrin  
47  
48 (Shandong Bangye Co, Ltd), earth of diatomea (Pflücker e Hijos SA, Peru) and rice flour  
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50 (Santa Lupe Rice Flour, María Carolina and Hnos. SAS Colombia).  
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### 56 2.1. Production of bacterial biomass 57 58 59

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4 1.0 mL of the cryopreserved strain was inoculated in 100 mL of nutritious broth at pH 9.0  
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6 for reactivation and incubated at 30 ° C for 48 hours, then scaling the strain to 500 ml and  
7  
8 then to 5 liters in a bioreactor Steel 8 liters capacity; in the same conditions described  
9  
10 above. The culture medium was nutrient broth with calcium oxalate at 2.3% w / v,  
11  
12 achieving raise the pH of production to 9.0 with potassium hydroxide. The growth curve of  
13  
14 each strain was established by regularly measuring its absorbance at 600 nm with a visible  
15  
16 722 model Kert Lab spectrophotometer, and was correlated by counting the CFU by plate  
17  
18 count using the official method of Association of Official Agricultural Chemists (AOAC)  
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20 966.23 (Cayra et al., 2017).  
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26  
27 To obtain the final biomass, the procedure followed by Chávez and Ledebøer was used,  
28  
29 with some modifications (Chávez and Ledebøer, 2007). During the exponential phase, the  
30  
31 cells were precipitated with a XC-2000 digital centrifuge at 4000 rpm for 30 min.  
32  
33 discarding the supernatant. Subsequently, the cells were washed with sterile saline (NaCl  
34  
35 0.85% w / v). Finally, they were resuspended in 50 mL of sterile saline and kept at 4 ° C,  
36  
37 until later use (Cayra et al., 2017).  
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## 42 *2.2. Microencapsulation*

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45 Five encapsulating formulations were prepared, F1: Caolin (29.5%) + CMC (5%), F2: skim  
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47 milk (26%) + maltodextrin (8%), F3: maltodextrin (20%) + Rice flour (14%), F4:  
48  
49 diatomaceous earth (34%) and F5: Rice flour (34%). In relation to the concentration of  
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51 inputs with preservation and stabilization capacities to drying processes. Each of these  
52  
53 formulations was completed at 41.5% total solids with calcium oxalate, sucrose,  
54  
55 carboxymethylcellulose and xanthan gum, as shown in Table 1. All formulations were  
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4 hydrated with sterile distilled water at room temperature and were homogenized with a  
5  
6 magnetic stirrer with SH23-2 heating plate. The sterile suspensions were inoculated with  
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8 the microbial biomass up to an initial concentration of  $9.2 \times 10^9$  CFU / mL on average for  
9  
10 each formulation; then they were homogenized with a magnetic stirrer with SH23-2 heating  
11  
12 plate for 3 hours at 30 ° C.  
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### 23 *2.3. Experimental design to evaluate the performance of the spray drying process*

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26 For each formulation a standardization of the yield of the process was made, by means of  
27  
28 the use of spray drying. For the experimental design a factorial model (3x2x2) of a block  
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30 considering only central with respect to temperature was used. It was taken as process  
31  
32 variables: the inlet temperature, the percentage of feed (flow of income from the sample to  
33  
34 the dryer) and the percentage of aspiration (air flow extracted). The process performance  
35  
36 (%) was considered as a dependent variable. The minimum temperature was 10°C above  
37  
38 the evaporation of water and the maximum value was considered 150°C (Müller et al.,  
39  
40 2000). Table 2 shows the values.  
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### 46 *2.4. Determination of the viability percentage of the strains:*

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48  
49 The concentration of viable microorganisms, before and after spraying in the trials of each  
50  
51 treatment, was calculated by plate count, applying the official method of AOAC 966.23. In  
52  
53 this procedure, for the encapsulating agent inoculated with the strain, before the spraying  
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55 process, 50 µL was measured and dissolved in 450 µL of sterile saline; and for the final  
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4 spray, a sample of 0.5 g was weighed and dissolved in 1.5 mL of sterile saline. Serial  
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6 dilutions were made up to a dilution of 1/10000, seeding 0.1 mL by dissemination on TSA  
7  
8 agar plates at pH 9.0; and incubating at 30 ° C for 48 hours.  
9

10  
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12 Finally, the following equation was used to calculate the percent (%) viability of the strains  
13  
14 (Schell and Beermann, 2014; Strasser et al., 2009).  
15

16  
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18 (1)

$$\% \text{ Viability} = \frac{\log (N.W)}{\log (N_0.V)} \times 100$$

19  
20  
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24  
25 Where, N is UFC / g after the spraying process, W is the final spraying weight, N0 is UFC /  
26  
27 mL before the spraying process and V is the volume of the encapsulating agent fed (Cayra  
28  
29 et al., 2017).  
30

### 31 32 33 *2.5. Determination of viability during storage:*

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36 The product obtained after the drying process was packed in ziploc-type polyethylene bags  
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38 and stored at a temperature of 18 ± 2 ° C protected from light (Schell and Beermann, 2014),  
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40 for a period of 90 days. In order to validate the survival rate of the encapsulations, the UFC  
41  
42 was counted periodically every 15 days. Samples were taken in duplicate for the trials of  
43  
44 each treatment.  
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### 47 48 49 *2.6. Statistical analysis:*

50  
51  
52 The analysis of the results was carried out using a simple variance test (ANOVA),  
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54 according to the univariate general linear model. Surface graphs were made in the STATA  
55  
56 program.  
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4 3. RESULTS AND DISCUSSION.  
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7 Table 3 shows the experimental conditions for the spray drying of the spores and the  
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10 respective yield of the 5 formulations.  
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12 In Formulation No. 3, the highest percentages of performance in general were presented  
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14  
15 with respect to the experimental work conditions. The maximum yield was 77.56% at 110  
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18 °C, 5 mL / min 100% for temperature, feed and aspiration respectively. These conditions  
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21 ensured easier removal from both the cyclone and the collection vessel. (Graph No. 1).  
22

23 The lowest yield was obtained in formula 4 with 33.47%. This could be due to the increase  
24  
25  
26 of the feeding speed, humidity in the drying chamber and in the cyclone caused by the  
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29 formed and not dried drops.  
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31 Each variable has different results on drying, that is, when the temperature difference  
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34 between the heating medium and the product to be dried is greater, the drying speed is  
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36  
37 more.  
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39 The feed determines the speed with which the sample enters the drying chamber; since, if it  
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41  
42 is low and maintaining constant pressure, then there will be a greater subdivision of  
43  
44  
45 droplets accelerating the drying and allowing a better thermal efficiency of the dryer, since  
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48 a larger area, provides more contact surface with the heating medium. The influence of  
49  
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51 aspiration is explained by the residence time of the sample inside the drying chamber, if it  
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53  
54 is low this time will be longer (Maa and Prestrelski, 2000).  
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56 The ANOVA analysis showed significant differences ( $p < 0.05$ ) among the five  
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59 formulations, in relation to the percentage of cell viability after the drying process. In  
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4 Formula 3 it shows a greater percentage of viability than the rest of the formulations, as  
5  
6 shown in Table N° 4. These data suggest that maltodextrin and rice flour have a positive  
7  
8 effect on the protection of *Bacillus spores. pseudofirmus* during the encapsulation process  
9  
10 obtained positive results with skim milk; comparing it with (Cayra et al., 2017), which used  
11  
12 10% skim milk, improving the survival of *Candida sake* yeast. In the case of our  
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14 experience; Although acceptable results were shown with the skim milk, the resistance  
15  
16 capacity of the bacterial spores contributes in the durability and stability, stabilizing with  
17  
18 good effects against the maldotextrina and rice flour. The cost of these inputs is also  
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20 considered, with the least cost prevailing since this formulation will have a potential use for  
21  
22 the construction industry; in which the construction material provides a large volume of  
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24 use.  
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32 The reduction of cell viability after drying is mainly due to the heat of the process (Silva et  
33  
34 al., 2011). Therefore, the greater survival shown by *Bacillus pseudofirmus* in the face of  
35  
36 high temperatures is due to the complexity of the reserve structure that it possesses; called  
37  
38 endospore and in which is responsible for protecting the genetic material and the precursors  
39  
40 of the metabolic machinery to germinate at the time of returning to favorable conditions.  
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45 On the effect of the encapsulating formulation on the viability of the strains during the  
46  
47 storage of the encapsulations, Graph 2 shows that the concentration of the strain,  
48  
49 encapsulated with F1, F4 and F5, decreased significantly in the first 45 days of storage;  
50  
51 while with F3 it reduced the cellular concentration little during the 90 days, maintaining its  
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53 concentration throughout the storage period. Therefore, after 90 days the number of  
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55 bacterial cells in F3 is greater than in the rest of the formulations, after being atomized with  
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4 the process conditions: Temperature of 110°C, 5 mL / min of feeding and 100% of  
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6 aspiration.  
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#### 9 10 4. CONCLUSIONS

11  
12 The formulation of rice flour and maltodextrin (F3), provided an improved protection in the  
13 stability of *Bacillus pseudofirmus* strain during storage. In addition, by means of the  
14 atomization method with the process conditions: Temperature of 110°C, 5 mL / min of feed  
15 and 100% of aspiration, high percentages of viability were obtained for the strain after the  
16 drying process, thus providing a new efficient technology for the microencapsulation of  
17 microorganisms.  
18  
19

20  
21 The self-healing of cement by microorganisms has proven to be a promising strategy.  
22 Although the formulations and stabilization processes are necessary for the effectiveness of  
23 the bacterial process, the calcium mineralization activity of the microorganism itself is  
24 crucial in the evaluations; for which this type of cellular stabilization processes and  
25 protocols seek to improve the CaCO<sub>3</sub> production capacity of the bacterial strain and its  
26 storage.  
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#### 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 5. ACKNOWLEDGMENTS

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**Figure 1**  
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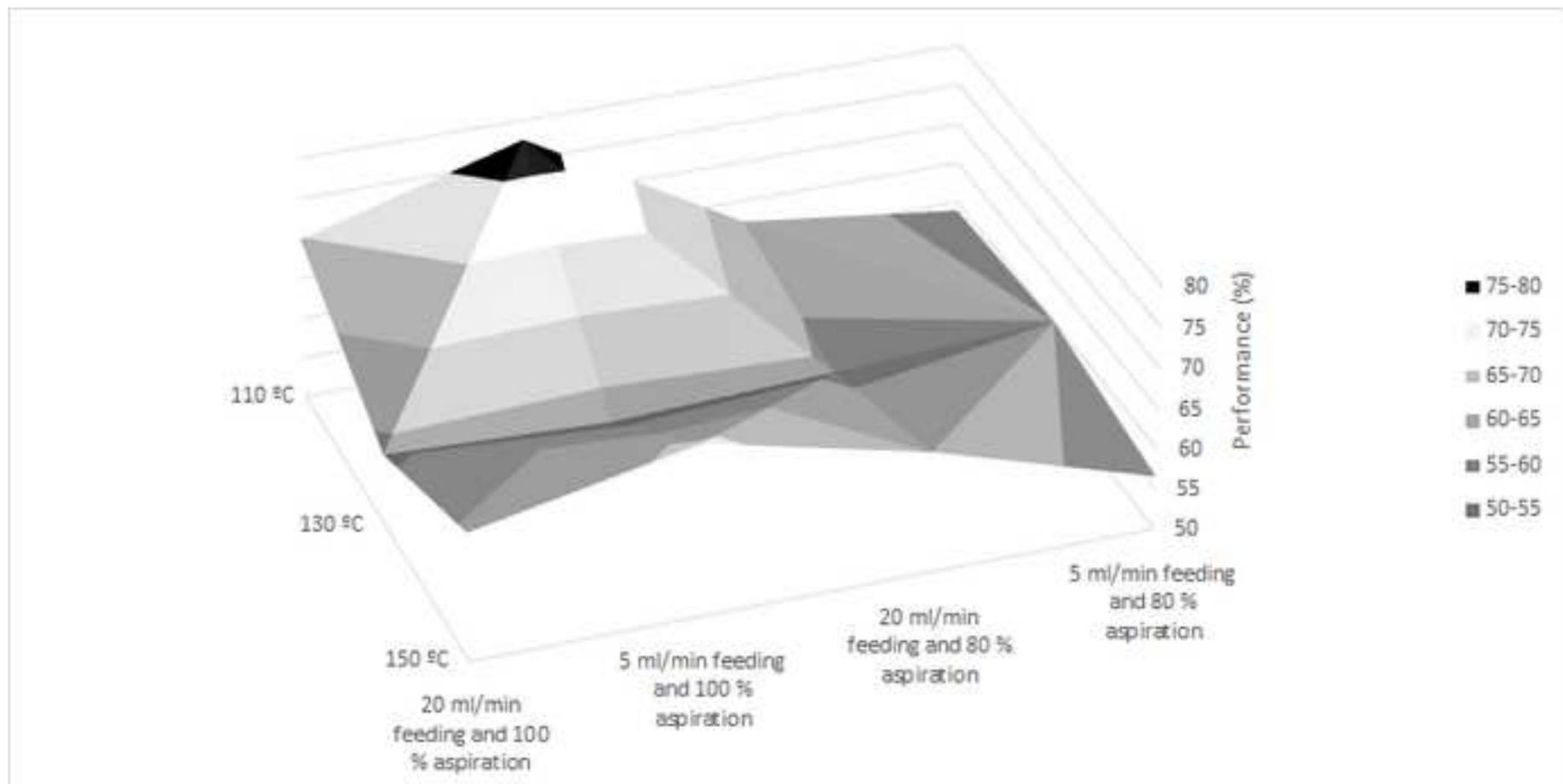
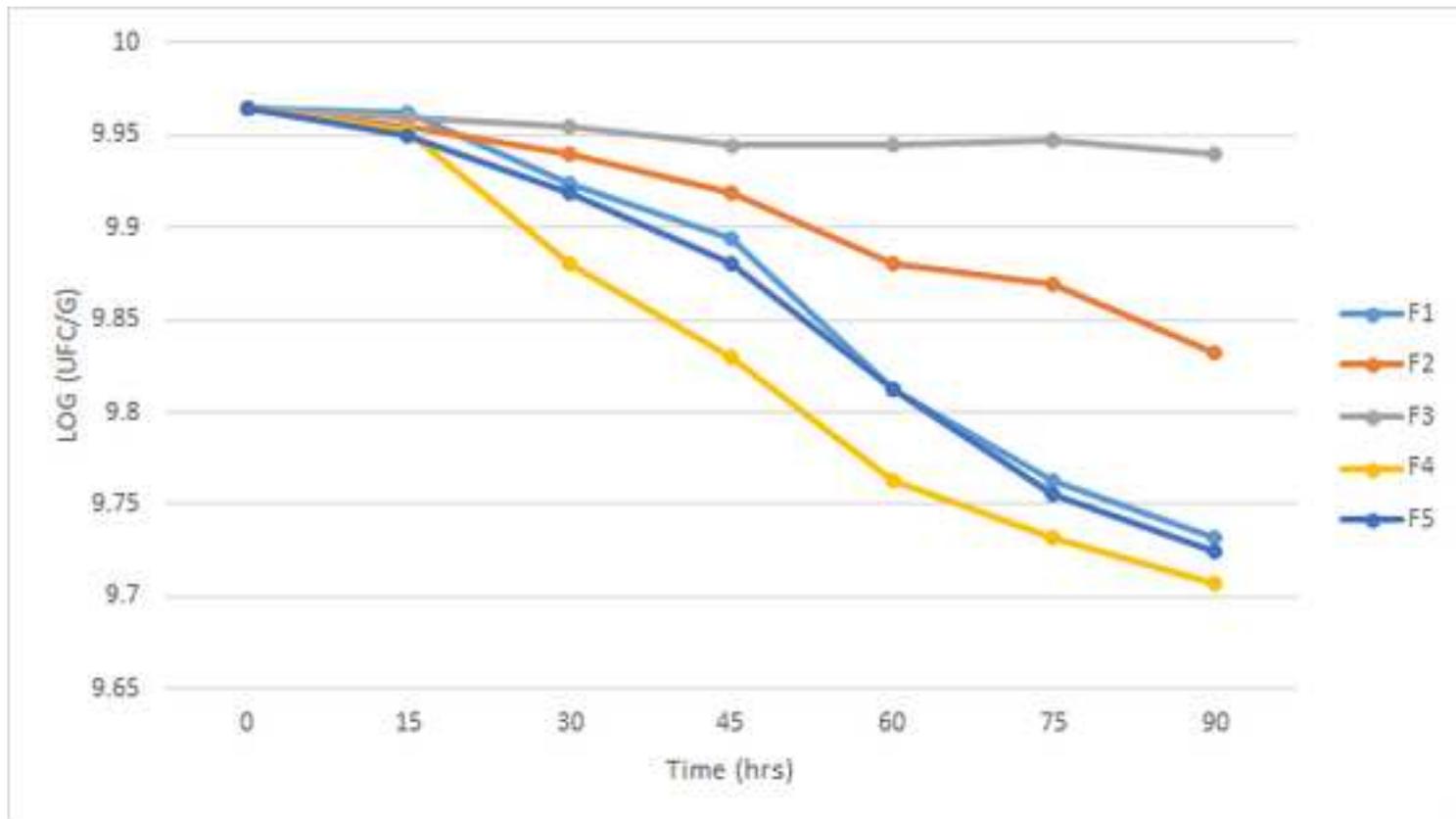


Figure 2  
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**Table1**

Table 1: Encapsulating formulations used in the study; the data are percentage (weight / volume).

	<i>Formulation 1</i> (F1)	<i>Formulation 2</i> (F2)	<i>Formulation 3</i> (F3)	<i>Formulation 4</i> (F4)	<i>Formulation 5</i> (F5)
Skim Milk (%)		26			
Maltodextrin (%)		8	<b>20</b>		
Sucrose (%)	5	5	<b>5</b>	5	5
Carboxymethylcellulose (%)	5	0.5	<b>0.5</b>	0.5	0.5
Xanthan gum (%)	0.5	0.5	<b>0.5</b>	0.5	0.5
Caolin (%)	29.5				
Diatomaceous earth (%)				34	
Rice flour (%)			<b>14</b>		34
Calcium oxalate (%)	1.5	1.5	<b>1.5</b>	1.5	1.5

Table 2: Maximum and minimum values of the independent variables in the study.

	<i>Upper level</i>	<i>Central level</i>	<i>Lower level</i>
A: Temperature (°C)	150	130	110
B: Feeding (mL/min)	20	-	5
C: Aspiration (%)	100	-	80

Table 3: Experimental conditions for the spray drying of the spores and the respective yield of the 5 formulations.

Experiment	Drying Temperature (°C)	Feeding (mL/min)	Aspiration (%)	Performance (%)				
				F <sub>1</sub>	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>	F <sub>5</sub>
V1	110	5	80	54.32	63.46	58.87	46.57	56.34
V2	150	5	80	45.62	56.36	56.55	43.35	54.39
V3	110	20	80	67.86	46.12	62.34	33.47	66.78
V4	150	20	80	65.37	68.73	64.87	54.66	64.98
<b>V5</b>	<b>110</b>	<b>5</b>	<b>100</b>	<b>67.33</b>	<b>66.36</b>	<b>77.56</b>	<b>57.34</b>	<b>69.87</b>
V6	150	5	100	59.25	66.22	61.21	54.24	64.64
V7	110	20	100	65.33	54.95	64.87	58.73	54.75
V8	150	20	100	59.89	67.56	65.74	53.34	57.98
V9	130	5	80	65.47	46.45	59.98	52.37	53.98
V10	130	20	80	67.22	58.54	58.72	56.53	61.09
V11	130	<b>5</b>	<b>100</b>	57.63	63.43	57.62	57.67	59.87
V12	130	20	100	47.77	56.60	58.79	53.40	64.98

Table 4: Feasibility of *Bacillus pseudofirmus* after the atomization processes in the five encapsulating agent formulations. Process chosen V5: Temperature of 110 °C, 5 mL / min of feeding and 100% of aspiration.

Formulation	Initial (Log UFC)	Final (Log UFC)	Viability (%)
<i>V5: Temperature of 110 °C, 5 mL/min feeding y 100% aspiration</i>			
F1	9.96 ± 0.03	9.73 ± 0.02	95.14 ± 0.13 <sup>a</sup>
F2	9.96 ± 0.03	9.83 ± 0.02	96.45 ± 0.18 <sup>b</sup>
<b>F3</b>	<b>9.96 ± 0.03</b>	<b>9.93 ± 0.02</b>	<b>98.50 ± 0.13<sup>c</sup></b>
F4	9.96 ± 0.03	9.70 ± 0.02	94.89 ± 0.13 <sup>c</sup>
F5	9.96 ± 0.03	9.72 ± 0.02	95.02 ± 0.15 <sup>c</sup>