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SPRAY DRYING OF CELLULASES PRODUCED BY *Bacillus* sp. SMIA-2

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Abstract

Microbial cellulases present biotechnological potential to be used in several industries, including food, brewery and wine, industrial waste for chemical feedstock, animal feed, pulp and paper, agriculture, textile and detergent production. In this work, cellulases produced by the thermophilic *Bacillus* sp. SMIA-2 in submerged cultures containing sugarcane bagasse, corn steep liquor and passion fruit rind flour were spray-dried, in an attempt to improve their stability for industrial purposes. The cellulases were spray dried and analyzed before and after the drying process and subsequent storage. A Central Composite Design (CCD) 2^3 was used to investigate the effect of different concentrations of arabic gum and microcrystalline cellulose, as well as the spray dryer inlet temperature on the cellulase spray drying process. The results evidenced that the combination of 1.0 % (w/v) arabic gum and 1.0% (w/v) microcrystalline cellulose, at inlet temperature of 70 °C, was effective in maintaining the activities of both avicelases (avicel-hydrolyzing enzymes) and carboxymethylcellulases (carboxymethylcellulose-hydrolyzing enzymes - CMCase). The dried avicelase was completely preserved when stored at 5ºC, while the CMCase retained 89% of its activity, which indicates promising potential for industrial uses, especially in detergent formulations.

Keywords: Additives. Avicelase. *Bacillus.* Carboxymethylcellulase. Spray drying.

1. Introduction

Thermophilic cellulases are ideal biocatalysts for modern biotechnology, due to their thermostability and improved yields under extreme operational conditions (Anbar and Bayer 2012; Azizi et al. 2015). The wide range of applications of thermophilic cellulases include the food, textile, chemical, pulp and paper, laundry detergents and chemical industries, besides second-generation ethanol production (Singh et al. 2017; Li et al. 2018; Listyaningrum et al. 2018).

Bacillus sp. SMIA-2, a thermophilic and thermostable enzyme-producing bacterium, was able to express a high level of cellulases in submerged cultures employing agricultural -products, such as sugarcane bagasse and passion fruit rind flour (Costa et al. 2017; Cruz et al. 2019). This feature, which has raised industrial interest, allows for the generation of high-value products based on sustainable production

processes. The cellulases from *Bacillus* sp. SMIA-2 have both CMCases (carboxymethylcellulose-hydrolyzing enzymes) and avilaces. However, avicelases are more active than CMCase (Ladeira et al. 2015).

Cellulases are a group of complex enzymes which catalyze the hydrolysis of cellulose and related cellu-oligosaccharide derivatives. They can form a multi-enzyme complex called cellulosome, in which they are anchored to a common structure, or work as non-complexed extracellular free cellulase systems that provide increased synergy and activity (Escuder-Rodríguez et al. 2018).

Bernardo et al. (2020) have recently reported that the SMIA-2 genome encoded 5 amylase genes, 13 loci for xylose metabolism, 55 protein degradation-associated loci and 3 cellulolytic enzyme loci, under a putative cellulosome complex. This complexed enzyme system may have great potential for the degradation of plant biomass (Chen et al. 2020).

The cellulases produced by *Bacillus* sp. SMIA-2 were able to work at high temperatures and pH levels and retained good stability in the presence of several surfactants, oxidizing agents and locally available detergents (Ladeira et al. 2015; Cruz et al. 2019). It indicates the potential for the use of this bacterium and its cellulases for various industrial applications, including the detergent industry.

They are often preserved in dry form in order to increase shelf life, ease storage and transport, reduce transportation cost and protect the biological activity of enzymes. Although freeze drying (lyophilization) is preferred for products such as enzymes, spray drying is considerably more economical, as it can process larger volumes and operate with greater efficiency (Schutyser et al. 2012; Jesus et al. 2014).

Therefore, several attempts have been made to optimize spray dried enzyme formulations with minimal loss of activity. Carbohydrates and polymers, for example, are often used as bulking agents and protective additives. These carrier agents are employed in spray-drying, as drying helps increase the overall glass transition temperature and product yield, overcome stickiness and agglutination, and protect heat-sensitive compounds against unfavorable ambient conditions (Do and Nguyen 2018).

The present work aimed to investigate the combined interactive effect of arabic gum (AG) and microcrystalline cellulose (MC) concentrations and the spray dryer inlet temperature on the spraying of the cellulases produced by cultures of *Bacillus sp*. SMIA-2 containing sugarcane bagasse, passion fruit rind flour and corn steep liquor, through Response Surface Methodology (RSM), also seeeking to strike a balance between them to avoid cellulase activity loss. In addition, the long-term stability tests were performed during 180 days, at 5 °C and 25 °C.

2. Material and Methods

Organism

The bacterial strain *Bacillus* sp. SMIA-2 was isolated from a soil sample collected in the city of Campos dos Goytacazes, Rio de Janeiro, Brazil (Souza and Martins 2001).

Enzyme Production

The culture medium used in this work for cellulase production contained (g L^{-1}): KCl - 0.3, $MgSO_4$ - 0.5, K_2HPO_4 - 0.87, $CaCl_2$ - 0.29, ZnO - $2.03x10^{-3}$, $FeCl_3.6H_2O$ - $2.7x10^{-2}$, $MnCl_2.4H_2O$ - $1.0x10^{-2}$, $CuCl_2.2H_2O$ - $8.5x10^{-4}$, $CoCl_2.6H_2O$ - $2.4x10^{-3}$, $NiCl_3.6H_2O$ - $2.5x10^{-4}$, H_3BO_3 - $3.0x10^{-4}$, sugarcane bagasse treated with alkali (81.05% cellulose, 18.75% hemicellulose, 5.45% lignin) - 0.3%, commercial corn steep liquor (Sigma Aldrich) - 0.3% and passion fruit rind flour - 0.3% (Costa et al. 2017).

The pH was adjusted to 7.2, with 1.0 M NaOH, and the medium was sterilized by steam-autoclaving, at 121 °C, 1 atm, for 15 minutes. The medium (50 mL in 250 mL Erlenmeyer flasks) was inoculated with 1 mL of a standard overnight culture (initial number of cells 10^4) and incubated at 50 °C, in an orbital shaker (Thermo Forma, Ohio, USA) operated at 150 rpm. After 168 h, the flasks were withdrawn, and the contents were centrifuged (HERMLEZ 382K, Wehingen, Germany), at 15.500 *g,* for 15 min, at 4 °C. The cell free supernatant was used as crude enzyme preparation.

Enzyme Assay

The cellulolytic enzyme activities were determined using the dinitrosalicyclic acid method (Miller 1959), which measures reducing sugars. The reaction containing 0.5 mL of 1% (w/v) substrate solution (carboxymethylcellulose sodium salt or avicel, PH-101) was prepared in 10 mM sodium phosphate buffer, pH 7.5, and 0.5 mL of appropriate concentration of enzyme solution and incubated at 70 °C. After 10 min of reaction, 1 mL of dinitrosalicyclic acid reagent was added and boiled in water bath for 5 min. The resulting samples were then cooled to room temperature, and the absorbance was measured at 540 nm.

When the activity was tested using avicel as substrate, the assay tubes were agitated during the assay to keep the substrate suspended. One unit (U) of activity toward the substrates mentioned above was defined as 1 µmol of glucose equivalent released per minute, under the above assay conditions, by using a glucose standard curve. Appropriate controls were conducted in parallel with all assays. Enzyme blank containing 0.5 mL of 10 mM sodium phosphate buffer and 0.5 mL of 1% (w/v) substrate solution was run.

A substrate blank containing 0.5 mL of 10 mM sodium phosphate buffer and 0.5 mL enzyme solution was also run to exclude the background of reducing sugars found in the enzyme supernatant from the results. The absorbance rates of the enzyme blank sets and the substrate blank were subtracted from the absorbance of the activity assay (Ghose 1987). All the samples were performed in triplicate, and the blanks, in duplicate.

Spray drying of crude enzyme solution

The powders were produced through the atomization of crude cellulase preparation (Figure 1) in a lab-scale spray dryer (LAB-PLANT, MODELO SD-04, England). During the operation, 100 mL of the prepared solution were fed at constant rate, 2.5 mL/min, with a peristaltic pump, to a nozzle, where atomization occurred by means of a pressurized air stream. The drying air entered the drying chamber in the same direction as the descending spray droplets. The process variables were the dryer air inlet temperature and the concentration of adjuvants (arabic gum and microcrystalline cellulose), according to Table 1. The outlet air temperature could not be controlled directly, but it depended on the dryer inlet temperature and the solution feed rate. The air flow rate was constant in all the experiments. According to the working inlet temperatures, namely, 50, 58, 70, 82 and 90 ^{o}C, the dryer outlet temperatures observed were 39, 41, 52, 61 and 67 ^{o}C, respectively. All drying experiments were carried out in duplicates. Arabic gum (AG) and microcrystalline cellulose (MC) were incorporated into the enzyme solution before spray drying. The dried particles were collected and stored at refrigeration temperature (15 ^{o}C).

The avicelase and CMCase activities were measured before and after drying. The cellulase activity after drying was measured by dissolving enzyme powder (0.01 g mL^{-1}), while the residual activities (%) were determined and compared with the activities of the crude enzyme solutions before drying and without any adjuvants.

Mass recovery (%)

The mass recovery was determined according to Belghith et al. (2001):

$$\text{Mass recovery (\%)} = \frac{Wt}{TSS} \times 100$$

Where TSS is the Total Soluble Solid of the crude cellulase solution in the presence of the adjuvant, and Wt is the weight of powder mass (g) obtained from the spray-drying process. TSS was determined from the crude extract cellulase solution, using a hand-held digital refractometer (Pocket Refractometer Pal-1, Atago Co. Ltd, Tokyo, Japan). Three measurements were recorded from each sample.

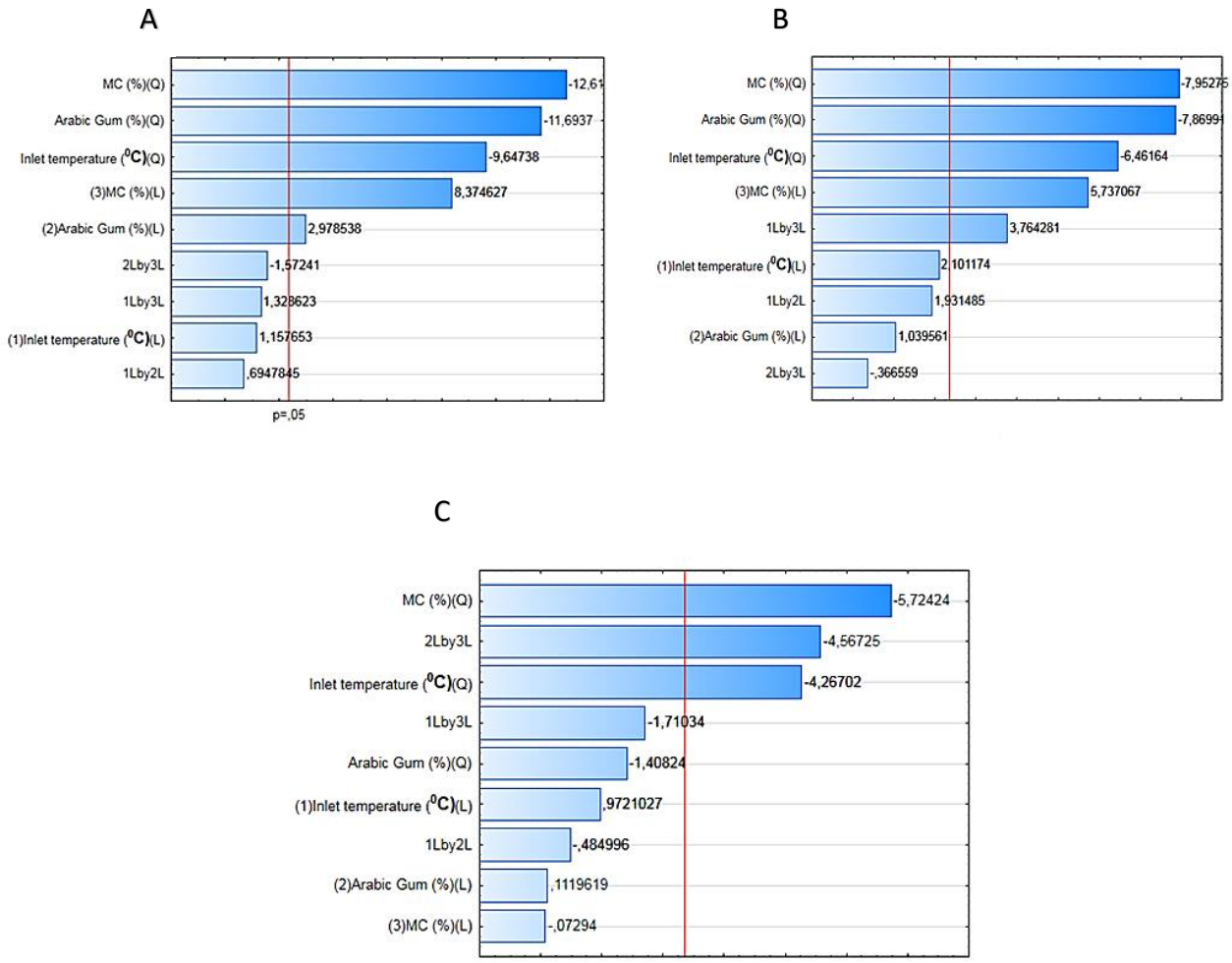

Figure 1. Effect of the independent variables and their interactions: A - Residual avicelase activity (%); B - Residual CMcase activity; C - Mass recovery, for a 95% confidence level ($p < 0.05$).

Stability of cellulase powder (%)

After drying, the storage stability of cellulase powders obtained at 5^0C and 25^0C was investigated for up to 180 days. Cellulase stability was determined by measuring cellulase activities immediately after drying and every 30 days, for up to six months of storage.

Water activity

The water activity (aw) in the dried samples was measured using a Rotronic Hygrolab (C1 Bench-top Indicator, Switzerland) by direct reading, during 6 months of storage.

Experimental Design and Statistical Analysis

A central composite design (CCD) 2^3 was constructed to evaluate the effects of arabic gum (AG) and microcrystalline cellulose (MC) concentration and the spray dryer inlet temperature on Residual cellulase activities (%). The factorial planning presented three central points and yielded a total of 17 treatments for each experiment. The factors and factor levels studied are described in Table 1.

The condition was optimized using CCD, and surface-response was produced with fixed central points of 70^0C, 1.0 % (w/v) AG and 1.0% (w/v) MC.

The results of the residual avicelase activity, CMCase residual activity and mass recovery obtained were submitted to regression analysis, initially calculating a polynomial equation and then evaluating its

lack of adjustment and the significance of each variable at $p < 0.05$. The predictive model was adjusted, considering only the significant parameters. The Response Surface graphs were drawn for the models with high correlation coefficient, using the Statistica software system, version 10.0.

Table 1. Factors and Levels studied in CCD.

Factors	Factor Level				
	-1.68	-1	0	+1	+1.68
Temperature (^{0}C)	50	58	70	82	90
AG (%, w/v)	0.00	0.40	1.00	1.60	2.00
MC (%, w/v)	0.00	0.40	1.00	1.60	2.00

The experimental model can be expressed as follows: (Equation 1):

$$Y = bo + \sum_{i=1}^{4} bi\ xi + \sum_{i=1}^{4} bii\ xi^2 + \sum_{i \neq j=1} \sum bij\ xi\ xj$$

Where y is the cellulase activity (response); xi and xj are indicated as the independence variables; and bo, bi, bii and bij means are the intercept terms, the linear, square and the interaction effect, respectively.

3. Results

The supernatant (crude cellulase solution, CCS) from the cultures of *Bacillus* sp. SMIA-2 (168 h) containing sugarcane bagasse treated with alkali (0.3%, w/v), commercial corn steep liquor (0.3%, w/v) and passion fruit rind flour (0.3%, w/v) presented avicelase activity and CMCase activity of 1.9191 U mL^{-1} and 0.5384 U mL^{-1}, respectively. The CCS was spray-dried, and the results obtained for the Residual Cellulase activities (%) and Mass Recovery (%) of powder are provided in Table 2, according to the experimental design adopted.

Table 2. Matrix of CCD 23 (real and coded values) used and its response (Residual avicelase activity, Residual CMCase activity and Mass Recovery).

Formulation number	Spray dryer inlet temperature (^{0}C)	AG (%, w/v)	MC (%, w/v)	Residual avicelase activity (%)	Residual CMCase activity (%)	Mass Recovery (%)
1	58 (-1)	0.4 (-1)	0.4 (-1)	65.30 ± 0.12	64.50 ± 0.14	15.22 ± 0.09
2	82 (+1)	0.4 (-1)	0.4 (-1)	61.40 ± 0.18	50.00 ± 0.15	28.00 ± 0.07
3	58 (-1)	1.6 (+1)	0.4 (-1)	70.80 ± 0.14	66.50 ± 0.12	31.25 ± 0.06
4	82 (+1)	1.6 (+1)	0.4 (-1)	69.40 ± 0.12	58.30 ± 0.11	38.48 ± 0.03
5	58 (-1)	0.4 (-1)	1.6 (+1)	81.90 ± 0.20	73.40 ± 0.11	35.81 ± 0.10
6	82 (+1)	0.4 (-1)	1.6 (+1)	83.10 ± 0.17	78.20 ± 0.14	35.65 ± 0.09
7	58 (-1)	1.6 (+1)	1.6 (+1)	80.60 ± 0.22	65.40 ± 0.12	21.67 ± 0.02
8	82 (+1)	1.6 (+1)	1.6 (+1)	85.00 ± 0.13	91.30 ± 0.13	21.21 ± 0.03
9	50 (-1.68)	1.0 (0)	1.0 (0)	76.50 ± 0.12	61.00 ± 0.12	23.75 ± 0.05
10	90 (+1.68)	1.0 (0)	1.0 (0)	83.70 ± 0.16	79.40 ± 0.12	21.33 ± 0.06
11	70 (0)	0 (-1.68)	1.0 (0)	69.80 ± 0.13	63.10 ± 0.14	31.67 ± 0.07
12	70 (0)	2.0 (+1.68)	1.0 (0)	80.40 ± 0.13	65.40 ± 0.15	33.95 ± 0.05
13	70 (0)	1.0 (0)	0 (-1.68)	65.10 ± 0.18	52.80 ± 0.13	18.06 ± 0.07
14	70 (0)	1.0 (0)	2.0 (+1.68)	80.60 ± 0.14	75.00 ± 0.16	16.55 ± 0.06
15	70 (0)	1.0 (0)	1.0 (0)	103.70 ± 0.16	99.80 ± 0.12	41.03 ± 0.10
16	70 (0)	1.0 (0)	1.0 (0)	104.10 ± 0.22	101.40 ± 0.17	41.03 ± 0.12
17	70 (0)	1.0 (0)	1.0 (0)	103.90 ± 0.12	99.60 ± 0.11	41.38 ± 0.10

Residual activity is expressed as a percentage of crude enzyme solution before drying operation (100% of the avicelase activity = 1.9191 U mL-1 and 100% of the CMCase activity = 0.5384 U mL-1).

Variation in Residual Avicelase activity (%) and Residual CMCase activity (%) was observed from 61.40 (Treatment 2) to 104.10 (Treatment 16) and from 50.0 (Treatment 2) to 101.40 (Treatment 16), respectively. Regarding Mass Recovery (%), the lowest value was observed in treatment 1 (15.22), and the

highest, in treatment 17 (41.38). The comparison between the parameters studied in these experiments indicates that the maximum retention of activity for both Avicelase and CMCase and Mass Recovery of the powder was obtained when 1.0 % (w/v) AG, 1.0% (w/v) MC and spray dryer inlet temperature of 70^0C were used.

The experimental results presented in Table 1 were submitted to analysis of variance in order to identify statistically significant variables. As shown in the Pareto graph (Figure 1A), the spray dryer inlet temperature, MC concentration and AG concentration generated a significant quadratic effect on residual avicelase activity (%), while the MC concentration and AG concentration resulted in a significant linear effect. Regarding the residual CMCase activity (%), the spray dryer inlet temperature, MC concentration and AG concentration caused a significant quadratic effect, while the MC concentration and the interaction between MC and AG concentration presented a significant linear effect (Figure 1B). As shown in Figure 1C, only the spray dryer inlet temperature had a significant quadratic effect on Mass Recovery (%). Furthermore, only the interaction between AG concentration and spray dryer inlet temperature presented significant linear effect.

Regression analysis was applied to the experimental results of residual avicelase activity, residual CMCase activity and mass recovery. First, a model containing all linear and quadratic effects was tested, as well as the interaction factors between the variables. Table 3 shows the results of Anova and Regression coefficient of the adjusted model.

Table 3. ANOVA for the variables of response surface quadratic model for Residual avicelase e CMCase activities and Mass recovery.

Variable	Sum of squares	(Degrees of freedom)	Mean square	Fcal	Ftab0.05	P<0.05
Residual avicelase activity (%)						
Regression	2803.31	9	311.48	37.02	3.11	0.000044
Residues	58.89	7	8.41			
Lack of adjustment	58.81	5	11.76	294.06	19.41	
Pure error	0.080	2	0.04			
Total error	2862.20	16	178.89			
				R^2 =	97.94%	
Residual CMCase activity (%)						
Regression	4084.11	8	510.51	22.76	3.11	0.000099
Residues	179.47	8	22.43			
Lack of adjustment	177.52	6	29.59	30.39	19.41	
Pure error	1.947	2	0.97			
Total error	4263.58	16	266.47			
				R^2 =	95.79%	
Mass recovery (%)						
Regression	1198.07	7	171.15	12.07	3.11	0.000629
Residues	127.63	9	14.18			
Lack of adjustment	127.55	7	18.22	446.23	19.41	
Pure error	0.082	2	0.04			
Total error	1325.70	16	82.86			
				R^2 =	90.37%	

The adjusted models were highly significant ($p < 0.05$), with a satisfactory value of determination coefficient (R^2). It was possible to state that 97.94%, 95.79% and 90.37% of the variability in the Residual Avicelase activity (%), Residual CMCase activity and Mass Recovered (%) response, respectively, could be accounted by the model, and that it was suitable to represent the real relationship among the independent variables studied. When the calculated F value is greater than the table for the adopted confidence level, there is sufficient statistical evidence to suppose the existence of a relationship between the variables. However, the also significant lack of adjustment suggests that the model was not ideal for its validation.

In order to carry out a comprehensive study on the effect of independent variables on Residual Avicelase activity, Residual CMCase activity and Mass Recovery, the experimental and predicted values were compared with empirical equations adjusted based on the analysis of variance (ANOVA). The equations obtained to predict the residual avicelase activity (Eq. 2), residual CMCase activity (Eq. 3) and the mass recovery (Eq. 4) of the obtained powder are presented below as a function of the independent variables of the coded terms.

$$Y = 103.8597 + 0.9086\ x_1 - 8.3342\ x_1^2 + 2.3378\ x_2 - 10.1019\ x_2^2 + 6.5731\ x_3 - 10.8974\ x_3^2 + 0.7125 x_{1.2} + 1.3625 x_{1.3} - 1.6125 x_{2.3}$$

$$Z = 99.9828 + 2.8517\ x_1 - 9.6523\ x_1^2 + 1.4109\ x_2 - 11.7559\ x_2^2 + 7.7863\ x_3 - 11.8797\ x_3^2 + 3.4250\ x_{1.2} + 6.6750\ x_{1.3}$$

$$H = 40.8102 + 1.1218\ x_1 - 5.4196\ x_1^2 - 1.7886\ x_2^2 - 7.2705\ x_3^2 - 0.7312\ x_1.x_2 - 2.5787\ x_1.x_3 - 6.8862\ x_2.x_3$$

Where x_1 is the Temperature (^{0}C), x_2 is the AG and x_3 is the MC concentration.

The response surface and contour plot figures obtained by the analysis of the experimental CCD data were used to determine the optimum conditions and study the interactive effects. They revealed a relationship between two variables at a time.

The effect of the interaction between AG concentration and spray dryer inlet temperature when the MC concentration remained constant at level 0 (1.0%, w/v) on Residual enzyme activities and Mass Recovery was depicted in the response surface plot of Figure 2A. The maximal retention of Avicelase and CMCase activities and higher Mass Recovery were obtained when a curvature occurred in the response, which was observed at concentrations between 0.75 and 1.25% (w/v) AG, at spray dryer inlet temperature range of 65-75^{0}C. The effect of the interaction between MC concentration and spray dryer inlet temperature when the AG concentration remained constant at level 0 (1.0%, w/v) on Residual enzyme activities and Mass recovery was depicted in the response surface plot of Figure 2B.

In this frame, a curvature occurred in the response, and the maximum retention in avicelase and CMCase activity and Mass recovery was achieved when concentrations of MC between 0.75-1.25% (w/v) and spray dryer inlet temperature between 65-75^{0}C were used in the process. The effect of the interaction between MC and AG concentration when the spray dryer inlet temperature remained constant at level 0 (70^{0}C) on Residual enzyme activities and Mass recovery was depicted in the response surface plot of Figure 2C. The maximal retention of avicelase and CMCase activity and Mass recovery was found when concentrations ranged between 0.75 and 1.25% (w/v) of both adjuvants. AG and MC were incorporated into enzyme solutions, prior to the spray drying process.

The water activity (aw) of the cellulase dried formulations is shown in Figure 3. With the exception of formulation 5 (F5), values below 0.6 were obtained for all other formulations.

A new fermentation process was carried out to confirm the optimized conditions for drying cellulases produced by *Bacillus* sp. SMIA-2, as described in section 2.2. The cellulase-rich extract obtained was dried in the presence of 1.0 % (w/v) AG, 1.0% (w/v) MC, at inlet temperature of 70 ^{o}C. The avicelase powder activity was completely preserved when it was stored at 5^{0}C, while the CMCase lost about 11% of its activity (Table 4). When the enzyme powders were stored at 25 ^{o}C, the avicelase and CMCase lost about 4% and 16% of their activities, respectively.

4. Discussion

Considering the great industrial relevance of thermostable cellulases, an attempt was made to increase the shelf life of cellulase solutions produced by the *Bacillus* sp. SMIA-2, by using the spray drying process. The cellulases were recovered from 168 hours of SMIA-2 submerged cultures containing sugarcane bagasse, passion fruit rind flour and corn steep liquor. The medium was optimized by Costa et al. (2017) and enabled the use of these agricultural by-products as novel and cost-effective culture media for the production of cellulases. Higher avicelase (avicel-hydrolyzing enzymes) amounts were found in the

supernatant, compared to the CMCase (carboxymethylcellulose- hydrolyzing enzymes). According to Ladeira et al. (2015), the cellulases secreted by the strain SMIA-2 were categorized as predominantly exoglycanases, with complementary and lower endoglucanase activities.

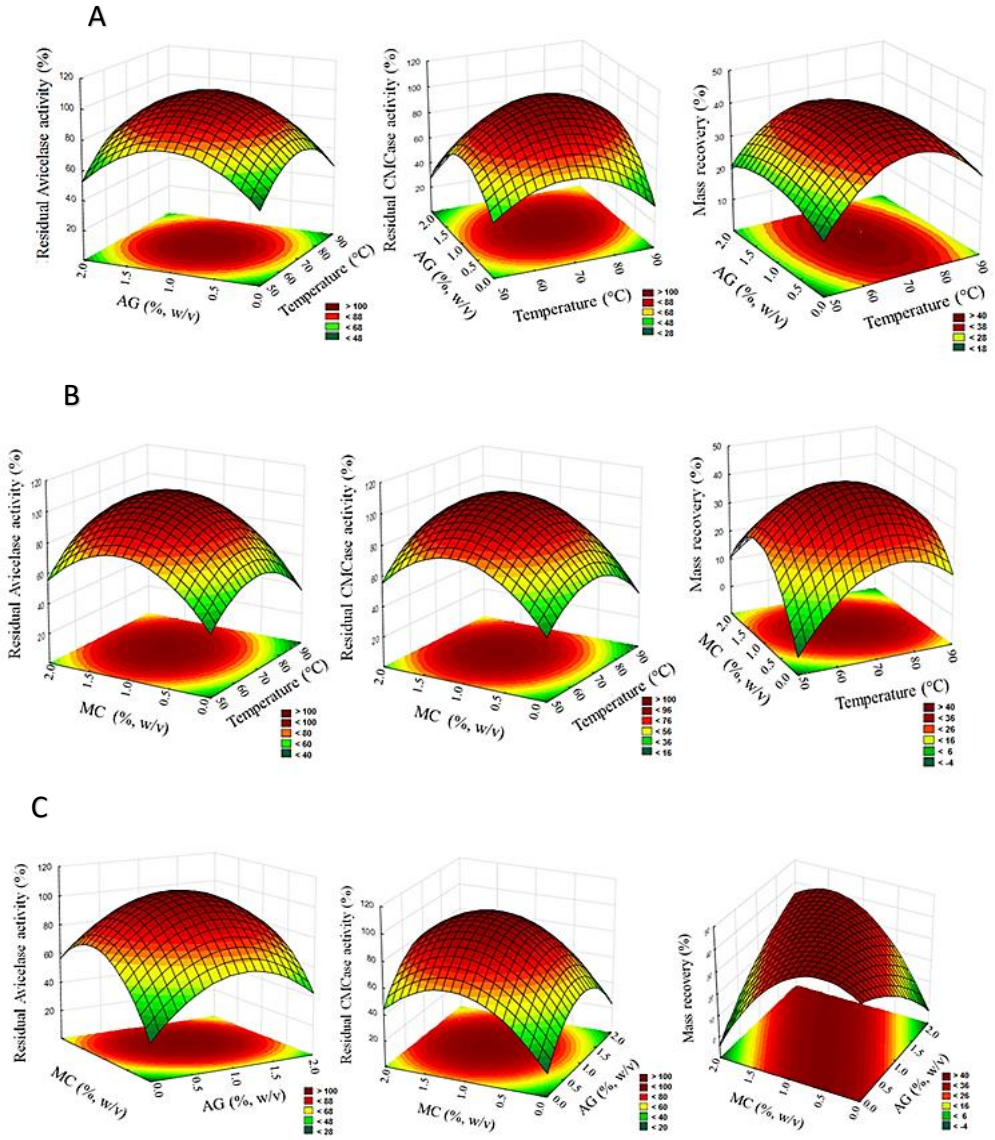

Figure 2. Three-dimensional response surface plots representing effects of A - AG concentration (%, w/v) and spray dryer inlet temperature (ºC) on Residual avicelase activity (%), Residual CMCase activity (%) and Mass recovery at a fixed MC concentration (1.0%, w/v); B - Spray dryer inlet temperature (ºC) and MC concentration (%,w/v) on Residual avicelase activity (%), Residual CMCase activity (%) and Mass recovery at a fixed AG concentration (1%, w/v); C - MC concentration (%, w/v) and AG concentration (%, w/v) on Residual avicelase activity (%), Residual CMCase activity (%) and Mass recovery at a fixed spray drier inlet temperature (70ºC). Dark red color indicates high Residual enzyme activity (%), while green and yellow indicate low Residual enzyme activity (%).

The enzymes recovered from SMIA-2 strain were not purified before the spray drying process, which explains why the crude cellulase extract obtained in this study contains by-products of the fermentation process (other proteins, carbohydrates and salts). On the other hand, several industrial processes employ enzymes in the presence of impurities originating from culture supernatants, as observed in detergent formulations (Niyonzima 2018).

Spray drying is a cost-effective drying method widely used to stabilize enzymes (Schutyser et al. 2012; Assadpour; Jafari 2019; Bajaj et al. 2021). However, when subjected to the high temperatures used in

the spray drying process, some enzymes may be denatured and, consequently, lose their catalytic activity (Hamin et al. 2014). A variety of excipients are often used as bulking agents and protective additives to stabilize the enzymes (Ohtake et al. 2011; Emami et al. 2018).

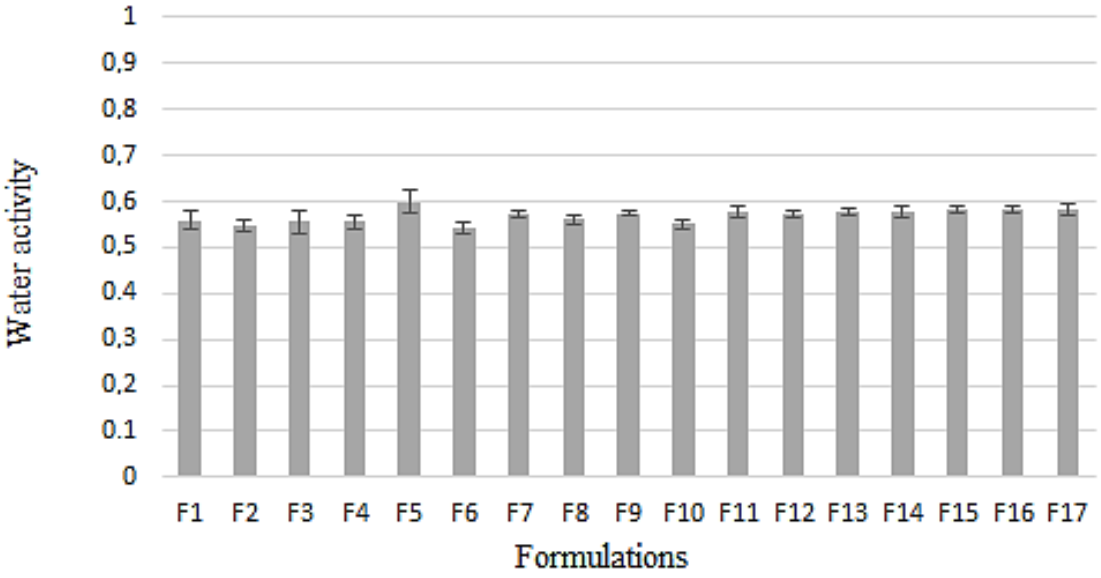

Figure 3. Water activity (aw) of the cellulases powders formulations.

According to Ladeira et al. (2015), the optimum temperature for both avicelase and CMCase activities produced by the *Bacillus* sp. SMIA-2 was 70 °C. At 80 °C, the avicelase activity was still suitable, but CMCase activity presented a sharp decrease. Thus, to minimize the thermal denaturation leading to the loss of cellulase activities, arabic gum (AG) and microcrystalline cellulose (MC) were incorporated into the cellulase solutions before spray drying. The interactive effect between its concentrations and the inlet air temperature (°C) were also studied, using a central composite design (CCD) 2^3, in order to obtain the maximal retention of the enzymes.

Selection was based on their function and safety. Arabic gum is a dry exudate obtained from the stems and branches of acacia trees. This ecological and highly soluble polymer is widely used in the food, pharmaceutical and numerous other industries (Musa et al. 2019). Microcrystalline cellulose has been used in the food and cosmetics industries, as a suspension stabilizer and water retainer (Nwachukwu and Ofoefule 2017). It is a strong, non-stick, absorbent, filler and thinner dry binder (Thoorens et al. 2014). The incorporation of 0.75-1.25% (w/v) of both adjuvants into cellulase solutions before spray drying, at inlet air temperature of 70 °C, provided powder with satisfactory levels of avicelase and CMCase activity, ease reconstitution in water and less powder adhesion on the internal walls of the drying chamber. As the culture supernatant generally contains a small number of solids, the use of excipients is justified not only to develop more stable enzyme formulations, but also to improve dry mass recovery (Libardi et al. 2020).

Several studies have approached the addition of carbohydrates to formulations containing enzymes. Siqueira et al. (2013) selected the adequate adjuvant and evaluated the best parameters to be used in the drying of *Trichoderma harzianum* enzymatic extract containing peptidases and cellulases. According to these authors, higher activity preservation was obtained when the additives were incorporated before the formulations were spray dried. They also found that the degree of improvement was dependent on the nature of the carbohydrate and its concentration. The use of spray drying technique with additives, especially maltodextrin, was considered an efficient method for drying abattoir's rumen fluid to produce an environmental friendly cellulase additive for animal feeding (Sarteshnizi et al. 2018). The concentrated cellulase extract from *Trichoderma harzianum* (TRIC03-LPBII) was dried in a spray-dryer, with the addition of maltodextrin, at 20% (w/v), which resulted in a powder enzymatic formulation with 85% stability, after 60 days (Libardi et al. 2020).

Water activity is fundamental for the maintenance and stability of enzyme activity. With the exception of formulation 5 (F5), all formulations presented water activity below the minimum value for the proliferation of decomposer microorganisms, which is a very desirable trait. Bacteria, yeast and filamentous fungi present water activity values of 0.9, 0.88 and 0.8, respectively, which are higher than those found in the spray dryer formulations reported in this work (0.55-0.62).

The cellulase-rich extract dried in the presence of 1.0 % (w/v) AG, 1.0% (w/v) MC and at inlet temperature of 70 °C was more stable during storage at 5 °C than at 25 °C. Therefore, when the enzymes were stored at low temperatures, it was possible to extend the shelf life of both Avicelase and CMCase.

5. Conclusions

The study demonstrated that the spray drying process used in this work was effective to dehydrate the cellulases recovered from the submerged cultures of *Bacillus sp.* SMIA-2, while preserving their enzymatic activity, when stored at 5°C, for 180 days. These findings will be extremely useful for industrial purposes, especially in detergent formulations.

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