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Eficácia de Celulases do *Bacillus* sp. SMIA-2 como um Aditivo em Formulações de Detergentes

Effectiveness of Bacillus sp. SMIA-2 Cellulases as an Additive in Detergent Formulations

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Enzymes with greater thermostability and alkaline stability have become the main choice as detergent additives. In this article, the compatibility of cellulases recovered from a thermophilic *Bacillus* sp. SMIA-2 in the presence of the surfactants, oxidizing and bleaching agents was studied. In addition, the Central Composite Design (CCD) was adopted to determine the range of the best concentrations of a mixture of three components: Triton X-100, Renex 95, and Hydrogen peroxide (H_2O_2) for preserving celulases activities. Finally, the impact of the cellulases in the presence of these three components to hydrolyze cellulosic fabrics was also evaluated. The results revealed that the celulases showed good compatibility with Triton X-100, Renex 95 and hydrogen peroxide to all concentrations tested (1.0, 4.0, and 8.0% w/v). In the presence of sodium perborate, the compatibility of cellulases decreased as the oxidant concentration increased from 1 to 8% (w/v). Although the cellulases were stable over a wide concentration range of Triton X-100, Renex 95, and Hydrogen peroxide, its performance to hydrolyze a cellulose fiber from the cotton fabrics was higher when any of the three components in the cleaning solution was used in lower concentrations, which is important considering the toxicity of these three components.

Keywords: *Bacillus*; cellulase; detergent; compatibility.

1. Introduction

Bacillus sp. SMIA-2 is a Gram-positive, aerobic, thermophilic, spore-forming bacterium capable of producing thermostable cellulases in submerged cultures employing agricultural byproducts such as sugarcane bagasse and passion fruit rind flour,[1,2] which opened perspectives to generate high-value products from sustainable production processes. According to Bernardo *et al.*,[3] the cellulase activities of SMIA-2 could be supported by three cellulolytic enzyme loci (e.g. endoglucanase) under a putative cellulosome complex. The cellulosomal cellulases from *Bacillus* sp. SMIA-2 have both carboxymethylcellulase (CMCase) (carboxymethylcellulose - hydrolyzing enzymes) and avicelase (avicel-hydrolyzing enzymes) activity; however, the 'cellulases' had a higher activity with avicels.[1]

Cellulases are used in detergents as softening, anti-pilling, and color-reviving agents. The process is environmentally friendly, and the use of cellulases and other detergent compatible enzymes diminish the utilization of toxic detergent constituents that are hazardous to humans.[4] The use of alkaline cellulase attracted the industrial sector as a potential detergent additive due to its ability to selectively contact cellulose inside the fibers and remove the soil in the interfibrillar spaces in the presence of the most conventional detergent ingredients.[5]

Bacillus sp. SMIA-2 produced cellulases capable of functioning at high temperatures and pH levels, retained good stability in the presence of several surfactants, oxidizing agents and locally available detergents,[1,6] which indicated the potential for the use of this bacterium and their cellulases for various industrial applications, such as in the detergent industry.

In this article, the compatibility of cellulases recovered from a thermophilic *Bacillus* sp. SMIA-2 in the presence of the surfactants Triton-100 and Renex 95 and the oxidant hydrogen peroxide was studied. Besides, the impact of the cellulases in the presence of these three components to hydrolyze cellulosic fabrics was studied to define general parameters for cellulases applications in detergents.

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2. Experimental

2.1. Organism and enzyme production

The present study used a thermophilic *Bacillus* sp. strain SMIA-2, previously isolated from a soil sample collected in the city of Campos dos Goytacazes, Rio de Janeiro, Brazil.[7]

The culture medium used in this work for cellulase production contained (g/L of distilled water) : 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 (SCB) (81.05% cellulose, 18.75% hemicellulose, 5.45% lignine)- 0.3%, commercial corn steep liquor (Sigma Aldrich) - 0.3% and passion fruit rind flour (obtained from a local market) - 0.3%.[8]

The pH of the medium was adjusted to 7.2 with 1.0 M NaOH, which is the optimal pH value for the growth of the microorganism and subsequently, the medium 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 then centrifuged (HERMLEZ 382K, Wehingen, Germany) at 15,500 g for 15 min, at 4 °C. Arabic Gum (1%, w/v) and microcrystalline cellulose (1%, w/v) were incorporated into the cell-free supernatant (enzyme solution) as bulking agents and protective additives, and this feed solution was then spray-dried, using a model SD-04 Lab-Plant Spray Dryer (inlet air temperature = 70±2 °C, outlet air temperature = 100±1 °C, pressure 5 Kgf /cm^2 and flow rate = 2 mL/min). The feed solution was constantly stirred using a magnetic stirrer to maintain homogeneity and introduced into the spray dryer by a peristaltic pump operating from 1 to 100 rpm with a 4 mm diameter silicone tube. The dried particles containing 240 Ug^{-1} avicelase and 55 Ug^{-1} CMcase were collected and stored at room temperature.

2.2. Enzyme assay

The cellulolytic enzyme activities were determined using the dinitro salicylic acid method,[9] which measures reducing sugars. The reaction mixture containing 0.5 mL of 1% (w/v) substrate solution (Carboxymethylcellulose sodium salt or avicel, PH-101) prepared in 10 mM sodium phosphate buffer, pH 8.0, and 0.5 mL of 1% (w/v) enzyme solution, was incubated at 70 °C. After 10 min of reaction, 1 mL of dinitro salicylic acid reagent was added and boiled in a 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 a 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 µmole 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 were run. To exclude the background of reducing sugars found in the enzyme supernatant from the results, a substrate blank containing 0.5 mL of 10 mM sodium phosphate buffer and 0.5 mL enzyme solution was also run. The absorbance of the enzyme blank and the substrate blank were subtracted from the absorbance of the activity assay. All of the samples were run in triplicate, while the blanks were run in duplicate.

2.3. Compatibility of cellulases with surfactants, oxidants and bleaching agents

The surfactants (Triton X-100, Renex 60, Renex 95 and Linear Alkylbenzene Sulfonate (LAS), oxidants (hydrogen peroxide and sodium perborate) and bleaching agents (Ethylenediamine tetraacetic acid (EDTA) and sodium carbonate) were incorporated into the reaction mixture (1, 4 and 8 %, w/v) and the reaction was carried out under standard assay conditions. The activity of the enzyme assayed in the absence of surfactants, oxidizing and bleaching agents was taken as 100%.

2.4.Cleaning solution

The cleaning formulated solutions (25mL) were prepared by mixing the components in this order: Triton X-100 (0, 4, 10, and 20% (w/v), Renex 95 (0, 4, 10, and 20% (w/v), hydrogen peroxide (0, 4, 10, and 20% (w/v), Na_2CO_3 as alkalinity agent (8% w/v) and water (up to 100%, w/v). The *Bacillus* sp. SMIA-2 cellulase concentration was fixed at 1% (w/v), following recommendations for formulating enzymatic detergents.[10] All solutions presented pH levels higher than 8.5.

2.5. Stability of cellulases with the cleaning solution

The stability of cellulases was determined by incubating the enzyme solution (1%, w/v) with the cleaning solutions at 45 °C. Aliquots (0.5 mL) were taken after one-hour incubation and the residual activity determined under standard assay conditions and compared with the control sample incubated at 45 °C without any ingredient.

2.6. Performance of cellulases to hydrolyze a cellulose fiber from the cotton fabrics

The cleaning solution (25mL) containing Triton X-100 (0, 4, 10, and 20% (w/v), Renex 95 (0, 4, 10, and

20% (w/v), hydrogen peroxide (0, 4, 10, and 20% (w/v), alkalinity agent (8% w/v), *Bacillus* sp. SMIA-2 cellulases (1% w/v) was added to 125mL erlenmeyer flasks at the required concentration in each assay. Small cotton fabrics (3.0 x 3.0 cm, standardly stained EMPA fabrics i.e. EMPA 111, TEXCONTROL) was added to the flasks containing the cleaning solution and the flasks were incubated at 45 °C under 150 rpm shaking on a Thermo Forma orbital shaker (Ohio, USA) for 60 minutes.

The hydrolysis of the cellulose fiber from the cotton fabrics was analysed by measuring the amount of solubilized reducing sugars by the dinitrosalicylic acid method with glucose as a standard.[11] After incubation, aliquots (0.5 mL) were taken and 1 mL of dinitro salicylic acid reagent,[9] was added and boiled in a water bath for 5 min. The resulting samples were then cooled to room temperature, and the absorbance was measured at 540 nm. The reducing sugars (mg/mL) were measured by using a glucose standard curve. Appropriate control was conducted in parallel with all assays. To exclude the background of reducing sugars found in the cotton fabrics from the results, a blank containing 0.5 mL of the water solution containing only the cotton fabrics was also run.

2.7. Experimental design and statistical analysis

The surface-response methodology (SRM) was used to obtain a model for Residual CMCase activity (Equation 2), Residual avicelase activity (Equation 3), and Reducing sugar groups released during the washing of test fabric (EMPA 111) with the cleaning solutions (Equation 4). Initially, a central composite design (CCD) 2^3 was constructed to evaluate the stability of CMCase and Avicelase in the presence of Triton X-100, Renex 95, and hydrogen peroxide (Equation 2 and Equation 3, respectively). Thereafter, a central composite design (CCD) 2^3 was also constructed to evaluate the performance of the cellulases in the presence of these three components to hydrolyze a cellulose fiber from the cotton fabrics (Equation 4). The factorial planning had three central points and yielded a total of 17 treatments for each experiment. The concentrations of Triton X-100, Renex 95, and hydrogen peroxide in the factorial design were determined so that factor level -1.68 represented 0% concentration and factor level 0 (central point) represented 10% concentration of the analyzed components. From these concentration values, the other factor levels (-1, +1 and +1.68) were defined according to Table 1.

The results were evaluated using the Statistica software system, version 10.0. In this context, the F test was used as a validation criterion of statistical significance of the models obtained at a confidence level of 95% (Experiment 1, 2 and 3).

Condition optimization was performed using CCD, and surface-response was produced for three experiments. The model of the experiment can be expressed as follows:

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

where *bo*, *bi*, *bii* and *bij* are the intercept terms, linear, quadratic coefficient, and interactive coefficient, repectively, and *xi* and *xj* are coded independent variables.

3. Results and Discussion

It is known that due to wear and tear in washing, fabrics, both made of cotton and those composed of artificial or synthetic fibers, produce an accumulation of fibers on the surface of the yarn, forming undesirable "little balls", which will generally cause a pronounced, unpleasant harshness in the fabric. Anti-pilling is a treatment that aims to eliminate these loose fibers in the fabric and prevent the formation of pills. Currently, cellulases are added to detergents not only to improve cleaning performance, but also because they have an exceptional anti-pilling effect.[12] The process is environmentally friendly, since diminishes the utilization of toxic detergent constituents that are hazardous to humans. In this context, the suitability of the *Bacillus* sp. SMIA-2 crude cellulases to be incorporated in a detergent formulation was investigated by testing its impact in the presence of detergent components to hydrolyze cellulosic fabrics.

The compatibility of the cellulases with surfactants, oxidants, and bleaching agents is shown in Table 2. The avicelase showed good compatibility with the surfactants Renex 95 and Triton X-100 to all concentrations tested (1.0, 4.0, and 8.0% w/v), but was drastically inhibited in the presence of Renex 60 and LAS. The CMCase showed good compatibility with Triton X-100 and lost about 20-30% of its activity in the presence of Renex 95. In the presence of Renex 60 and LAS the enzyme lost about 60% of its activity. The CMCase maintained its activity when incubated in the presence of EDTA to all concentrations tested, but the activity of avicelase was inhibited at higher concentrations.

The celulase from *Paenibacillus barcinonensis* showed stability with 0.5% concentration of various surfactants such

Table 1. Factors and Levels studied in CCD

Factors	Factor Level				
	-1.68	**-1**	**0**	**+1**	**+1.68**
Triton X-100	0%	4%	10%	16%	20%
Renex 95	0%	4%	10%	16%	20%
Hydrogen peroxide	0%	4%	10%	16%	20%

as SDS, Tween 20, and Triton X-100. In the presence of EDTA, the enzymatic activity was decreased.[13] According to Saraswat *et al.*[14], the effect of surfactants on enzyme activity varies depending on the type of enzyme, but in general, nonionic surfactants are more benign to an enzyme than anionic surfactants. However, the cellulases from *Paenibacillus* sp. IHB B 3084[15] and *Bacillus* sp. CY8[16] were slightly inhibited by nonionic surfactants.

In the presence of sodium carbonate, the avicelase activity was stimulated in all three concentrations tested. The CMCase maintained about 72% of its activity when incubated in the presence of 1% (w/v) sodium carbonate. The enzymes were compatible with hydrogen peroxide to all concentrations tested (1.0, 4.0, and 8.0% w/v). In the presence of sodium perborate, the compatibility of cellulases decreased as the oxidant concentration increased from 1 to 8% (w/v).

Taking into account the results presented in Table 2, a central composite design (CCD) 2^3 was constructed to study the effect of cleaning solutions containing combined concentrations of Renex 95, Triton X-100, and hydrogen peroxide on cellulases residual activities. In addition, the performance of the formulated cleaning solutions on cellulose fiber from the cotton fabrics was also evaluated.

According to the results presented in Table 3, the activity of CMCase was lower, when compared with avicelase. Avicelase and CMCase activity is a measurement of the ability of the enzyme to hydrolyze microcrystalline cellulose and carboxymethylcellulose, respectively. Ladeira *et al.*[1] reported that the cellulosomal cellulases from *Bacillus* sp SMIA-2 has both CMCase and avicelase activity; however, the 'cellulases' had a higher activity with avicels.

The amounts of Triton X-100, Renex 95, and hydrogen peroxide affected the residual activities of both, CMCase and avicelase. The highest Residual CMCase (0.65 $U.mL^{-1}$) and avicelase (2.61 $U.mL^{-1}$) were observed in the treatments 16 and 12, respectively.

The performance of cellulases to hydrolyze the fiber of cotton fabric was evaluated by analyzing the reducing sugars released in the cleaning solution after cotton fabric washing. The highest amount of reducing sugar released into the cleaning solution after cotton fabric washing, was found in the treatment 3 (1.29 mg/mL) and 12 (1.22 mg/mL), suggesting that in these treatments the simultaneous action of CMcase and avicelase enzymes was more efficient in removing microfibrillar material with reducing power. Therefore, the variation of reducing end groups (reducing power of cotton) in cotton fibers after fabric washing showed that CMCase and avicelase activities in the crude cellulase mixture were affected by different concentrations of Triton X-100, Renex 95, and hydrogen peroxide used to formulate cleaning solutions.

The statistical significance of the model equation was assessed by an F-test (ANOVA) and the data are shown in Table 4. An equation for Residual CMCase activity (Eq. 2), Residual avicelase activity (Eq. 3) and for Reducing sugar amount (Eq. 4) was developed based on a regression analysis of the following experimental data:

$$X = 0.639322 - 0.068152_{X1} - 0.097204_{X1}^2 + 0.006267_{X2} - 0.050288_{X2}^2 - 0.017915_{X3} - 0.036977_{X3}^2 - 0.037337_{X1X2} + 0.023463_{X1X3} + 0.010812_{X2X3} \quad (2)$$

$$Y = 2.373116 + 0.261635_{X1} - 0.507103_{X1}^2 + 0.140951_{X2} - 0.073240_{X2}^2 - 0.099740_{X3} - 0.103787_{X3}^2 + 0.201250_{X1X2} - 0.218225_{X1X3} + 0.092600_{X2X3} \quad (3)$$

$$Z = 0.566321 + 0.028654_{X1} - 0.040319_{X1}^2 + 0.144571_{X2} + 0.118120_{X2}^2 - 0.023516_{X3} + 0.173116_{X3}^2 - 0.259427_{X1X2} - 0.123623_{X1X3} - 0.192118_{X2X3} \quad (4)$$

where x_1 is the Triton X-100, x_2 is the Renex 95 and x_3 is the Hydrogen peroxide concentration.

Table 2. Effect of surfactants, oxidizing, and bleaching agents on residual cellulases activities. (100% of the avicelase activity = 2.3541 U. mL^{-1} and 100% of the CMCase activity = 0.624 $U.mL^{-1}$)

	CMCase[1]							
	Surfactants				**Oxidizing agents**		**Bleaching agents**	
	Triton X-100	**Renex 95**	**Renex 60**	**LAS**	**H_2O_2**	**Sodium perborate**	**EDTA**	**Sodium carbonate**
1 % (w/v)	92.1 dC	79.1 eA	45.5 gB	44.7 gB	113.7 bA	102.4 cA	124.6 aB	71.6 fA
4 % (w/v)	93.7 cB	70.2 eC	42.5 gC	42 gC	111.7 bB	73.2 dB	124.4 aB	58.1 fB
8 % (w/v)	96 cA	75.7 dB	46.5 gA	47.3 gA	113.3 bA	57.9 eC	127.0 aA	52.0 fC
	Avicelase[1]							
	Surfactants				**Oxidizing agents**		**Bleaching agents**	
	Triton X-100	**Renex 95**	**Renex 60**	**LAS**	**H_2O_2**	**Sodium perborate**	**EDTA**	**Sodium carbonate**
1 % (w/v)	99.3 dB	104.0 bA	38.9 fA	9.2 gB	102.4 cB	75.4 eA	102.0 cA	110.3 aB
4 % (w/v)	100.1 dA	101.1 cB	21.5 gB	12.9 hA	106.9 bA	62.6 eB	49.1 fB	113.7 aA
8 % (w/v)	95.8 cC	96.2 cC	12.0 fC	8.7 gC	100.6 bC	57.4 dC	46.0 eC	113.0 aA

[1]Means with the same lowercase letters on the same line do not differ significantly at $p \leq 0.05$, according to the Tukey Test; Means with the same capital letters in the same column do not differ significantly at $p \leq 0.05$, according to Tukey's test.

Table 3. Matrix of CCD 2^3 (real and coded values) used and its response (CMCase and Avicelase activity and Reducing sugar)

Treatments	Triton X-100%	Renex 95%	Hydrogen peroxide	Residual activity (UmL^{-1})		Reducing sugar groups (mg/mL)
				CMCase	Avicelase	
1	(-1) 4%	(-1) 4%	(-1) 4%	0.55	1.04	0.36
2	(+1) 16%	(-1) 4%	(-1) 4%	0.40	2.32	0.95
3	(-1) 4%	(+1) 16%	(-1) 4%	0.63	1.12	1.29
4	(+1) 16%	(+1) 16%	(-1) 4%	0.34	2.32	1.18
5	(-1) 4%	(-1) 4%	(+1) 16%	0.40	1.52	0.61
6	(+1) 16%	(-1) 4%	(+1) 16%	0.35	1.04	1.05
7	(-1) 4%	(+1) 16%	(+1) 16%	0.53	1.08	1.11
8	(+1) 16%	(+1) 16%	(+1) 16%	0.33	2.30	0.17
9	(-1.68) 0%	(0) 10%	(0) 10%	0.46	0.97	0.30
10	(+1.68) 20%	(0) 10%	(0) 10%	0.31	1.19	0.54
11	(0) 10%	(-1.68) 0%	(0) 10%	0.53	1.99	0.52
12	(0) 10%	(+1.68) 20%	(0) 10%	0.51	2.61	1.22
13	(0) 10%	(0) 10%	(-1.68) 0%	0.54	2.37	0.87
14	(0) 10%	(0) 10%	(+1.68) 20%	0.57	2.07	1.18
15	(0) 10%	(0) 10%	(0) 10%	0.63	2.35	0.54
16	(0) 10%	(0) 10%	(0) 10%	0.65	2.33	0.57
17	(0) 10%	(0) 10%	(0) 10%	0.64	2.40	0.60

Table 4. ANOVA for the variables of response surface quadratic model for Residual CMCase activity, Residual avicelase activity and Reducing sugar

Variable	Sum of squares	(Degrees of freedom)	Mean square	Fcal	F statistic
Residual CMCase activity					
Regression	0.210	3	0.0671	45.216	3.41
Residues	0.019	13	0.0015		
Lack of adjustement	0.019	11	0.0017	17.347	19.4
Pure error	0.0002	2	0.0001		
Total error	0.220	16	0.0138		
				R^2 =	91.26%
Residual avicelase activity					
Regression	5.196	2	2.606	37.094	3.74
Residues	0.984	14	0.070		
Lack of adjustement	0.981	12	0.082	62.881	19.4
Pure error	0.0026	2	0.0013		
Total error	6.0892	16	0.381		
				R^2 =	83.85%
Reducing sugar (mg/mL)					
Regression	1.772	4	0.443	20.675	3.26
Residues	0.257	12	0.021		
Lack of adjustement	0.255	10	0.025	28.363	19.4
Pure error	0.0018	2	0.0009		
Total error	2.057	16	0.128		
				R^2 =	87.50%

The models showed significant regression at the 95% confidence level (F_{cal} higher than F_{tab}) with R^2 equal to 0.9087, 0.8399 and 0.8768 for Residual CMCase activity, Residual avicelase activity and Reducing sugar amount respectively, showing that it was suitable to represent the real relationship among the independent variables studied.

The lack of adjustment for Residual CMCase activity was not significant (F_{cal} less than the F_{tab}), indicating that the experimental data adjusted to the obtained models. However, for Residual avicelase activity and Reducing sugar amount the lack of adjustment significant for the models suggests the need for research on a more accurate mathematical model to predict the Residual avicelase activity and Reducing sugar amount in function of Triton X-100, Renex 95 and Hydrogen peroxide concentrations used to formulate the cleaning solution.

The response surface and contour plot figures obtained by the analysis of the experimental data of CCD showed a relationship between two variables at a time. The non-explicit variables were fixed at the central point (level 0) for the surface construction.

The effect of the interaction between Renex-95 and Triton X-100 concentration when the Hydrogen peroxide concentration remained constant at level 0 (10%, w/v) on Residual cellulases activities and Reducing sugar amounts is showed in the response surface plot of Figure 1A, 1D,

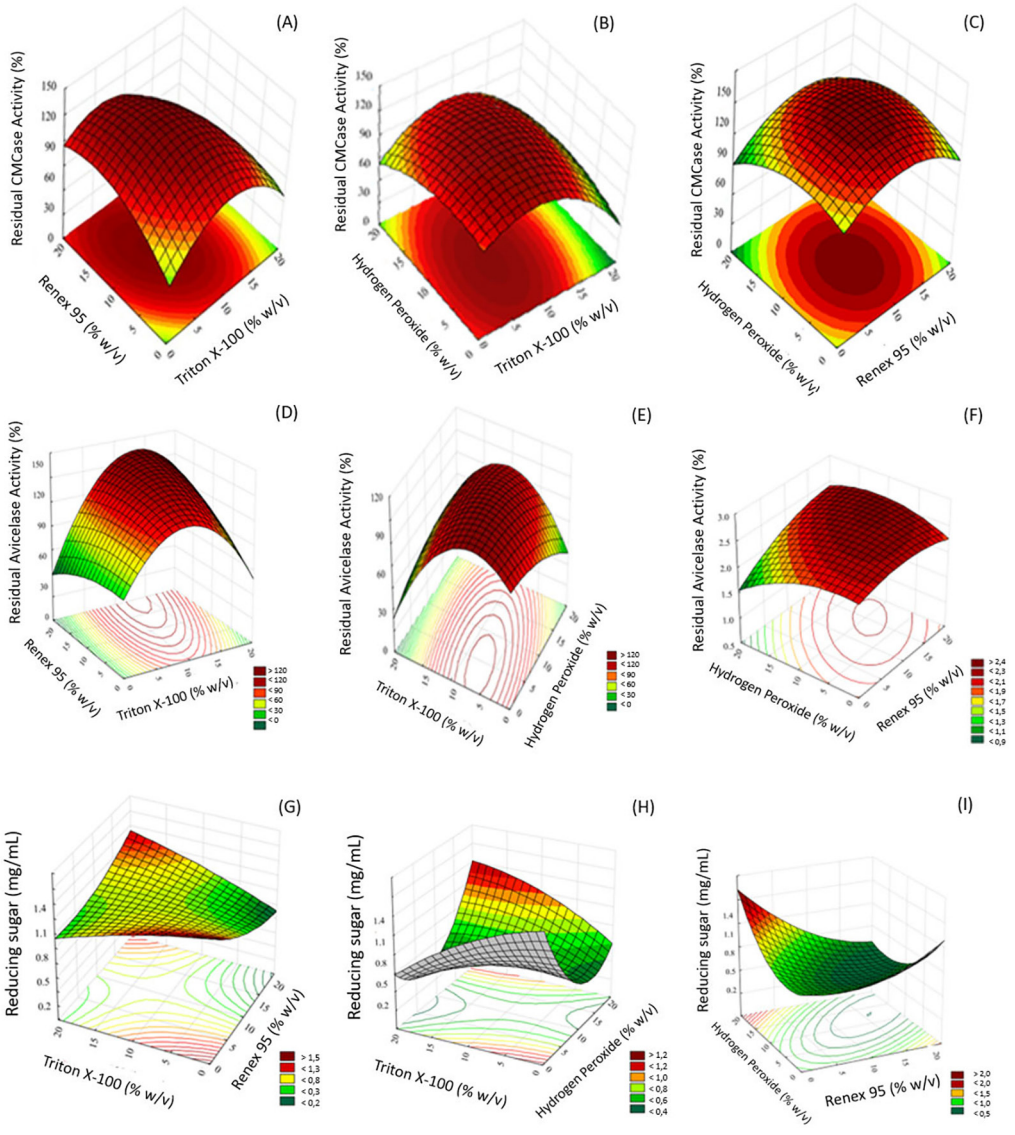

Figure 1. Three-dimensional response surface plots representing effects of (A, D) - Renex-95 and Triton X-100 concentration (%, w/v) on Residual CMCase activity (%) and Residual avicelase activity (%) at a fixed Hydrogen peroxide concentration (10%, w/v); (B, E) - Hydrogen peroxide and Triton X-100 concentration (%, w/v) on Residual CMCase activity (%) and Residual avicelase activity (%) at a fixed Renex-95 concentration (10%, w/v); (C, F) - Hydrogen peroxide and Renex-95 concentration (%, w/v) on Residual CMCase activity (%) and Residual avicelase activity (%) at a fixed Triton X-100 concentration (10%, w/v); (G) - Renex-95 and Triton X-100 concentration (%, w/v) on Reducing sugar (%) at a fixed Hydrogen peroxide concentration (10%, w/v); (H) - Hydrogen peroxide and Triton X-100 concentration (%, w/v) on Reducing sugar (%) at a fixed Renex-95 concentration (10%, w/v); (I) Hydrogen peroxide and Renex-95 concentration (%, w/v) on Reducing sugar (%) at a fixed Triton X-100 concentration (10%, w/v). Dark red color indicates high Residual enzyme activity (%), while green and yellow indicate low Residual enzyme activity (%).

and 1G. The maximal CMCase activity was observed when concentrations between 5-15% (w/v) Triton X-100 and 10-15% (w/v) Renex-95 were incorporated into cleaning solutions. The combination of Triton X-100 between concentrations of 5-15% (w / v) with any concentration of Renex-95, within the studied range, allowed obtaining good levels of avicelase activity. Regarding the amount of reducing sugars, higher amounts were found when the cleaning solution contained between 15-20% (w/v) Renex 95 and lower concentrations of Triton X-100 (<5%, w/v) or when the cleaning solution contained between 15-20% (w/v) Triton X-100 and lower concentrations of Renex-95 (< 5%, w/v). The effect of the interaction between Hydrogen peroxide and Triton X-100 concentration when the Renex-95 concentration remained constant at level 0 (10%, w/v) on Residual cellulases activities and Reducing sugar amounts is showed in the response surface plot of Figure 1B, 1E, and 1H. Higher residual CMCase activity was found when concentrations of Triton X-100 between 5-10% (w/v) and Hydrogen peroxide concentrations between 5-15% (w/v) were incorporated into cleaning solutions. The combination of any Hydrogen peroxide concentration within the studied range with concentrations of Triton X-100 between 5-10%, allowed obtaining good levels of avicelase activity. Higher values of Reducing sugar were obtained when higher concentrations of Hydrogen peroxide (20%, w/v) and lower concentrations of Triton X-100 (<5%, w/v) were used to formulate the cleaning solution or when higher concentrations of Triton X-100 and lower concentrations of Hydrogen peroxide were used in the cleaning solution.

Figures 1C, 1F and 1H presents the interactive effect between Hydrogen peroxide and Renex-95 concentrations on Residual CMCase activities, when the Triton X-100 concentration remained constant at level 0 (10%, w/v). Higher residual CMCase activities were found when H_2O_2 and Renex 95 concentrations between 5-15% (w/v) were used in the cleaning solution and higher levels of residual avicelase activity were found when Renex 95 concentrations between 10-15% (w/v) and Hydrogen peroxide between 5-10% (w/v) were incorporated into cleaning solution. As for the Reducing sugars, higher amounts were found when higher concentrations of Hydrogen peroxide in combination with very lower concentrations of Renex 95 were used in the cleaning solution or when higher concentrations of Renex 95 were used in combination with lower concentrations of Hydrogen peroxide.

An overall analysis of the results suggested that the experimental design approach used in this study was efficient in optimizing the concentration ranges of Triton X-100, Renex 95 and Hydrogen peroxide in the cleaning solution in order to obtain higher levels of CMCase and avicelase activities. Although the cellulases were stable over a wide concentration range of the three components used to formulate the cleaning solution, its performance to hydrolyze a cellulose fiber from the cotton fabrics was higher when any of the three components Triton X-100 (4%, w/v), Renex 95 (10%, w/v) or Hydrogen peroxide (4%, w/v) in the cleaning solution was used in lower concentrations. This was important because anionic and nonionic surfactants and oxidizing and bleaching agents can be toxic and cause environmental pollution. Thus, use of a low concentration of them in cleaning solution is necessary.

4. Conclusions

The cellulases of *Bacillus* sp. SMIA-2 (1%, w/v) can be combined with Triton X-100 (4%, w/v), Renex 95 (10%, w/v) and Hydrogen peroxide (4%, w/v) in a single composition so that the benefits of these three components in the hydrolysis of cellulose fiber in cotton fabrics can be achieved by improving the quality of the fabric, maintaining whiteness, brightening the colors and the rough lumps of cotton.

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