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Response Surface Methodologies to Investigate the Pretreatment of Sugarcane Bagasse via Alkaline Hydrogen Peroxide

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This study aims to assess a large number of information related to the process of sugarcane bagasse delignification process using alkaline hydrogen peroxide (H_2O_2). For this purpose, it was used a central composite rotatable design (CCRD) 2^3, with three central and six axial points, totalling 17 runs This matrix was applied in the temperature (25.0 to 60.0 ºC), H_2O_2 concentration (2.0 to 15.0 %) and pH (10.0 to 13.0) optimization, using the percentage of insoluble lignin analyzed by the Klason Method as response. The experiments were performed in duplicate. The statistical modelling was confirmed by analysis of variance (ANOVA) and the model was able to predict the response with 92.16% certainty. Through the investigation of response surface and contour curves, it was identified that the conditions optimized for the process are temperature of 60 °C, H_2O_2 concentration of 10 % m/v and pH of 11.5.

1. Introduction

The production of chemical compounds from lignocellulosic residues is a green chemistry strand. Sugarcane bagasse is a vegetal biomass that has much potential for use, because of their three structural elements: cellulose, hemicellulose and lignin. The pretreatment process with H_2O_2 leads to delignification of sugarcane bagasse. In other words, this process is mainly involved in effective separation of these complex interlinked fractions and increase the accessibility of each individual component. Shimizu et al., 2018 studied acid (H_2SO_4) pretreatment, alkaline (NaOH) pretreatment and pretreatment using H_2O_2 to remove lignin from banana pseudostem to increase the accessibility of cellulose. In this context, even without an experimental design, pretreatment using H_2O_2 proved to be more efficient in reducing the lignin content (from 17% to 7%), as well as alkaline pretreatment. Therefore, the cellulose content increased from 60% to 75% when pretreatment using H_2O_2 was applied. The acid pretreatment did not result in any improvement. However, the alkaline pretreatment generates several intermediate residues. Considering the principles of green chemistry, the pretreatment using H_2O_2 in sugarcane bagasse becomes more interesting, since it is a compound that dissociates in oxygen and water, without high environmental risk. According to Karagoz et al., 2012, pretreatment using H_2O_2 is more effective in solubilizing lignin, improving the digestibility of lignocellulosic biomass. With the demand for more efficient processes, with lower costs and environmental impacts, it becomes necessary to use computational tools to achieve optimal solutions. Aiming at the application of optimization methods, it is relevant to build a mathematical model that adequately describes the process to be studied. Ramli et al., 2017 used the response surface methodology with central composite design in order to evaluate the effects of the amino acid fermentation operation for isobutanol production. The evaluated parameters were temperature, pH, agitation speed and inoculum size. From the experimental results, it was possible to build a prediction model of the produced isobutanol yield. The use of the response surface allowed the identification of optimum values of the process parameters suited so that the yield of isobutanol was maximum. It is important to emphasize that the methodology for the quantification of residual lignin in the biomass after pretreatment is delayed, so that the result can only be acquired after 48 h of procedure. Thus,

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the construction of a statistical model capable of predicting the insoluble lignin content in biomass after pretreatment using H_2O_2, given the process parameters evaluated, is of high importance. Therefore, it is proposed in this study the use of experimental design to obtain the mathematical model to predict insoluble residual lignin content in sugarcane bagasse after pretreatment with H_2O_2. In addition, it was proposed, based on the developed model, to obtain the response surface and, through this analysis, to determine the optimized conditions, knowing the statistical significance of the responses.

2. Materials and Methods

2.1 Experimental design

A CCRD with three independent variables (temperature, H_2O_2 concentration and pH) was used to evaluate the parameters of the biomass delignification process. In total, 17 tests were performed: 8 factorial tests, 6 axial point to test the second order model and 3 tests in the central condition, indispensable to evaluate the quality of process repeatability.

Table 1 describes the levels in which the parameters were analyzed. The variables were coded as x_1, x_2 and x_3, respectively.

Table 1: Values used in the experimental design for the sugarcane bagasse delignification process.

Variables	Code	-1.68	-1	0	+1	+1.68
Temperature (°C)	x_1	25.0	32.1	42.5	52.9	60.0
Hydrogen Peroxide Concentration (% m/v)	x_2	2.0	4.6	8.5	12.4	15.0
pH	x_3	10.0	10.6	11.5	12.4	13.0

The statistical model used in the insoluble lignin content prediction in biomass after pretreatment shows a relation between the independent variables and the response, according to the polynomial equation shown in Eq(1).

$$y= \beta_0+\beta_1x_1+\beta_2x_2+\beta_3x_3+\beta_{12}x_1x_2+\beta_{13}x_1x_3+\beta_{23}x_2x_3+\beta_{11}x_1^2+\beta_{22}x_2^2+\beta_{33}x_3^2 \qquad (1)$$

The parameter y is the insoluble lignin content in biomass. x_1, x_2 and x_3 represent the variables coded for linear effects. x_{12}, x_{13} and x_{23} represent the interaction between coded variables. In addition, the other terms represent the parameters of the regression model, which were estimated using the least squares method.

The quality of the model for the insoluble lignin content prediction was evaluated by the F-value obtained from the analysis of variance (ANOVA), performed at software STATISTICA version 10.0 and by the observed regression coefficient.

2.2 Process of sugarcane bagasse delignification using alkaline hydrogen peroxide

Based on Rabelo et al., 2014 the sugarcane bagasse used in this work was washed under a stream of tap water, dried in an oven at 45 °C for 48 h to reduce bagasse moisture content. The lignocellulosic biomass was homogenized in an electric crusher in order to reduce particle size, fragmented into small fine particles and stored in a dessicator with no moisture for later use in the delignification experiments with H_2O_2 pretreatment. All the experiments were carried out from samples of this same biomass.

The process of lignin extraction from the biomass was carried out as Valim et al. (2017). The reactions were processed in 250 mL Erlenmeyer flasks placed on a shaker under constant temperature and agitation for 1 h after the addition of 2 g of biomass and 50 mL of an alkaline H_2O_2 solution at different solution concentrations. The values used for the temperature, pH and H_2O_2 concentration in the experiments were as defined in the experimental design step. The pH was adjusted to the values stipulated with KOH 5 M.

At the end of the reaction time, the mixtures (treated sugarcane bagasse and lignin solutions) were filtered. The liquid containing the lignin was collected and the bagasse was washed with distilled water and stored to later analyses. After 24 h, H_2SO_4 1 M was added to the lignin solution until the pH of the solution reached the value 2.0 for lignin precipitation. After a further 24 h, the acid solution containing the precipitated lignin was centrifuged, the supernatant was discarded, and the precipitate was washed with distilled water and dried for futther analysis. All tests were performed in duplicate.

2.3 Insoluble lignin determination

The standard methodology NREL (60) was used to determine the content of lignin insoluble (Sluiter et al., 2008). An aliquot of 300 mg was removed from the biomass obtained after delignification. This sample was hydrolysed with H_2SO_4 72 % (m/v) for approximately 2 h. The acid was diluted to 4 % (m/v) and the sample was placed in an autoclave at 121 °C for 1 h. A vacuum filtration was performed in the autoclave solution using pre-weighed crucibles. The crucible with the filtered solid was placed in an oven at 105 °C for 12 h. The insoluble lignin determination was performed in duplicate. Consequently, the result presented corresponds to the mean of the 4 samples results for the same condition studied.

Eq(2) shows the calculation of the lignin insoluble percentage in biomass, where m_1 corresponds to the mass in grams of the empty crucible and m_2 corresponds to the mass in grams of the crucible with the filtrate, after the autoclave stage.

$$\%\ \text{Insoluble Lignin} = \frac{(m_2 - m_1)}{0.3} \text{x} 100 \qquad (2)$$

3. Results and Discussion

Temperature, H_2O_2 concentration and pH are factors that directly influence the lignin extraction process of the biomass. Using CRRD, 17 experiments were performed with different values for each of the variables, in order to study the interaction effects between them in the process. Figure 1 shows the Pareto chart for the model obtained by the least squares method. It is possible to observe that the effects of the quadratic temperature and the linear relations between temperature with H_2O_2 concentration and temperature with pH are not significant for the model.

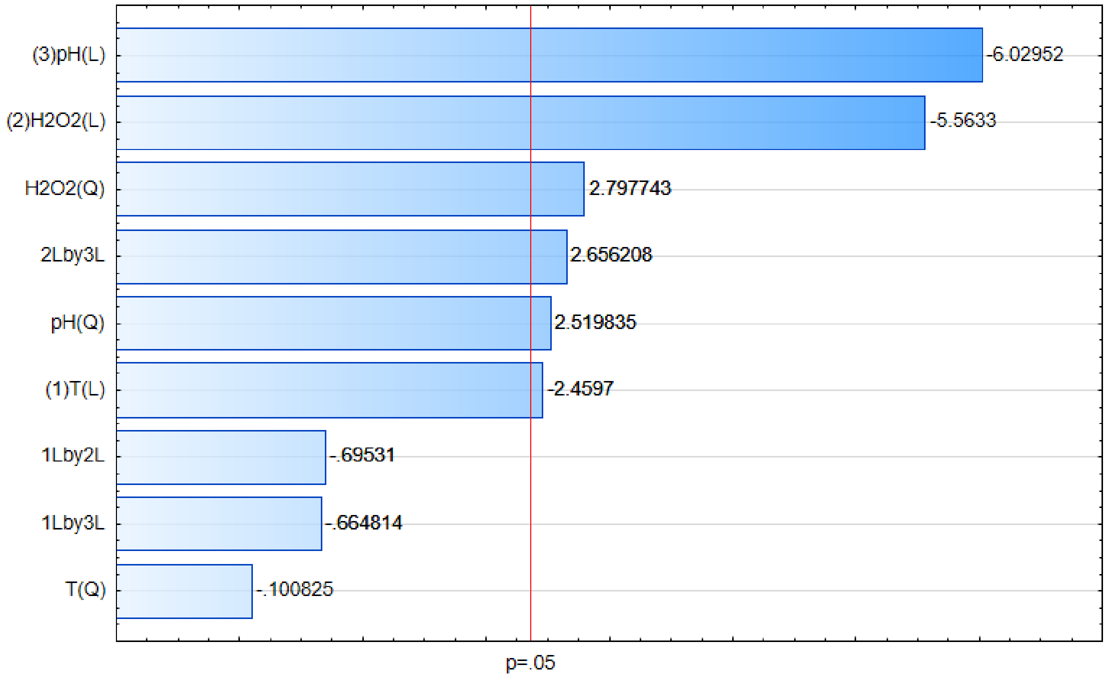

Figure 1: Pareto chart for the model obtained from experimental design developed.

Eq(3) describes the reparametrized mathematical model capable of predicting residual lignin content in sugarcane bagasse after pretreatment with H_2O_2.

$$y = 362.084 - 0.1236x_1 - 8.5357x_2 - 50.1036x_3 + 0.517x_2x_3 + 0.1083x_2^2 + 1.8345x_2^2 \qquad (3)$$

The model efficiency was analyzed using analysis of variance (ANOVA). Table 2 shows the F-value for each variable. The theoretical F-value for this case is 3.22. The coefficient of determination R^2 of 92.16% indicates that the statistical model is suitable for representing the relationship between the selected variables.

Table 2: Analysis of variance (ANOVA) for the prediction model of insoluble lignin in biomass.

Model Terms	Sum of Square	DF	Mean Square	F-value
x_1	22.5883	1	22.5883	7.6240
x_2	115.5541	1	115.5541	39.0019
x_2^2	32.5831	1	32.5831	10.9975
x_3	135.7328	1	135.7328	45.8126
x_3^2	26.4919	1	26.4919	8.9415
x_2x_3	26.3417	1	26.3417	8.8909
Error	29.6278	10	2.9628	-
Total	378.2404	16	-	-

Table 3 shows the mean of the experimental results and the value predicted by the generated model to predict the insoluble lignin content in the biomass.

Table 3: Comparisons between the experimental and predicted values for insoluble lignin content in the biomass after hydrogen peroxide pretreatment.

Runs	Variable			Insoluble Lignin (%)	
	Temperature (°C)	Hydrogen Peroxide Concentration (%)	pH	Experimental	Predicted
1	32.1	4.6	10.6	19.37	21.38
2	52.9	4.6	10.6	21.31	18.81
3	32.1	12.4	10.6	14.25	11.91
4	52.9	12.4	10.6	9.18	9.34
5	32.1	4.6	12.4	12.27	11.42
6	52.9	4.6	12.4	7.29	8.85
7	32.1	12.4	12.4	9.31	9.21
8	52.9	12.4	12.4	7.53	6.64
9	25	8.5	11.5	10.70	11.22
10	60	8.5	11.5	6.13	6.90
11	42.5	2	11.5	18.98	18.51
12	42.5	15	11.5	7.22	8.77
13	42.5	8.5	10	17.21	18.46
14	42.5	8.5	13	8.09	7.91
15	42.5	8.5	11.5	7.64	9.06
16	42.5	8.5	11.5	10.70	9.06
17	42.5	8.5	11.5	9.28	9.06

Figure 2 shows the 3-D surface plots representing the obtained model. The interaction between the temperature and H_2O_2 concentration variables can be understood and localized using the response surface plane.

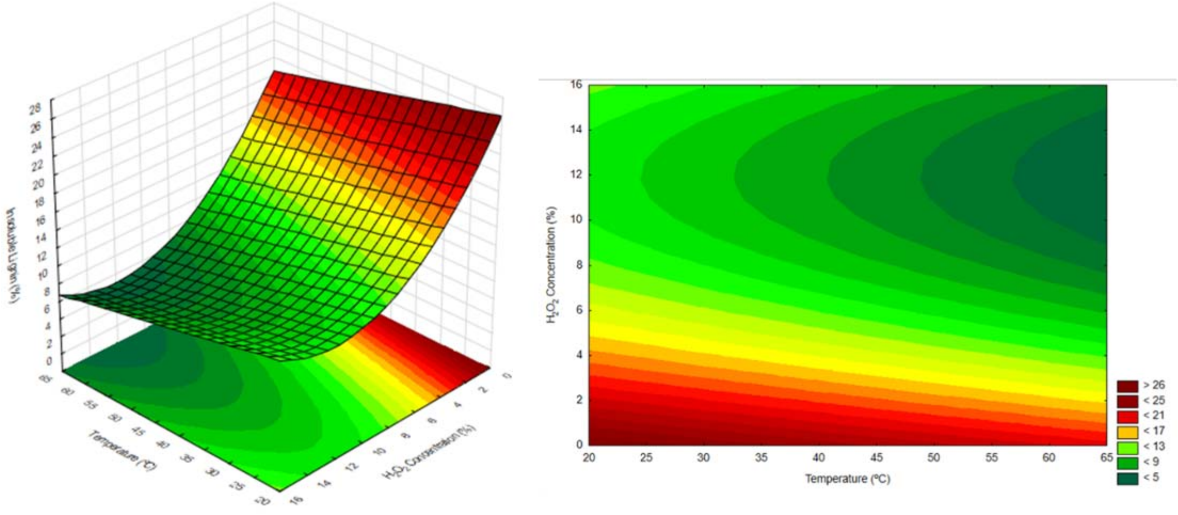

Figure 2: Effects of the temperature and H_2O_2 concentration variables on the predicted model of the insoluble lignin content in the biomass for the pH fixed at the central point.

It is possible to observe that the decrease of the H_2O_2 concentration leads to the higher value of insoluble lignin content in the biomass, regardless of the temperature used. However, for higher values of temperature and H_2O_2 concentration, the surface shows the trend that leads to the lower residual lignin content, which indicates that at that point, the sugarcane bagasse delignification process was successful.

Similarly, Figure 3 shows the interaction between the temperature and pH variables. According to the response surface graph, the decrease in pH values leads to an increase in the insoluble lignin content in biomass, regardless of the temperature used, indicating that lignin extraction in this condition is not satisfactory. However, the formation of a valley, resulting in a lower insoluble lignin content, is observed in processes that use higher values of pH and temperature.

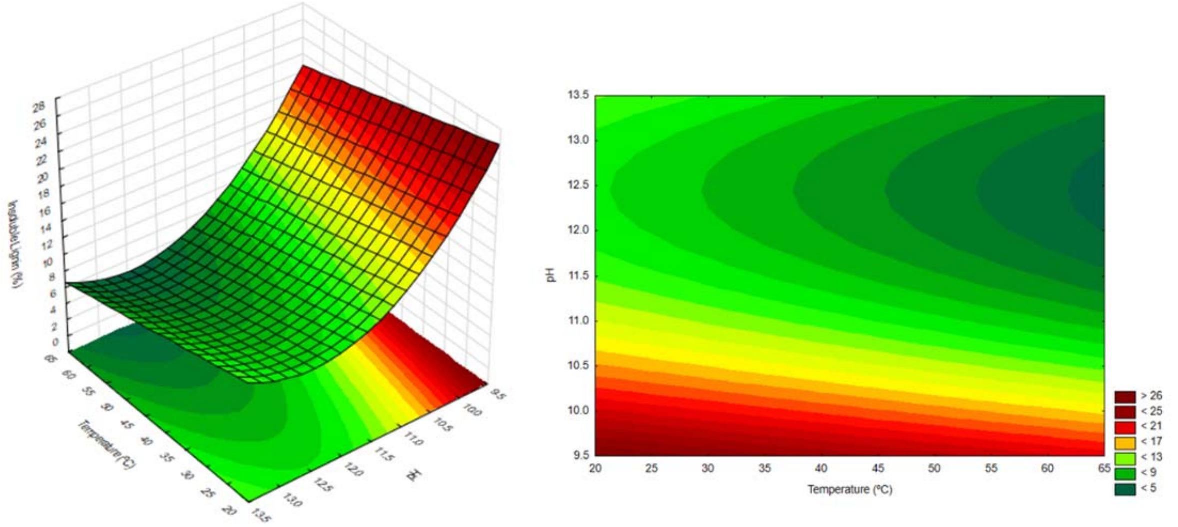

Figure 3: Effects of the temperature and pH variables on the predicted model of the insoluble lignin content in the biomass for the H_2O_2 concentration fixed at the central point.

Figure 4 shows the interaction between the H_2O_2 concentration and pH variables. In this case, it is possible to observe the formation of a well-defined valley, indicating that the ideal values for H_2O_2 concentration and pH to reduce the insoluble lignin content in the biomass delignification process would be around 8 to 14% m/v and around 11.5 to 13, respectively.

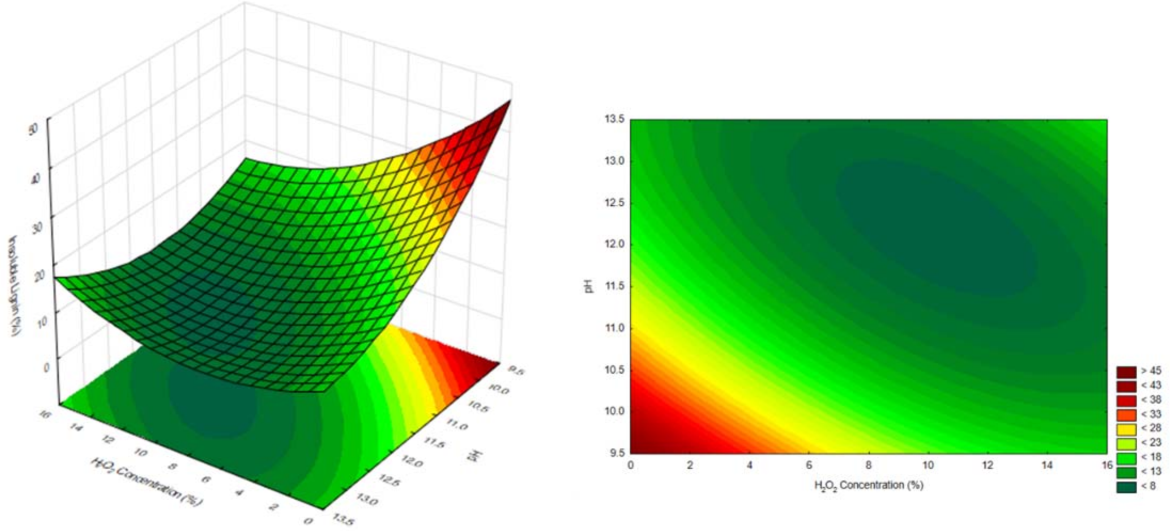

Figure 4: Effects of the H_2O_2 concentration and pH variables on the predicted model of the insoluble lignin content in the biomass for the temperature fixed at the central point.

4. Conclusions

This study revealed the influence of the variables temperature, H_2O_2 concentration and pH on the sugarcane bagasse delignification process. From the experimental design, it was possible to build a mathematical model to evaluate the insoluble lignin content in the biomass, a methodology that takes more than 2 days to be carried out and that is important for the step of quantifying extracted lignin in the studied process. The model was satisfactory in the prediction of the residual lignin content, with a correlation coefficient of 92.16%. The response surface methodology was used to optimize the process conditions. The biomass delignification process is satisfactory when lower insoluble lignin content in the material is observed. Thus, the optimized process condition is a temperature of 60 °C, a H_2O_2 concentration of 10% m/v and a pH value of 11.5.

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