

## Adsorption and Desorption of Cellulases NS 50013 Onto/From Avicel PH 101: A Simple Functional Model

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### Abstract

A functional model based on adsorption pattern suggested by Langmuir was proposed to explain the effect of initial cellulases concentration ( $E_0$ ) on the concentration of cellulases adsorbed ( $E_a$ ). A number of experiments (triplicates) were conducted to determine the equilibrium adsorption values for various  $E_{0s}$  (125, 141, 163, 183, 220, 250, 262  $\mu\text{g mL}^{-1}$ ). The maximum  $E_a$  for the  $E_0$  of 262  $\mu\text{g mL}^{-1}$  was around 117  $\mu\text{g mL}^{-1}$ . Langmuir model predicted  $E_a$  as 99.29  $\mu\text{g mL}^{-1}$  and the response surface methodology (RSM) Model predicted it as 109.30  $\mu\text{g mL}^{-1}$  while the experimental value of  $E_a$  was 107.70 for an  $E_0$  of 183. The proposed predictive RSM model for adsorption provided less percent error (i.e. 0.2) than the Langmuirian model, hence, RSM was used to develop a model for desorption of cellulases from Avicel. The variables considered for desorption of cellulases NS 50013 were temperature (40, 50, 60 oC), pH (7, 8 and 9) and cellulases adsorbed ( $E_0$ ) from  $E_0$ . The proposed desorption model was validated for  $E_0$  values of 175  $\mu\text{g mL}^{-1}$ , 190  $\mu\text{g mL}^{-1}$ , and 210  $\mu\text{g mL}^{-1}$ . The error between the predicted and experimental values of  $E_a$  was around 4-8% for  $E_0$  values of 175 to 210  $\mu\text{g mL}^{-1}$ . The cellulases desorption model is first time presented. The cellulases desorption model will bring certainty in estimation for the amount of cellulases to recycle and corresponding reduction in the initial cellulases loading and hence decrease in bioethanol production price.

**Keywords:** Langmuir; Adsorption; Desorption; Model; Response surface methodology; Cellulases

### Introduction

The desire for safe environment and clean energy motivated the public to demand for biofuel. Biofuel is a fuel produced from biomass. Biomass is a generic term used for all biological materials (except fossil fuels) encompassing agricultural crops, forestry and marine products and organic wastes. Among the various kinds of biomass, woody biomass was used traditionally as an energy source for a long time, and even to this day it is being used in the form of firewood or charcoal. It is, however, difficult to use firewood or charcoal as an alternative fuel for commercial equipment and industrial processes where fossil fuels, in particular oil, are used at present. It is necessary to develop technologies which make possible conversion of biomass to a more suitable form, such as liquid or gas.

In Canada, since 1991, the grain-based ethanol production process exists, representing 92% of actual production capacity. Canadian ethanol plants are presently using corn 68%, wheat 29.9%, municipal waste 1.8%, forestry waste 0.2%, agriculture waste 0.1% [1]. Canada produced 26.8 million tons of wheat in the year 2009-2010 [2]. Depending upon the ethanol production process employed, commercial ethanol could yielded from 340 to over 500 liters per ton of wheat, depending on the type of wheat [3]. Though Canada is among top ten producers of wheat in the world [4] yet the use of food grain for fuel is not appreciated. Because our world cannot sustainably feed a population expected to reach 9.6 billion by 2050. Using food grains (corn and wheat) for biofuels, biofuels production competes with food, making this goal (food sustainability) even more difficult. The low cost ethanol can be produced by using waste lignocellulosic materials such as crops residues, grasses, saw dust, wood chips and animal waste. In last decade research has been done on the conversion of lignocellulosic materials to ethanol [5-8]. The viability of wheat straw which is an agricultural waste and a renewable source was chosen to investigate in this study to produce a valuable product such as bioethanol. The average yield of straw is 1.3-1.4 kg per kg of wheat grain, which means that Canada has capability to produce 37.52 million tons of straw

per year. Wheat straw like all other lignocellulosic materials mainly contains, cellulose, lignin and hemicellulose. In its structure, cellulose is a linear polymer, comprised of polymer of  $\beta$ -D-glucose units. Lignin is a complex aromatic polymer and it is a natural protection of cellulose. Techniques have been developed to remove lignin in order to utilize this huge amount of cellulose. The exposed cellulose is available to react with enzymes. Cellulases are the enzymes to give efficient conversion of cellulose to glucose which is further hydrolyzed to bioethanol. Instead of working on cellulose prepared from lignocellulosic materials scientist prefer to work on pure cellulose (such as Avicel) to develop theoretical understanding of adsorption / desorption behavior of enzymes (cellulases).

Adsorption isotherms give the adsorption pattern and capacity of the adsorbent based on the ratio between the quantity adsorbed and the remaining in solution at fixed temperature at equilibrium [9]. The adsorption isotherms have history for being used in representing adsorption of gases on liquids [10,11] and liquids on solid surfaces [12,13]. A number of researches have been conducted for removal of dyes from the industrial wastewaters on various types of adsorbents. Similarly, the studied adsorption of heavy metals using multicomponent adsorption isotherms. Recently, some reports are available on cellulases adsorption on various lignocellulosic substrates, such as microcrystalline cellulose [14-16], corn stover [17], steam-exploded Douglas fir [18], pretreated hardwood [19]. In all the reports, adsorption patterns and adsorption capacity of adsorbents were studied using various adsorption isotherms. Langmuir isotherm was found reported more

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than others isotherms because of its simplicity and theoretical background. Desorption of enzymes (cellulases) from a lignocellulosic material with the help of desorption model was never studied.

Response Surface Methodology (RSM) has been applied previously for optimizations of cellulase production [20] and ethanol production during the simultaneous saccharification and fermentation of lignocellulosic biomass [21]. RSM was used to optimize enzymatic hydrolysis residues using a small center composite design [22]. The small center composite design has been frequently used in conjunction with RSM [23-26]. Statistical tools of Design of Experiments have latest inclinations in bioprocess advancements. Response surface methodology (RSM), developed by Box and his collaborators, is an assembly of mathematical and statistical techniques for building models, and evaluating the relative significance of several affecting factors in the situations of complex interactions [27,28]. The objective of this work was to apply RSM to determine a functional model for adsorption of cellulases onto Avicel and desorption of cellulases from Avicel. Hence, a functional model for adsorption of cellulases NS 50013 is presented for the first time. Desorption of cellulases NS 50013 from a cellulosic substrate was never been modeled before. The importance of this desorption model is that it is designed on the basis of initial cellulases loading which is also a novel approach. The proposed desorption model will help bioethanol producing industry to estimate the amount of cellulases desorbed (means the amount of cellulases which can be recycled). This will help in controlling cost for the production of bioethanol.

## Materials and Methods

### Materials

Avicel PH 101 (analytical grade, 50  $\mu\text{m}$ , 100% solids) was purchased from Sigma Aldrich, Canada. Avicel PH 101 was a microcrystalline cellulose, and a cellulose analog for wheat straw. Cellulases NS 50013 was a gift from Novozymes, Denmark. It was composed of approximately, EG I 10%, EG II 10%, CBH I 60%, CBH II 15% and  $\beta$ -glucosidase 2% [29], with an activity of 53 FPU/ml.

### Adsorption of cellulases

5 ml of cellulases solution,  $V$ , (citrate buffer solution at  $\text{pH}$  5) was added to 100 mg of the cellulose substrate,  $M$ . Mixing in 10 ml glass tubes was done in an incubator shaker at 100 rpm. After adsorption each tube was centrifuged at 4000 rpm for 4 minutes and the supernatant were decanted off. The concentration of free unbound cellulases in the supernatant,  $E_f$ , was measured by modified Lowry method using Biochrom Libra S50 UV/Vis Spectrophotometer. The concentration of cellulase that remained adsorbed onto a solid substrate was determined as the difference between the total concentration of cellulase initially applied and the concentration of free cellulase in the decanted supernatant solution. Triplicates were used for each of the 7 contact times. The solid residues (thick slurry) remaining after the centrifugation step was immediately used for desorption study. The cellulases adsorbed per mg of substrate were calculated by:

$$E_0 = (E_0 - E_f) * \frac{V}{M} \quad (1)$$

$$E_e = (E_a) * \frac{V}{M} \quad (2)$$

### Desorption of cellulases

5 ml of distilled water with to a pH value varying from 7 to 9 was added to thick slurry from the centrifugation. The reaction mixture was

then placed in an incubator for 20 minute at temperature 40°C, 50°C and 60°C. Then centrifuged for 4 minutes at 4000 rpm. The concentration of free unbound cellulases, here called desorbed cellulases, was measured in the decanted supernatant, as before.

### Design of Experiment (DOE)

The response surface methodology employed was derived from one factor design with a total of 7 runs for adsorption. Similarly, RSM applied was from central composite design (CCD) with 20 runs for desorption. The results were adjusted to quadratic polynomial which was followed by analysis of variance (ANOVA), regression analysis and finally concentration of cellulases adsorbed on Avicel in case of adsorption, and concentration of desorbed cellulases in the case of desorption study was obtained. The data from the CCD method can be fitted into the following quadratic function, which is a second order equation:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^{j-1} \sum_{i=i+1}^k \beta_{ij} X_i X_j + \sum_i \beta_{ij} X_i^2 + e_i \quad (3)$$

Where  $Y$ ,  $X_i$ ,  $X_j$ ,  $\beta_0$ ,  $\beta_i$ ,  $\beta_{ij}$ ,  $\beta_{ii}$ ,  $k$  and  $e_i$  represent: predicted response, independent response, independent variables, constant coefficient, the influence of independent variable, the influence of interaction among variables, quadratic effect, the number of variables and error residual.

## Results and Discussion

### Cellulases adsorbed

The concentration of cellulases adsorbed is the basic unit for study of adsorption isotherms. In Figure 1, the concentration of cellulases adsorbed  $[E_a]$  on Avicel at room temperature, 100 rpm is given along y-axis for varying adsorption time from 0 to 90 minutes. Each data point was measured individually. All the results are triplicates. In the start increasing time increased adsorption which achieved its maximum at 20 minutes after that further increasing adsorption time the amount of cellulases adsorbed almost remained the same.

However, the time of adsorption on Avicel was taken 30 minutes for conducting experimentation for the study of isotherms. The maximum concentration of cellulases adsorbed  $E_a$  for initial cellulase  $E_0$  262  $\mu\text{g mL}^{-1}$  taken on 100 mg of Avicel PH 101 was at the average of 117  $\mu\text{g mL}^{-1}$ .

The adsorption data obtained from Figure 1 was tested using a physical model which may be described as a hyperbolic function similar to Langmuir adsorption isotherm

$$E_e = \frac{k_a E_a E_f}{1 + E_a E_f} \quad (4)$$

Equation 4 can be written in a linear form, called, Scatchard regression (Equation 5), proposed 1949.

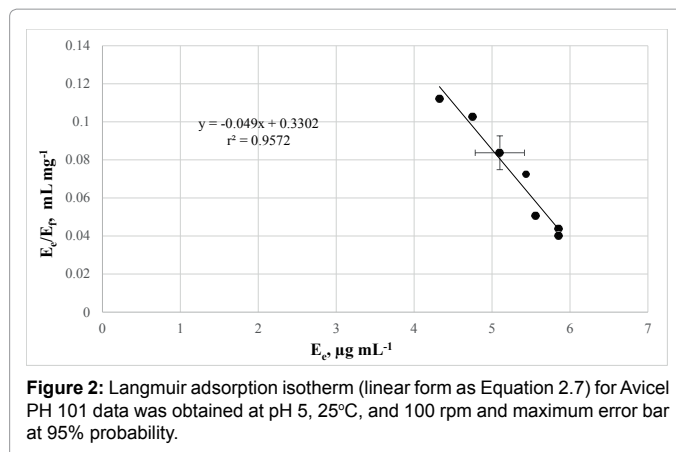
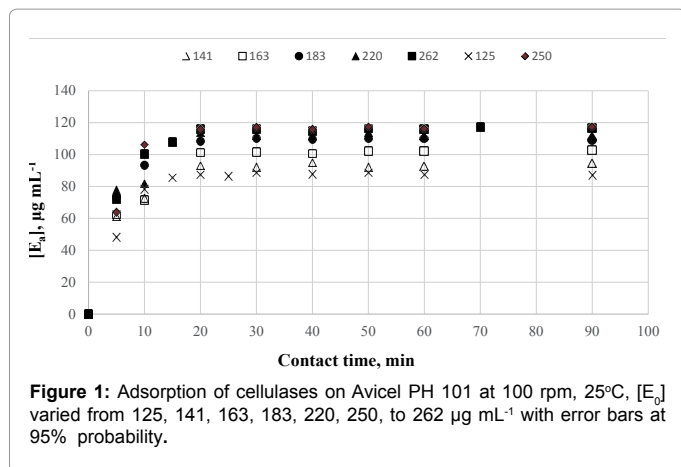
$$\frac{E_e}{[E_f]} = K_a [E_a] - K_a E_e \quad (5)$$

$E_e$  = cellulases adsorbed,  $\mu\text{g}$  cellulases per mg of substrate<sup>-1</sup>

$E_f$  = concentration of free cellulases present in supernatant at equilibrium,  $\mu\text{g mL}^{-1}$

$K_a$  = capacity constant

Adsorption data for Avicel according to Langmuir isotherm is shown in Figure 2. The ratio of specific adsorbed cellulases to non-adsorbed cellulases (y-axis) versus the specific adsorbed cellulases



(x-axis). The plot gave a straight line with a slope of ( $E_e$ ) and an intercept of ( $K_a E_a$ ). The model equation obtained from the plotted data was:

$$\frac{E_e}{E_f} = -0.049 E_e + 0.3302 \quad (6)$$

The non-adsorbed cellulases  $E_f$  were directly proportional to the initial cellulases concentration  $E_0$ .

The results of Langmuirian adsorption also indicated that the cellulases adsorbed on the surface of Avicel PH 101 homogeneously and all adsorption was monolayer type. Similar results were observed by other researchers [30,31] as well while working on cellulose fibers and Avicel respectively.

The adsorption data obtained from Figure 1 was subjected to analysis of variance (ANOVA) and regression analysis and the model was adjusted to a quadratic polynomial model. The polynomial obtained for Avicel was:

$$E_a = -47.95 + 1.535(E_0) - 3.964 E - 003(E_0)^2 \quad (7)$$

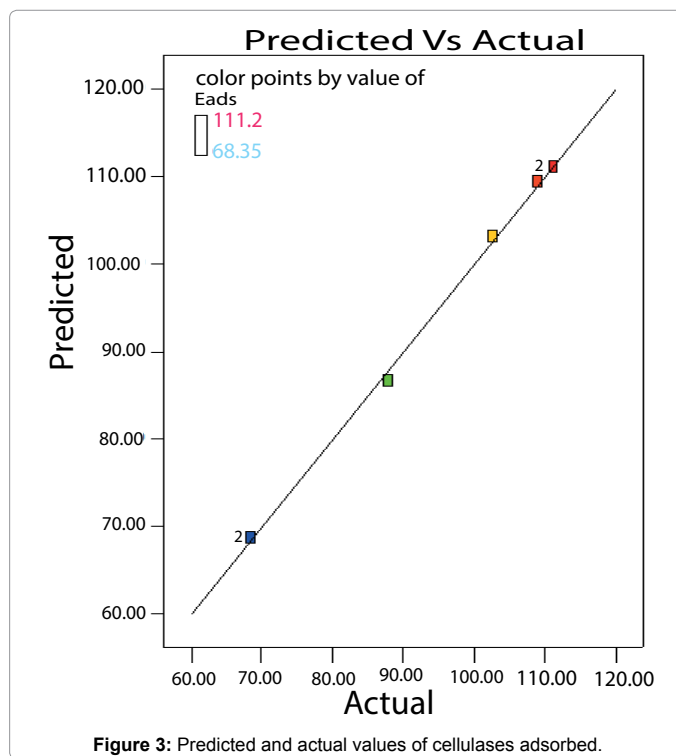
$E_a$  = concentration of cellulases adsorbed,  $\mu\text{g mL}^{-1}$

$E_0$  = initial concentration of cellulases,  $\mu\text{g mL}^{-1}$

Figure 3 indicate that actual values (experimental values) are quite in line with the predicted values from Equation 7. It is to be noted that all the five points lies on a line. As given in Table 1 the % error calculated varies from 0.0 to 0.2 which is a proof of appropriate model.

The calculated concentration of cellulases adsorbed on Avicel PH 101 by using regressions equation from the plot of Langmuir in Figure 2 (Equation 6) is compared with the actual  $E_a$ . The % error found was less than 10. The  $r^2$  value equal to 0.9572. Thus, 96% of the specific adsorbed cellulases were directly accounted for initial cellulases concentration. Table 1 gave a comparison of concentration of cellulases adsorbed obtained from experiments and predicted values from Equation 6 and Equation 7.

Therefore, it was concluded that in our experiments, the action of cellulases was a surface phenomenon (on the basis of % error and  $r^2$  value), with mechanism of adsorption in control. The constant agitation at 100 rpm, and relatively small particle size of Avicel PH 101 (50  $\mu\text{m}$ ) could have reduced substantially the resistance to mass transfer and minimized its importance in adsorption. The influences of cellulases loading using RSM was also studied. Based on the results of the sum of squares (Table 2) and the calculated statistics for all model



terms, a quadratic model was suggested. The model obtained using RSM was given in Equation 7. The results of the ANOVA response surface quadratic model are shown in Table 2. The significance of each coefficient was ensured using F-test and its allied probability, P value. Values of “Prob > F” (<0.0001 which is less than 0.050) indicate that the model terms are significant. Thus, the model F-value of 2068.45 implies it is significant and there is only a 0.01% chance that a “Model F-value” this large could occur due to noise. The significance of the model was also determined using the “Lack of fit” test to measure the failure of the model at data points that were not included in the regression analysis. The model shows statistically insignificant “Lack of fit”. The insignificant “Lack of fit” F-value shows the validity of the predictive model which can be used to calculate cellulases adsorbed from Equation 7.

Adequate precision measures the signal to noise ratio. The ratio 87.970 indicates an adequate signal (a ratio greater than 4 is desirable). A coefficient of determination ( $r^2$ ) value of 0.9990 shows that the

$E_0$ $\mu\text{g mL}^{-1}$	$E_a$ $\mu\text{g mL}^{-1}$	Langmuirian		RSM	
		Ea	% Error	Ea	% Error
125.0	86.50	91.07	5.020	86.20	0.003
141.0	95.00	90.04	5.500	95.14	0.001
160.0	102.9	97.80	4.280	104.1	0.020
185.0	107.7	99.29	8.500	109.3	0.015
220.0	112.1	121.1	7.403	110.9	0.010
250.0	117.0	115.8	0.998	104.9	0.103
262.0	117.0	126.3	7.359	100.6	0.139

Note: Langmuirian Ea by using Equation 6; RSM Ea by using Equation 7.

Table 1: Comparison of predicted cellulases adsorbed on to Avicel.

Source	Sum of Squares	df	Mean Squares	F value	p-value Prob>F
Model	2242.81	2	1121.41	2068.45	<0.0001
Coefficient of determination, $r^2= 0.9990$ Adjusted $r^2= 0.9986$ Predicted $r^2= 0.9978$ Adequate Precision= 87.970					
Initial concentration of enzymes, $E_0$	2012.99	1	2012.99	3712.98	<0.0001
$E_0^2$	222.95	1	222.95	411.23	<0.0001
Residual	2.17	4	0.54		
Lack of fit	2.17	2	1.08		
Pure error	0.000	2	0.000		
Correlation total	2244.98	6			

Table 2: Analysis of variance for adsorption.

model is highly reliable; the regression model could explain 99.90% of the variability in the response and only 0.10% of the total variation could not be attributed to the variables. However, the predicted  $r^2$  of 0.9978 is as close to the adjusted  $r^2$  of 0.9986 as one might normally expect. The significance of regression coefficients was also determined on the basis of their P value. The smaller the P value, the bigger the significance of the corresponding coefficient [32]. However, the effect of cellulases concentration on adsorption (P value <0.0001) is higher than the other factors. The importance of the variables and their effects can be also explained by the magnitude and sign of the coefficients, accordingly [33]. Therefore, the quadratic effects of cellulases loading can be clearly visualized in the response surface, which has significant effect on adsorption. The quadratic RSM model represent adsorption of cellulases onto Avicel PH 101 better than that of Langmuir model.

### Desorption

The CCD technique of DOE was used and 20 runs of experiment were formed by Design Expert 8.0.0 and a full quadratic model was proposed. The statistical treatment combinations of the test variables (i.e. initial cellulase loading, temperature and pH) along with the measured values were used. The experiments, which were subjected to regression analysis, were run in random order to give randomly distributed variables and minimize the effects of unexplained variability in the observed results.

To evaluate the influences of three factors including cellulases adsorbed, temperature and pH, the design matrix of experimental conditions fitted to a polynomial model. Based on the results of the sequential model sum of squares and the calculated statistics for all model terms, a quadratic model was suggested. Therefore, the mathematical equation proposed for this response (in terms of actual factors) was:

$$\begin{aligned} \text{Desorbed} = & -529.40 + 11.806 * E_a - 4.766 * \text{Temperature} \\ & - 4.904 * \text{pH} + 5.00010^{-3} * E_a * \text{Temperature} \\ & + 0.050 * E_a * \text{pH} + 0.400 * \text{Temperature} * \text{pH} \end{aligned} \quad (8)$$

The cellulases desorbed value was predicted by using Equation 8 and compared with the experimentally desorbed value. Figure 4 gave a comparison between predicted and actual values. By picking the X values of the corresponding Y values can be found and they are matching and showing a good agreement in the predicted and actual values of the cellulases desorbed.

The ANOVA results of the regression model obtained for desorption of cellulase from Avicel are shown in Table 3. The response of % cellulases desorbed, as a function of cellulase adsorbed, temperature, and pH, was evaluated in CCD. All experiments were carried out under these conditions, pH 7, 8 and 9, temperature 40°C, 50°C and 60°C, substrate 100 mg, and agitation 100 rpm. Using Design Expert 8.0.0, an analysis of variance was conducted for evaluation of the effects of the variables and their probably existed interactions. Coefficients of the full model were analyzed for their significance and insignificance.

The values of 'Prob>F' less than 0.0500 indicate model terms are significant. In this case cellulases adsorbed, temperature, pH, temperature with pH and square of temperature are significant terms. The 'Prob>F' values obtained were less than 0.0001 for all factors (sources) except the square of temperature which was 0.0176.

The variable cellulases adsorbed ( $E_a$ ) had significant positive effect on response according to Table 3. It has proved that adsorption is the first step in controlling hydrolysis which is associated with desorption. It can be speculated that more adsorption would lead to more desorption. The cellulases NS 50013 is produced from *T. reesei* which is the main source of most commercial cellulose [34]. The factor temperature was found as significant for desorption of cellulases. The regression sum of squares (Table 3) is the variation attributed to the relationship between

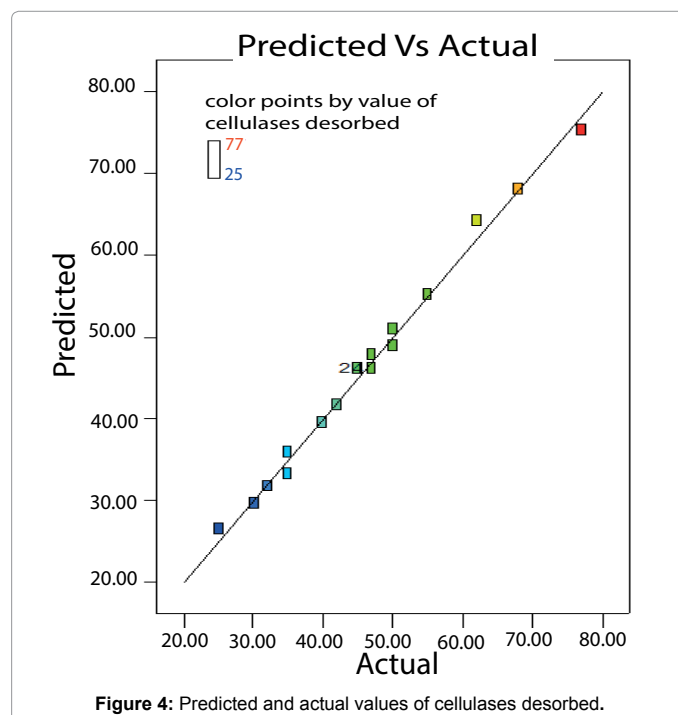


Figure 4: Predicted and actual values of cellulases desorbed.



Source	Sum of Squares	df	Mean Squares	F value	p-value Prob>F
Model	2983.27	9	331.47	144.58	<0.0001
Coefficient of determination, $r^2= 0.9924$ Adjusted $r^2= 0.9855$ Predicted $r^2= 0.9296$ Adequate Precision= 45.578					
A= $E_a$	96.100	1	96.10	41.92	<0.0001
B=Temperature	2402.50	1	2402.50	1047.88	<0.0001
C=pH	336.400	1	336.40	146.72	<0.0001
AB	0.500	1	0.50	0.22	0.6505
AC	0.500	1	0.50	0.22	0.6505
BC	128.00	1	128.00	55.83	<0.0001
A <sup>2</sup>	5.460	1	5.46	2.38	0.1538
B <sup>2</sup>	18.460	1	18.46	8.05	0.0176
C <sup>2</sup>	2.270	1	2.27	0.99	0.3429
Residual	22.930	10	2.29		
Lack of fit	17.590	5	3.52	3.30	0.1081
Pure error	5.330	5	1.07		
Correlation total	3006.2	19			

Table 3: Analysis of variance for desorption.

the cellulases adsorbed and % cellulases desorbed. The larger this value is (i.e. 2402.5), the better is the relationship explaining % cellulases desorbed as a function of cellulases adsorbed. The effect of pH and temperature on desorption of cellulases is shown in Figure 5.

The red plots showed cellulases desorbed at pH 9 while the black lines were for desorption at pH 7. The interaction of pH and temperature was significant for desorption of cellulases with a sum of squares value of 128.00. Though individually, the factor pH was found to be less significant for desorption of cellulases than that of temperature with sum of square value was 336.40.

On the other hand, those insignificant factors were: square effect of  $E_a$  (Prob>F = 0.1538); square effect of pH (Prob>F = 0.1538); and two interactive effects:  $E_a$  \* temperature (Prob>F = 0.6505),  $E_a$  \* pH (Prob>F = 0.6505) respectively. Based on the ANOVA analysis, those insignificant factors whether square or interaction effect can be eliminated. Although these effects were insignificant, but they were still maintained in the model to retain the model originality [35].

According to Table 3, the  $r^2$ -value was 0.9924 in good agreement with the adjusted  $r^2$ -value of 0.9855. The vicinity of adjusted  $r^2$  to  $r^2$  means a good adjustment of the predicted values to the experimental data by the model. Meanwhile the “Lack of fit” was insignificant and the  $r^2$ -value was high (0.9924) indicating that the model was well adapted to the response [36]. So the model was suitable to predict the experimental data for cellulases desorbed under different conditions of  $E_a$ , temperature, and pH. Coefficients and p-values of the model indicated that  $E_a$ , temperature and pH significantly affected the cellulase desorption. The interaction effect of temperature and pH was significant as compared to their corresponding interactions with  $E_a$ .

### Validation of model

Cellulases desorbed, given in Equation 8, were evaluated on the basis of cellulases adsorbed. In order to estimate cellulases desorbed on the basis of cellulases initially loaded, the term  $E_a$  was replaced by Equation 7. The new equation was:

$$\text{Desorbed} = -529.40 + 11.806 * [-47 + (1.535 * E_0) - 3.69410^{-3} * E_0^2] - 4.766 * \text{Temperature} - 4.904 * \text{pH}$$

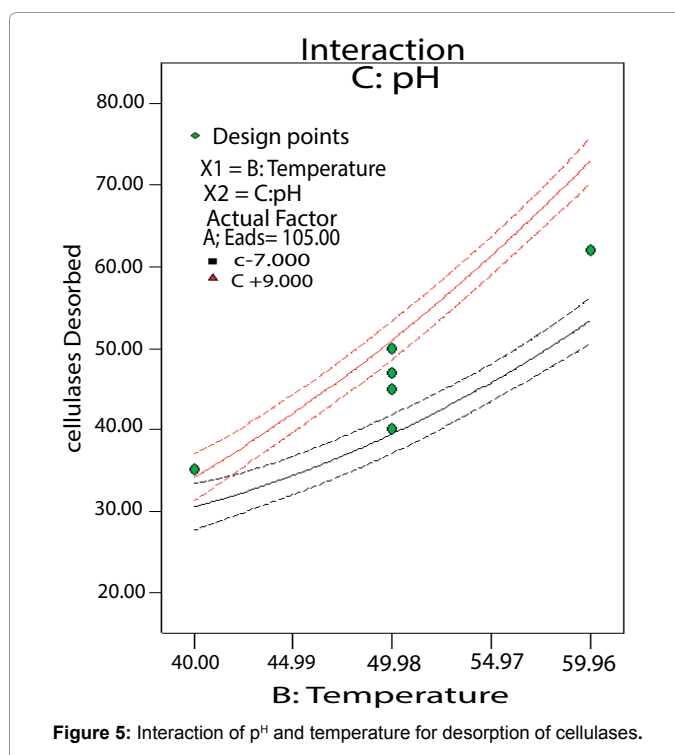


Figure 5: Interaction of pH and temperature for desorption of cellulases.

$$\begin{aligned} &+5.00010^{-3} * [-47 + (1.535 * E_0) - 3.69410^{-3} * E_0^2] * \text{Temperature} \\ &+0.050 * [-47 + (1.535 * E_0) - 3.69410^{-3} * E_0^2] * \text{pH} \\ &+0.400 * \text{Temperature} * \text{pH} \\ &-0.056 * [-47 + (1.535 * E_0) - 3.69410^{-3} * E_0^2]^2 \\ &+0.0259 \text{Temperature}^2 - 0.909 * \text{pH}^2 \end{aligned} \quad (9)$$

A comparison of cellulases desorbed predicted by RSM Equation 9 with the actual desorbed cellulases is given in the following Table 4.

There was a high degree of similarity between predicted and

Temp. °C	pH	[E <sub>0</sub> ] µg mL <sup>-1</sup>	RSM Model	
			Predicted	% Error
40	7	32	35.35	9.91
50	7	40	44.74	10.59
60	7	55	59.24	7.16
40	8	35	38.28	8.57
50	8	47	51.45	8.65
60	8	62	70.08	11.54
40	9	35	39.55	11.51
50	9	50	56.77	11.92
60	9	77	79.19	2.76

Note: Temp : Temperature; [E<sub>0</sub>]: cellulases desorbed.

Table 4: Prediction accuracy of RSM model for desorption.

experimental values, showing validity of the response surface model as shown in Table 4. The error values obtained were less than 12% which can be further reduced by removing the insignificant variables from the Equation 8. The model Equation 9 was validated for E<sub>0</sub> values of 175 µg mL<sup>-1</sup>, 190 µg mL<sup>-1</sup>, and 210 µg mL<sup>-1</sup>. The percent error for E<sub>0</sub> 175 and 210 µg mL<sup>-1</sup> was around was only a 4-8% error between the predicted and experimental values calculated by Equation 9. The closeness of the predicted response for cellulases proved the validity of the model Equation 9.

## Conclusion

The Central Composite Designs (CCD) from design of experiment (DOE) combined with the response surface methodology (RSM) was applied to estimate the cellulases desorbed from the cellulosic component (Avicel PH 101) of lignocellulosic materials. The effects of initial cellulose loading, temperature and pH on desorption of cellulases were investigated. After analyzing the experimental data by statistical tests (ANOVA) to yield polynomial regression model, response surface model was used to determine the concentration of cellulases desorbed. Accordingly, minimum % error (2.7) was achieved by working at pH 9 and 60°C. A validation assay confirmed the predictive response value under the aforementioned conditions. Therefore, the response model based on CCD was adequate for reflecting the expected optimization and desorption [37-39].

As a consequence, through enzymatic adsorption, an environmentally friendly process, cellulases desorbed was estimated which has promising potential for applications in the field of bioethanol production. The desorbed cellulases will help to manage cost of bioethanol production process through recycling of active desorbed cellulases.

There is a potential to further refine the RSM model (Equation 9) for desorption of cellulases by removing insignificant interactions from the model Equation 9.

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