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Thermodynamics of adsorption/desorption of cellulases NS 50013 on /from Avicel PH 101 and Protobind 1000

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Abstract: *Insight from thermodynamic parameters of enthalpy (ΔH), entropy (ΔS) and Gibbs free energy (ΔG) was used to predict conditions for desorption of cellulases from wheat straw. The analogues of the cellulose and lignin components of wheat straw used were Avicel PH 101 and Protobind 1000, respectively. The ΔH_a for adsorption on Avicelat pH 5 was -16.10kJmol^{-1}, ΔS_a was -50.10 Jmol^{-1}K^{-1} which indicated an increase in order due to adsorption and ΔG_a was negative only from 298K to 323K. Results of adsorption on Protobind 1000 were pretty much the inverse over the same range of temperature, proving the great affinity of cellulases for lignin. Over 298K to 333K, desorption from Avicel resulted in a ΔH_dincrease from 17.50 to 26.20 kJmol^{-1}, and a ΔS_dincrease from 46.89 to 75.65Jmol^{-1}K^{-1}when pH increased from 6 to 9indicating an enthalpy-driven, non-spontaneous desorption. For Protobind, the positive ΔH_d (9.65 to 6.90 kJmol^{-1}) with small positive ΔS_d (21.70 to 9.03 Jmol^{-1}K^{-1}) indicated that disorder was less than that of Avicel. For both the substrates ΔG_d decreased with rise in temperature in given temperature range. The minimum $\Delta G_d$of Avicel than that of Protobind proved that it was more difficult to desorb cellulases from Protobind.For bioethanol producing industries, using lignocellulosic material (e.g., wheat straw) where cellulose is embedded in lignin, removal of lignin is recommend along with adsorption/hydrolysis to be conducted at 323 K and desorption from used material at 333 K and pH 9.*

Keywords: *Adsorption, Avicel PH 101, Cellulases, Desorption, Protobind 1000, Thermodynamics*

I. Introduction

Lignocellulosic materials are used as a source of cellulose for production of bioethanol. These materials consist of lignin, cellulose and hemicellulose, which are interweaved in the cell walls. Cellulose and hemicellulose in the lignocellulosic materials release glucose and xylose that can in turn be fermented to bioethanol. Cost-effective liberation of fermentable sugars from lignocellulosic resources is still the largest obstacle to large-scale commercialization of bioethanol Process[1,2]. The main problem associated with the process is presence of lignin whichpreferentially adsorb cellulases instead of celluloseand resist to desorb cellulases(reusability), hence, increase product bioethanol cost [3,5].

The solution to problem is to remove the lignin and expose more cellulose and hemicellulose from lignocellulosic materials. However, due to the cost of some of these pretreatments, health and/or environmental concerns, the potential for economical bioethanol is not promising. For example, when using steam explosion, high temperature and pressure resistant fabrication material for the reactor is required [6-8]. Ammonia fiber expansion (AFEX) needs pressure resistant and corrosion resistant fabrication material for reactors and it may trigger asthma in workers [9]. Similarly, cellulose solvent(concentrated phosphoric acid) and organic solvent-based lignocellulose fractionation (COSLIF), as well as ionic liquids or organosolv processesrequire expensive corrosion resistant fabrication materials and may harm workers [10-12].In our lab we have successfully removed 90 % of lignin by a novel technique which is a sequential use of water and ozone [13]. All reactions occurred at normal temperature and pressure, no expensive material would be required to construct a reactor.In another technique new insoluble substrate was simply added so that cellulases could readsorb on fresh added substrate [14-15]. However a buildup of lignin rich residues would ultimately increase capability of cellulases to adsorb on the substrate reduce the capability of cellulases to desorb from the substrate because of strong binding on lignin. Hence, removal of lignin reduced cost due to loss of cellulases by nonproductive adsorption.

Some methodologies were also evaluatedto try to decreasethe costs of using cellulases in bioethanol production: (i) decreasing cellulases loading by using low lignin or no lignin containing substrates [16,17],(ii) recycling costly cellulases[14],(iii) increasing cellulases performance (activity)by genetic engineering [18], (iv) increasingdesorption with the addition of an agents such as alkaline media [19,20], Tween [21], urea [3], glycerol [22], and Triton X-100 [23] added as diluent in desorption stage.

A change in pH and /or temperature can be utilized to facilitate adsorption/desorption. Otter *et al*. (1984) showeda recovery of up to 65% with 45% of cellulases activity at pH 10 and reported that theenzymes were completely inactive at pH 10.5. The high cost and/or high dosage of desorbent needed made this approach impractical on a large scale.Although some work has been done on the effect of pH oncellulases adsorption [24-26], less attention was given to see the temperature dependence of the adsorption process. The studies to determine the effect of temperature have shown two aspects: i) small data was used to derive results. Some of the researches reported the results of 2 or 3 selected temperature[27-29] which representedonly that narrow area of study but not a general principle. ii) controversial results for the adsorption process were reported. It was stated that cellulases adsorption on lignocellulose was an exothermic, enthalpy-controlled reaction and the amount of cellulases adsorption decreased as the temperature increased [27,30, 31]. On the contrary, Hoshino *et al*. (1992) and Creagh*et al*. (1996)demonstrated that cellulases adsorption on cellulose was an endothermic and entropy-driven reaction [32, 33]. The temperature dependency and thermodynamics of desorption, however, was rarely reported in the literature.Thermodynamics study could indicate feasibility of the adsorption and desorption reactions from the obtained values of the thermodynamic parameters. The van't Hoff equation canbe adapted to represent the adsorption and desorption processes of cellulases to /from adsorbents like Avicel PH 101 and Protobind 1000. It relates the main thermodynamics parameters such as enthalpy (ΔH) and entropy (ΔS) to the equilibrium distribution coefficient (K) of the adsorbed or desorbed species between an aqueous solution and an adsorbent, as shown in Equation 1:

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad \ldots \quad 1$$

R is the universal gas constant (8.314 $JK^{-1}mol^{-1}$), and T is the absolute temperature.The other thermodynamic parameter Gibbs free energy (ΔG)is given by Equation 2:

$$\Delta G = \Delta H - T\Delta S \quad \ldots \quad 2$$

The objectives of this project were to settle the controversy on temperature dependency of adsorption of cellulases onto lignocellulosic components (cellulose and lignin)and to develop a temperature dependence for desorption. Adsorption, a prerequisite for desorption was conducted at pH 5 only as is currently being done in the fuel ethanol industry [34] because it gave high adsorption of enzymes in the process. Desorption studies were done at pH 6, 7, 8, and9, over the same temperature range(298K to 343K). To the best of my knowledge, the thermodynamic parameters for enzymatic desorption are being reported first time in this study.

II. Material and Methods

2.1 Materials

Avicel PH 101 (analytical grade, 50µm, 100% solids) was purchased from Sigma Aldrich, Canada. Avicel PH 101 was a microcrystallinecellulose,and a celluloseanalog for wheat straw. Protobind 1000 (analytical grade, 98% solids, 2% ash) a lignin analog of wheat straw, was kindly donated by the GreenValueEnterprises LLC,USA. Protobind 1000 was the. Cellulases NS 50013 was a gift from Novozymes, Denmark. It was composed of approximately, EGI 10%,EGII 10%, CBH I 60%, CBHII 15% and β - glucosidase 2%) [35], with an activity of 53 FPU/ml.

2.2 Adsorption of cellulases

5 ml of cellulases solution (citrate buffer solution at pH 5)was added to 100 mg of the cellulose or lignin substrate. 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 weredecanted off.The concentration of free unbound cellulases in the supernatant [E_{fa}] 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 supernatantsolution. Triplicates were used for each of the 12 contact times and 5 temperatures (298K-343K). The solid residues (thick slurry) remaining after the centrifugation step was immediately used for desorption study.

2.3 Desorption of cellulases

4.9 ml of distilled water with to a pH value varying from 6 to 9 was added to thick slurry from the centrifugation. The reaction mixture was then placed in an incubator for 20 minute temperature varying from 25 to 70^{o}C. Then centrifuged for 4 minutes at 4000 rpm. The concentration of free unbound cellulases here called desorbed cellulases and measured in the decanted supernatant, as before.

III. Results and Discussion

Fig. 1 shows temporal plotsof unbound(free) cellulases present in the supernatant after contact with Avicel PH 101at pH5and temperatures of 298, 313, 323, 333 and 343K.

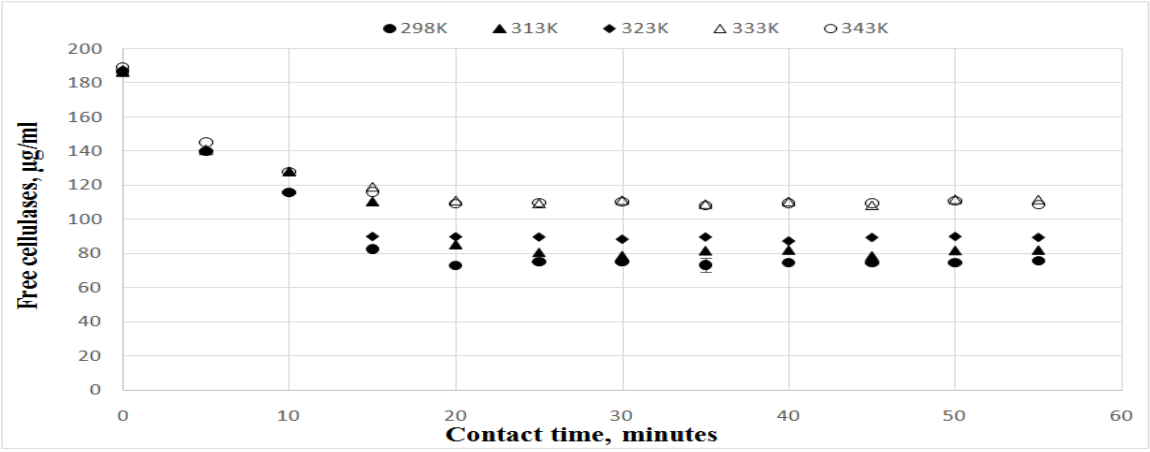


Figure 1: Free cellulases on after contact with Avicel PH 101 at various temperatures at pH 5 also shown with maximum error bar at 95 % probability

Within the first 10 minutes about 60 percent of the initial cellulases were adsorbed on Avicel.Equilibrium was achieved after about 20 minutes for all temperatures. Consequently, a contact time of 30 minutes was chosen for all adsorption studies used for desorption purposes. Since almost one and half times more cellulases were adsorbed at 298K and 313K than at 333K and 343K, 298K was chosen to maximize adsorption prior to desorption studies. When cellulases were placed in contact with Protobind similar trends of adsorption to those of Avicel were obtained. However, equilibrium was reached about after 40 minutes. Consequently a contact time of 45 minutes was chosen for adsorption from lignin. Long adsorption time of cellulases experienced for lignins versus cellulose can be explained by considering:

i) the functional groups taking part in this interaction. Cellulose contains a large number of hydroxyl groups [36] to interact with cellulases. The functional groups of lignin are, carboxylic, carbonyl, aliphatic hydroxyl, and phenolic hydroxyl groups [37-40]. Therefore, cellulases adsorption ability of lignin involved more complex factors other than just the interaction with the hydroxyl groups. More functional groups in lignin offers more attraction to cellulases. When a number of cellulases approaches to lignin molecule, electrostatic repulsion came into play between the cellulases themselves and they need time to rearrange [41, 42].

ii) a repulsion between positively charged amino acids from cellulases and carbonyl from lignin may allow cellulases to rearrange in order to adsorb on lignin and cause delayed adsorption.

iii) Since more cellulases adsorb on lignin than on Avicel, hence it took longer time

Plotting the equilibrium adsorption results for both solid substrates as a van't Hoff relation (Equation 1) linearity of adsorption is observed over the temperature range of 298K ($1/T = 3.35 \times 10^{-3}\ K^{-1}$)to 343K ($1/T = 2.92 \times 10^{-3}\ K^{-1}$) (Fig. 2). The left side of the plot represents higher temperature.

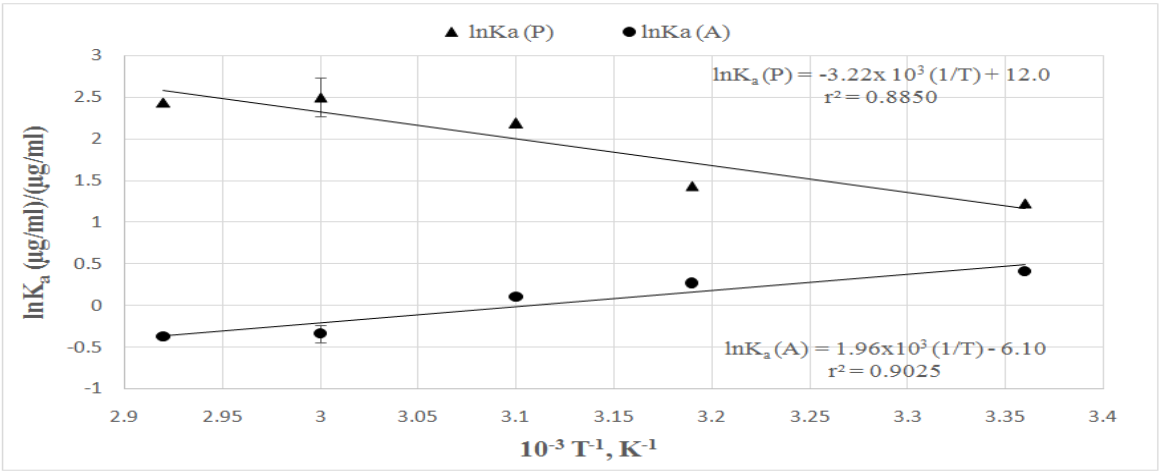


Fig.2: Van't Hoff plots for adsorption on Avicel PH 101 and on Protobind 1000 at pH 5 with maximum error bar at 95% probability

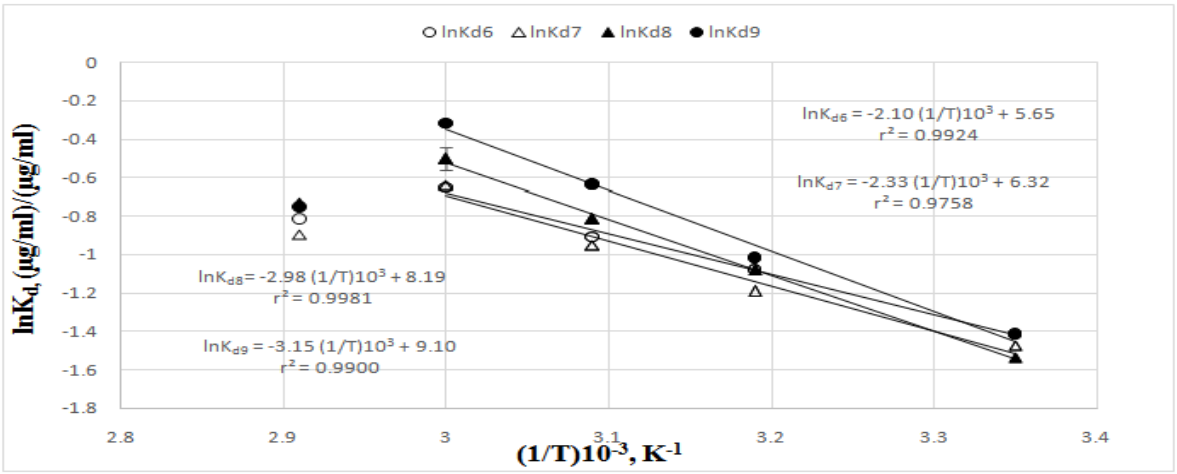
For Avicel, $\ln K_a$ decreased with an increase in temperature, there was a small decrease (10-12 %) in adsorption at 323 K compared to 298 K, and at 343 K the adsorption decreased by 33 %, as was expected from published literature [40, 43-45]. Other research groups reported that some cellulases started denaturing around 323K [19,43,45, 46].The Novozymes cellulases used in this study surpassed this limit by 10 K. On the other hand, Baker's group [47] proved that individual cellulases maintain their activities up to 333K. The observed $\ln K_a$ at lower temperature may be associated to a reduced translational energy androtationalenergy (vibrational energyis negligible) of cellulases and celluloseallowingopposite charges on the cellulose-bindingdomain (CBD) of cellulases and on the Avicelto align themselves. The van't Hoff model gave $\ln K_a = 1.96x10^3$ (1/T) - 6.10. The change in enthalpy (ΔH_a) value obtained from the slope of the equation was found to be-16.1 $kJmol^{-1}$, whichwas expected from the results of cellobiohydrolases I (CBH I) on Avicel [44] who reported -18.2$kJmol^{-1}$ at 288 K to 303 K. The negative ΔH_a of exothermic adsorption means that non-covalent interactions (such as electrostatic, van der Waals, hydrogen bonding, etc.are significant [48-50] between cellulases and Avicel. The ΔS_a value obtained from intercept of the plot was -50 $Jmol^{-1}K^{-1}$. The negative entropy indicated that the mobility of the adsorbed cellulases on the surface of Avicel was restricted. Negative values of ΔS_a were also observed foradsorbed enzymes during other studies [31, 51-52] and for ionic adsorption [53-55]. Another reason for negative ΔS_a value could be unfolding (conformational changes) of cellulases. The unfolding of endoglucanases from *Aspergillus niger* and α-amylase from *Bacillus licheniformis*, DNA ligase and xylanasewere reported where the ΔS value was negative [56-58]. Violet and Meunier (1989) presented the formation of an intermediate compound (X) on the pathway between the natural (N) and the denatured (D) enzyme as (i.e. $N \rightarrow X \rightarrow D$). They noticed that the intermediate state (adsorption of enzymes on substrate) is more ordered structure than the starting state i.e. ΔS_a is negative. According to the second law of thermodynamics, any spontaneous process the overall $\Delta S \geq 0$. Therefore, a negative change in entropy does not contradict the second law, because the adsorption of cellulases on Avicel have a sufficiently large negative ΔH_a (over 320 times of that ofΔS_a) results in a sufficiently large increase in entropy so that the overall change in entropy is positive.

The Gibbs free energy (ΔG_a) value increased from -1170$Jmol^{-1}$to 1080$Jmol^{-1}$ as temperature increased from 298K to 343K.A negative ΔG_a, expected for spontaneous adsorption occurred only from 298 K to 323K.Consequently, only this temperature range is recommended for adsorption of cellulases on Avicel PH 101.For bioethanol producing industries, using wheat straw where cellulose is a component, adsorption can be performedon delignified wheat straw between the temperature range of 298 K to 323 K. Since fermentations are conducted in most of the industries around 313K therefore, adsorption can also be performed at the same temperature for the ease of process.

In the case of Protobind,the experimental data points plotted between 298Kand 343K showed $\ln K_a$ increased with an increase in temperature.The trend line equation (regression equation)was $\ln K_a = -3.22x10^3$ (1/T) + 12.0. Therefore,ΔH_awas26$kJmol^{-1}$, which indicates an endothermic reaction.Theamount of cellulases adsorbed increased from 75% to 94% of the initial cellulases concentration whentemperature increased from 298K to 333K. The ΔS_a for the adsorption of cellulases on Protobind 1000 was positive (100 $jmol^{-1}K^{-1}$) which means that disorder of the system was increased [59].In accordance with the second law of thermodynamics since $\Delta S_a > 0$, the adsorption of cellulases on lignin appears to be an irreversible process.The ΔG_a decreased from -2.90$x10^3$ $Jmol^{-1}$ to -7.40$x10^3$ $Jmol^{-1}$ as temperature increased from 298K to 343K, which becomes the suitable adsorption temperaturerange for Protobind 1000.By choosing 298 K, 20% less cellulase would be adsorb on ligneous component than at 343 K and 30% more cellulases will adsorb [delignified wheat starw].Therefore, a temperature closer to 298 K is recommended for adsorptionpart of bioethanol production process. Reuse of adsorbed enzymes to save cost of enzymatic hydrolysis is important, therefore, entropy and enthalpy of desorbed cellulases from was studied next.

Desorption from Avicel PH 101and from Protobind 1000

Van't Hoff plots were constructed for the desorption of cellulases from Avicel over a temperature range of 298K to 343 K and a pH range of 6 to 9 (Fig. 3) for desorption time 20 minutes (data not shown). The curves were drawn up to $T \leq 333K$ because there appears to be denaturing of cellulases occurring after 333 K [60-61]. The denaturing was similarto what happened for adsorption but was much more pronounced.



Desorptionregression equations are given below:

$$lnK_{d6} = (-2.10)\frac{1}{T}10^3 + 5.65 \quad \ldots \quad 5$$

$$lnK_{d7} = (-2.33)\frac{1}{T}10^3 + 6.32 \quad \ldots \quad 6$$

$$lnK_{d8} = (-2.98)\frac{1}{T}10^3 + 8.19 \quad \ldots \quad 7$$

$$lnK_{d9} = (-3.15)\frac{1}{T}10^3 + 9.10 \quad \ldots \quad 8$$

Since all slopes of the van't Hoff equationswere negative, all ΔH_d values were positive.There was a slight increasing trend in $\Delta H_d$from pH 6 to pH 9:17.5, 19.4, 24.1, 26.2kJmol^{-1}, respectively.This meant that heat energywas gained by the cellulasesdesorption system,which resulted in the decrease invander Waals interactions and hydrogen bonding between cellulases and cellulose. The ΔH_d values obtained from the last two point 333K to 343K for all pH values were negative as -15.8, -24.6, -21.5, and -41.5kJmol^{-1} respectively. The negative signs of enthalpy usually mean that the heat is being released by the occurring reaction.The changes in entropy, ΔS_d, for all pH values were (46 to 75 Jmol^{-1}K^{-1}) positive. [Entropy of adsorption was ΔS_a was -50]This indicated decreased randomness which favored desorption. At 333K to 343K the ΔS_d was -52, -79, -68, and -127 Jmol^{-1}K^{-1}for pH6 to pH 9 which indicatedfurther decreased desorption. The values of ΔG_d obtained from Equation 2are given in Table 1.

Table 1: ΔG_d obtained for desorption of cellulases from Avicel PH 101 for pH 6 to pH 9 at temperatures 298K to 343K

T K	ΔG_{d6} Jmol^{-1}K^{-1}	ΔG_{d7} Jmol^{-1}K^{-1}	ΔG_{d8} Jmol^{-1}K^{-1}	ΔG_{d9} Jmol^{-1}K^{-1}
298	3.54 x10^3	3.77 x10^3	3.80 x10^3	3.60 x10^3
313	2.84 x10^3	2.98 x10^3	2.80 x10^3	2.48 x10^3
323	2.37 x10^3	2.12 x10^3	2.10 x10^3	1.72 x10^3
333	1.90 x10^3	1.94 x10^3	1.45 x10^3	0.970 x10^3
343	2.31 x10^3	2.52 x10^3	2.05 x10^3	2.10 x10^3

ΔG_d values were all positive and decreased with increase in temperature. Gibbs free energy (ΔG_d) decreased with the increase in temperature which means at pH 9 and 333K the cellulases showed minimum affinity (adsorption interest) for the substrate. The maximum desorption of cellulases was achieved at almost 333K for all pH values. Theoretically,the maximum desorption achieved should be at 343K since high temperature support desorption. The decrease in desorption while moving from 333K to 343K can be attributed to the configurational changes of cellulases which render the cellulases to desorb. The ΔG_d values calculated for temperature 343K were large and positive as compare to the values at 333K represented a less desorption the temperature(last row of Table 1).The positive and large ΔH_d, smallΔS_d value, with positive ΔG_d values indicated that desorption process was favorable for pH ranging from 6 to 9 and the temperature ranging from 298K to 333K only. Beyond T ≥333K, ΔH_d was negative ΔS_d was negative and $\Delta G_d$was very large which resulted in decrease in desorption due to denaturing of cellulases.

Desorption of cellulases NS 500013 from Protobind, in similar conditions as that for, Avicel is shown in Fig. 4. The maximum error barof all individualized experiments is at pH8 and 313K (3.19 x 10^{-3} K^{-1}). Again data for T ≥ 333K showed incompatibility with rest of the data because of very low lnK_d values observed (low desorption from the Protobind surface).

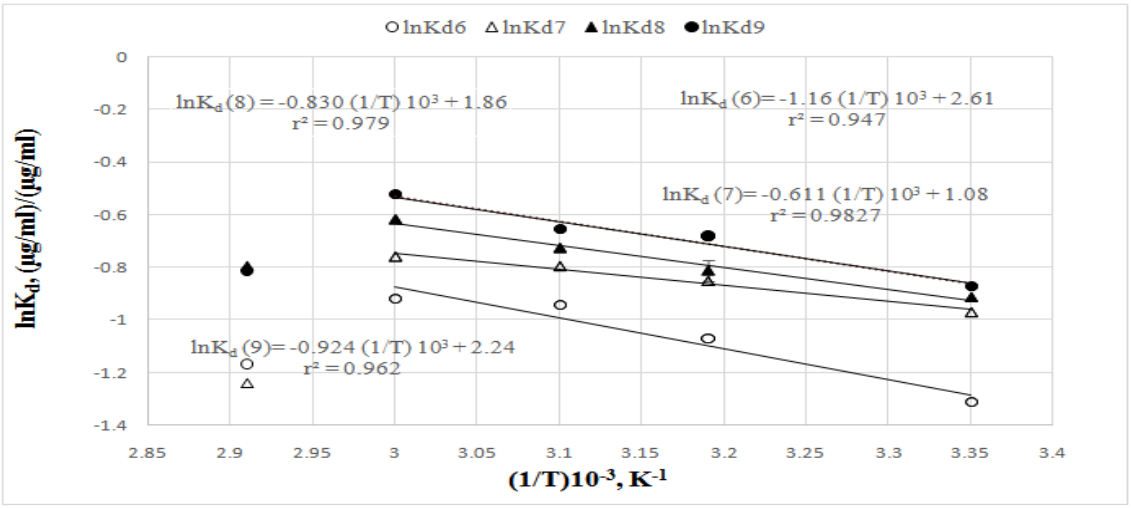


Figure 4: Desorption of Cellulases NS 50013 from Protobind 1000at various pH and temperatures with maximum error bar at 95% probability

The lnK_d increased with an increase in temperature from 298K to 333K and the corresponding regression equations are given below:

$$lnK_{d6} = (-1.16)\frac{1}{T}10^3 + 2.60 \quad \ldots \quad 9$$

$$lnK_{d7} = (-0.610)\frac{1}{T}10^3 + 1.00 \quad \ldots \quad 10$$

$$lnK_{d8} = (-0.830)\frac{1}{T}10^3 + 1.80 \quad \ldots \quad 11$$

$$lnK_{d9} = (-0.920)\frac{1}{T}10^3 + 2.20 \quad \ldots \quad 12$$

As with Avicel, all slopes for desorption from Protobind had negative values, although their values for Protobind were in the range of 0.610Kto 1.160K, 2-4 times less than Avicel at the respective pH values, leading to positiveΔH_dvalues of 9.60, 5.10, 6.90 and 7.70kJmol^{-1}respectively.The positive ΔH_d values indicated that the supplied heat enrgy was consumed in weakening the interactions between cellulases and Protobind. The large negative ΔH values were observed between 333K to 343K andwas attributed to a conformational change in enzymes.The small ΔS_d values of 21.70, 9.00, 15.40, and 18.60 Jmol^{-1}K^{-1} for all desorption from Protobind signify that all reactions are not entropy driven rather enthalpy-driven. This small ΔS_d in case of desorption could be the result of an indirect decrease in entropy due to the influence of water molecules in the vicinity of non-polar cellulases residues which are broken down at higher temperatures. The decrease in the entropy of desorption may be due to the decrease in the conformational flexibility of cellulases [62].The small ΔS_d implying that the cellulases-substrate complex has restricted flexibility (highly ordered), therefore less desorption.For a process that involves a decrease in entropy and a small change in enthalpy, a positive free energy change, ΔG, means that will not occur spontaneously [63]. ΔG_d values calculated for pH 6, 7, 8 and 9 over the range of temperature 298K to 333Kare presented in Table 2. They remainedpositive and almost the same throughout the range of temperature and pH

Table 2: ΔG_d obtained for desorption of cellulases from Protobind 1000 for pH 6 to pH 9 at temperatures 298K to 343 K

T K	pH 6 $Jmol^{-1}K^{-1}$	pH 7 $Jmol^{-1}K^{-1}$	pH 8 $Jmol^{-1}K^{-1}$	pH 9 $Jmol^{-1}K^{-1}$
298	3.18×10^3	2.38×10^3	2.30×10^3	2.14×10^3
313	2.86×10^3	2.25×10^3	2.08×10^3	1.86×10^3
323	2.64×10^3	2.16×10^3	1.93×10^3	1.67×10^3
333	2.40×10^3	2.07×10^3	1.78×10^3	1.48×10^3
343	3.29×10^3	3.47×10^3	2.24×10^3	2.28×10^3

ΔG_d values decreased from 2.40, 2.07, 1.78and 1.48$kJmol^{-1}K^{-1}$as pH increased from pH 6, 7, 8, and 9 at 333K and it also decreased with increase in temperature up to 333K. Minimum energy is required to desorb at pH 9 and 333K. ΔG_d was increased for all pH at 343K. The ΔG_d values calculated for temperature 343K were very large and positive as compare to the values at 333K represented a less desorption the temperature (last row of Table 2).Themaximum energyto desorb cellulases from Protobind was obtained at 343K for all pH values as given in the last row of Table 2.Desorption from the cellulases-substrate complexwas restricted due to unfolding of cellulases.

Positive ΔH_d and small ΔS_d were obtained from the plots for both Avicel and Protobind for desorption of cellulases over a range of temperature from 298 to 333K. Negative ΔH_d and small ΔS_d were obtained for both Avicel and Protobind at 343K. Similarly, ΔG_d decreased moving from pH 6 to pH 9, and further decreased increasingtemperature from 298K to 333K. ΔG_d was minimum at pH 9and 333K for both substrates. For lignocellulosic materials containing both cellulase and lignin desorption of cellulasescan be performed at pH 9 with 333K.

IV. Conclusion

Change in enthalpy for adsorption of cellulases on the surface of the Avicel was observed which decreased with the increase in temperature and indicated an exothermic reaction. A positive ΔH_a value for adsorption of cellulases on Protobind indicated an endothermic reaction. The ΔS_a value for Avicel was negative which means disorder of cellulases-cellulose system was decreased on adsorption. The contribution of entropy to the adsorption of cellulases on Protobind 1000 was opposite to that to the Avicel. Change in free energy of adsorption for Protobind was negative for all temperatures range while ΔG for Avicel change from negative to positive at 323K. It means that the 298K to 323K temperature ranges was spontaneous for adsorption on lignocellulosic materials. Lignocellulosic material may have a positive or negative change in enthalpy for adsorption of cellulases depending upon the composition of the substrate.

The positive ΔH_d for both Avicel and Protobind favored desorption. The positive ΔS_d for both Avicel and Protobind indicated that the increased randomness also favored desorption. A positive and minimum ΔG_d indicated that cellulases have less affinity for substrate at pH9 and 333K where cellulases showed maximum on desorption.

Since wheat straw is a combination of cellulose and lignin, the thermodynamic study offers a strategy for using wheat starw for ethanol production. The negative ΔH_a ,ΔS_a and ΔG_a indicated that maximum temperature for adsorption suitable is 298K to 323K. In depth study showed that maximum adsorption on Avicel was between temperatures 298K to 323K and maximum adsorption on Protobind was on temperature 333K to 343K. To avoid adsorption on lignin (in addition to optimum removal of lignin) low temperatures such as 298K to 323K should be used for adsorption because at these temperature thermodynamic conditions give less support for adsorption on lignin. For desorption of cellulases from wheat straw, the positive ΔH_d, ΔS_d and ΔG_d indicated favored desorption at pH 9 temperatures 323k and 333K.

V. Acknowledgement

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