International Journal of Waste Resources

Baig et al., Int J Waste Resour 2016, 6:2
DOI: 10.4172/2252-5211.1000222

Research Article Open Access

Looking at Adsorption of Cellulases NS 50013 onto Avicel PH 101 and Protobind 1000 through Isotherms and Thermodynamics

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Received date: June 07, 2016; **Accepted date**: June 10, 2016; **Published date**: June 17, 2016

Abstract

Understanding adsorption characteristics of cellulases can help to control the mechanism of adsorption of cellulases onto wheat straw. Desorption and reuse of cellulases is a way to decrease cost of production of bioethanol which can be perfected with knowledge of adsorption characteristics of cellulases. Adsorption of cellulases NS 50013 onto microcrystalline cellulose (Avicel PH 101) and wheat straw lignin (Protobind 1000) was studied in batch reactors.

Protobind adsorbed twice the amount of cellulases as did Avicel PH 101 and the rate of adsorption was higher than that of Avicel PH 101. A comparison of three (most used) adsorption isotherms was conducted to see: i) A correlation between cellulases adsorbed and initial cellulases loading, ii) Is it a monolayer adsorption, iii) Adsorption capacities of the substrates. It was observed that Langmuir isotherm was a good representation of adsorption for both Avicel and Protobind on correlation coefficient of 0.9572 and 0.9880. The Gibbs free energy changes obtained by using van't Hoff equation indicated that the adsorption was mainly spontaneous. However for Avicel, the process was spontaneous up to 220 $\mu g.mL^{-1}$ and the spontaneity decreased with the cellulases concentration. For initial concentrations between 220 $\mu g.mL^{-1}$ and 250 $\mu g.mL^{-1}$ the cellulases adsorption process became non-spontaneous. While ΔG was quite opposite for Protobind, as it was non spontaneous at 100 $\mu g.mL^{-1}$ and for further increase in concentration it was found spontaneous till 262 $\mu g.mL^{-1}$.

Among all three, Langmuir adsorption isotherm appeared to be the best represented of the cellulosic adsorption pattern. Hence, implying homogenous, monolayer adsorption. The reversible part of Langmuirian adsorption theory is in question in recent enzymatic literature therefore, a detailed study on desorption has been suggested by authors.

Keywords: Protobind 1000; Lignin; Cellulose; Langmuir; Isotherms

Introduction

In search of alternative source of energy, discovery to utilize biomass for production of bioethanol was an important milestone in solving fuel problems of mankind. Biomass is an abundant and a renewable source. Biomass is mainly consists of cellulose, lignin and hemicellulose. In its structure, cellulose is a linear polymer, comprised of polymer of β-D-glucose units. Anhydroglucose units are bonded via 1,4-β-glycosidic linkages. The 1,4-β-glycosidic linkage enables an intense intramolecular H-bonding among the groups around the glycosidic bond [1]. Lignin is a complex aromatic polymer, with varying side-chains allowing for different chemical interactions. Lignin is a natural protection of cellulose. Techniques have been developed to degrade or remove lignin in order to utilize this huge amount of cellulose. The exposed cellulose is available to react with enzymes in enzymatic hydrolysis. Cellulases are the enzymes to give efficient conversion of cellulose to glucose.

All of the lignin present in natural biomass is not completely removed by any feasible delignification technique. Therefore, the subsequent enzymatic hydrolysis involves adsorption of the cellulases enzymes on both the cellulose and lignin surfaces [2]. The substrate-cellulases system is heterogeneous and consists of the water-insoluble cellulose and the water soluble enzymes. The first step in cellulolysis is binding (adsorption) of the cellulases onto the substrate. Equilibrium relationships between cellulases and substrates are described by adsorption isotherms. Adsorption isotherms give the capacity of the adsorbent based on the ratio between the quantity adsorbed and the remaining in solution at fixed temperature at equilibrium [3]. The adsorption isotherms have history for being used in representing adsorption of gases on liquids [4] and liquids on solid surfaces [5,6]. Recently, some reports are available on cellulases adsorption on various lignocellulosic substrates, such as microcrystalline cellulose [7,8], corn stover [9], steam-exploded Douglas fir [10], pretreated hardwood [11], isolated lignin from softwood [12] and lignin preparations from lodgepole pine [13]. The information about adsorption of cellulases onto lignin is very rare. For wheat straw lignin none could be found. Adsorption of cellulases on cellulose and lignin under similar experimental conditions was never studied although both will be present on lignocellulosic substrate at the same time. The experiment determined that adsorption of cellulases onto microcrystalline cellulose can be represented by Langmuir model. Some of the researchers have suggested Freundlich isotherm for adsorption of cellulases [13,14]. Few reports used both Langmuir and Freundlich isotherms to represent adsorption of cellulases [15-17]. A dedicated study on the adsorption of cellulases on cellulose and lignin was required. Adsorption isotherms are usually the ratio between the quantity adsorbed and the remaining in solution at fixed temperature at equilibrium. Adsorption is the ability of cellulases to stay on a

substrate. The adsorption studies also provide insight about the structure of adsorbed layers, the type of interaction of cellulases with the substrates and, hence about desorption because desorption is dependent on surface coverage. The aim of the present work was to investigate the adsorption of cellulases on microcrystalline cellulose (Avicel PH 101) and wheat straw lignin (Protobind 1000) under similar experimental conditions. The adsorption pattern to best describe cellulases adsorption was investigated by using Nernst, Langmuir and Freundlich isotherms. This study will also help to understand about which of Langmuir or Freundlich represent adsorption of cellulases. Furthermore, this is the first report on the study of cellulases adsorption on Protobind 1000.

Theory

Adsorption is the adhesion of atoms, ions, or molecules (adsorbate) to a surface (adsorbent/substrate). Adsorption is mostly explained using isotherms, i.e., the amount of adsorbate on the adsorbent as a function of its concentration at constant temperature. The quantity adsorbed is standardized by the mass of the adsorbent to compare different materials. In determining suitable isotherm for data representation only two factors are considered: i) The accuracy of interpretation/description, and ii) The ease with which the isotherm can be incorporated into adsorption calculations.

Nernst's adsorption isotherm

Nernst's adsorption isotherm is also known as linear adsorption isotherm. Nernst adsorption isotherm is linked to the distribution of a solute between two immiscible solvents. According to Nernst isotherm equation, enzymes (cellulases) irrespective of their total amount, distribute themselves between two layers (adsorbed or unadsorbed) in a constant ratio, at constant temperature. The ratio, equal to the constant in Equation 1, is referred to as the distribution or partition ratio or coefficient (K_N) [18]:

$E_e = K_N [E_f]$ … 1

where,

E_e = Amount of cellulases adsorbed on substrate, μg.mg^{-1}

$[E_f]$ = Concentration of cellulases in the bulk solution, μg.mL^{-1}

K_N = Partition coefficient given by the slope of plot between E_e Vs $[E_f]$, (μg.mg^{-1})/

(μg.mL^{-1})

Interactions contribute to Nernst partitioning adsorption are hydrophobic interaction, van der Waals forces [19]. The Nernst adsorption isotherm (Equation 1) is considered as a limit case of Freundlich and Langmuir adsorption isotherms [20].

Langmuir adsorption isotherm

The Langmuir adsorption model is a semi-empirical isotherm. It is used due to its simplicity and its ability to fit a variety of adsorption data. It is based on four assumptions: i) All of the adsorption sites are equivalent and each site can adsorb one molecule, ii) The adsorbent is energetically homogeneous and adsorbate do not interact, iii) No phase transitions occur during adsorption, iv) At the maximum adsorption, only a monolayer is formed. For an adsorption system (adsorbent, adsorbate, carrier), all these assumptions are occasionally true because adsorbent surface may have imperfections, adsorbate molecules may not be inert, and the mechanism of adsorption may be different for the very first molecules to adsorb to adsorbent as for the last. A number of researches used the following equation to determine amount of cellulases adsorbed on lignocellulosic substrates:

$$E_e = [E_f] \frac{K_a [E_a]}{(1 + K_a[E_f])} \ldots 2$$

where,

$[E_a]$ = Maximum cellulases adsorbed (μg of cellulases mg of substrate^{-1})

K_a = Constant (μg/ml$^{-1)}$

E_e = Cellulases adsorbed, μg cellulases mg of substrate^{-1}

The Equation 2 is a hyperbolic equation, the rearrangement of Equation 2 is called Scatchard regression (Equation 3), proposed in 1949.

$$\frac{E_e}{[E_f]} = K_a E_a - K_a E_e \ldots 3$$

A plot of $\frac{E_e}{[E_f]}$ Vs Ee was used to determine adsorption.

Freundlich adsorption isotherm

The Freundlich isotherm has a place in colloid chemistry for characterising the adsorption of molecules onto an interface [21,22]. It is a purely empirical formula for adsorption (Equation 4) where K_f and m are empirical constants for each adsorbent-adsorbate pair at a given temperature. As the temperature increases, the constants K_f and m are changed to reflect the empirical observations made.

Freundlich isotherm has been used to describe adsorption of α-amylase to starch granules. Starch granules are heterogeneous with difference in molecular structures amount and type of molecular organisation, shape, size and surface features [23]. Freundlich isotherm for adsorption to heterogeneous surfaces is given as follows:

$$E_e = K_f [E_f]^{\frac{1}{m}} \ldots 4$$

where,

K_f = Freundlich constant for cellulases-substrate complex, distribution constant, μg mg^{-1}

m = Power term of the Freundlich Isotherm (m>1)

m and K_f are empirical parameters.

The linear form of Equation 4 can be written as:

$$\ln E_e = \frac{1}{m} \ln [E_f] + \ln K_f \ldots 5$$

Material and Methods

Materials

Avicel PH 101 (analytical grade, 100% solids, Sigma-Aldrich Corp., Missouri, and USA) is a white powder cotton-source microcrystalline cellulose. Protobind 1000 (analytical grade, 100% solid) is an aromatic polyphenolic material derived from wheat starw lignin during its soda

pulping was generously donated by Dr. J. Lora from Green Value Enterprises LLC. Cellulases NS 50013 was in a tetenal polyethylene bottle was a gift from Novozymes, Denmark. This concentrated solution was diluted with 0.05 mM sodium citrate buffer and distilled water to prepare solutions of 100 μg.mL^{-1} to 265 μg.mL^{-1} (used as [E_0's]). The concentration of cellulases in these solutions was measured by Lowry method [24].

Procedure for adsorption of cellulases

Adsorption of cellulases onto a substrate (Avicel PH 101 or Protobind 1000) was done by allowing the cellulases [E_0] to interact with the substrate. In fact, 5 mL of each predetermined ([E_0]) diluted cellulases solution was placed in contact with 100 mg of substrate in 10 mL screwed tight glass tube. The glass tubes were incubated in an incubator shaker 'INNOVA 40' (New Burnswick Scientific-Nederland) at 100 rpm, room temperature, and PH 5 for 5 min to 90 min contact time in triplicates. At each predetermined time the sample tube was taken out, centrifuged for 4 min and decanted off free (bulk or unbound cellulases) [E_f]. The cellulases adsorbed [E_a] were measured from the difference in initial [E_0] and concentration of cellulases in the supernatant [E_f]. The equilibrium uptake of cellulases was calculated by Equation 6:

$$E_e = \frac{(E_0 - E_f)\ V}{m} \ldots 6$$

E_e = Amount of cellulases adsorbed on substrate, (μg cellulases adsorbed) (mg of substrate)-1

m = Mass of substrate

V = Volume of adsorption system

Results and Discussion

In this study, adsorption of cellulases NS 50013 on microcrystalline cellulose (Avicel PH 101) and lignin (wheat straw lignin, Protobind 1000) with three (3) relations, Nernst, Langmuir and Freundlich, were examined. The data for isotherms was obtained by studying batch adsorption at room temperature (25°C), PH 5 and 100 rpm.

Cellulases adsorbed

The concentration of cellulases adsorbed is the basic unit for study of adsorption isotherms. In Figure 1, the concentration of cellulases adsorbed [Ea] 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 except that at 160 μg.mL^{-1} was 8 times. 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 (Figure 1).

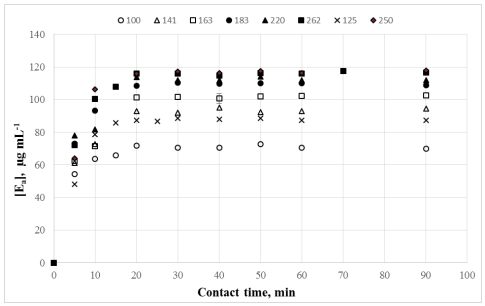

Figure 1: Adsorption of cellulases on Avicel PH 101 at 100 rpm, 25°C, [E_0] varied from 100, 125, 141, 163, 183, 220, 250 to 262 μg.mL^{-1} with error bars at 95% probability.

Similarly, adsorption of cellulases on Protobind 1000 was studied for amount of cellulases adsorbed for varying adsorption time under same conditions as Avicel was given in Figure 2. The maximum adsorption was achieved at 40 minutes of adsorption time.

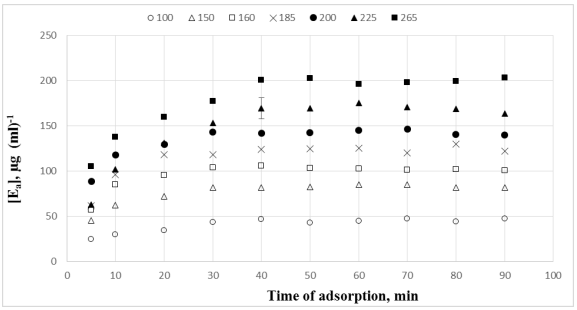

Figure 2: Adsorption of cellulases on Protobind 1000 at 100 rpm, 25°C, [E_0] varied from 100, 150, 160, 185, 200, 225, to 265 μg.mL^{-1} with error bars at 95% probability.

However, the time of adsorption on Avicel was taken 30 minutes for conducting experimentation for the study of isotherms. The experiments conducted to determine the amount of cellulase adsorbed on Protobind for adsorption equilibrium studies was taken as 45 minutes. The maximum concentration of cellulases adsorbed [Ea] for initial cellulase [E_0] 265 μg.mL^{-1} taken on 100 mg of Avicel PH 101 was 108-115 μg.mL^{-1}. The maximum amount of cellulases adsorbed on 100 mg of Protobind 1000 was 197-207 μg.mL^{-1}. Hence, Protobind has almost twice the adsorption capacity of Avicel.

Adsorption Isotherms

Nernst adsorption isotherm

Nernst adsorption isotherm is also known as linear adsorption isotherm was shown in Figure 3.

In Figure 3, amount of cellulases adsorbed on unit amount of Avicel PH 101 'E_e' was plotted along Y-axis and amount of cellulases present free in the supernatant at adsorption [E_f] was plotted along X-axis. All the data appoints are average of minimum 5 replicated conducted with initial enzyme concentration [E_0] of 100–265 g/ml. Error bars were

obtained at 95% probability. Each data point was measured separately. Total experiments conducted were 37. According to the Nernst theory, irrespective of the initial (total) cellulases concentration [E_0], cellulases distribute themselves in [E_a] and [E_f] in a constant ratio. The model equation obtained by plotting E_e against [E_f] according to the Nernst theory was: E_e = 0.0167 [E_f] + 3.694. In contrary to expectations did not pass through origin according to Equation 1. Therefore, the linear isotherm model did not so well fit the data obtained. The term E_e in the Nernst model suggest that there are limited sites (concentration of substrate was fixed) available for adsorption while direct (linear) relationship did not limit the amount of adsorption.

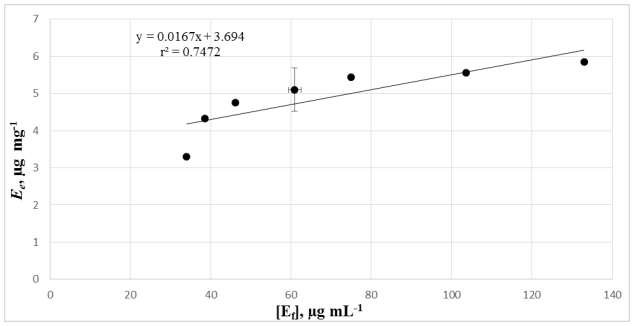

Figure 3: Adsorption of cellulases on Avicel PH 101 following Nernst adsorption with error bars at 95% probability.

The linear isotherm model did not fit so well with the obtained data. Because there are limited sites (concentration of substrate is fixed) available for adsorption and a direct (linear) relationship did not limit the amount of adsorption. The slope (distribution coefficient, K_N) obtained was 0.0167. The r^2 of 0.7472 indicated that adsorption on Avicel PH 101 almost 75% obeyed this relationship. The plot showed that with the increase in E_e, the [E_f] also increased and a linear relationship was obtained but the plotted line did not pass through the origin while the model stated that Ee was proportional to [E_f]. Therefore Nernst isotherms cannot be used to represent adsorption of cellulases on Avicel PH 101.

Linear adsorption pattern for Protobind 1000 was shown in Figure 4. The concentration of cellulases adsorbed [E_a] on Protobind 1000 in equilibrium with concentration of cellulases remaining in the solution [E_f] for an initial concentration of cellulases [E_0] between 100-265 µg.mL^{-1}, similarly with same experimental conditions as that of Avicel PH 101. The plot indicated that as E_e increased the [E_f] was increased. For a known value of [E_f] the corresponding [E_a] can be found by the model equation obtained, [E_a] = 0.249 [E_f] – 8.818.

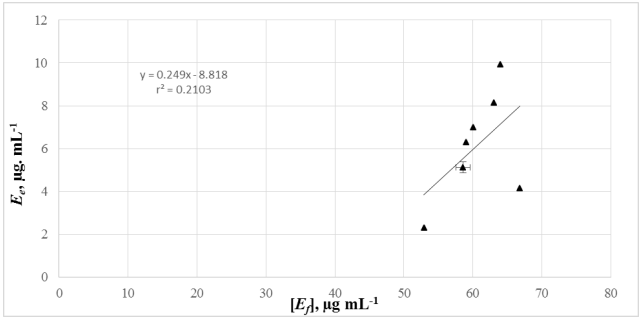

Figure 4: Adsorption of cellulases on Protobind 1000 following Nernst adsorption with error bars at 95% probability.

The slope showed that the as cellulases [E_f] increased the corresponding cellulases adsorbed on the Protobind 1000 increased. The data is clustered between 50 µg.mL^{-1} to 70 µg.mL^{-1} which indicated that the increase in specific adsorption E_e is much more than the corresponding increase in [E_f]. The both parameters E_e and [E_f] were dependent parameters on [E_0]. The r^2 value obtained was 0.2103 which suggest that Nernst adsorption model was not a good representation of adsorption of cellulases on Protobind 1000.

Langmuir Adsorption Isotherm: Adsorption data for Avicel according to Langmuir isotherm is shown in Figure 5. The ratio of 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 (K_a) and an intercept of (K_a, E_a). As [E_e] increased the corresponding (E_e/[E_f]) decreased. Eliminating one outlier (at E_e equal to 3.41 µg.mg^{-1}), rest of the seven data points gave the existing plot with r^2 value equal to 0.9572 (approximately 0.96). Thus, almost 96% of the adsorbed cellulases follow the Langmuirian adsorption pattern. The model equation obtained from the plotted data was: E_e/ [E_f] = -0.049 E_e + 0.3302.

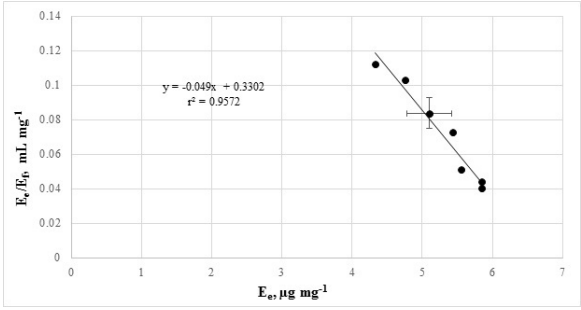

Figure 5: Langmuir adsorption on Avicel PH 101 with error bars at 95% probability.

The non-adsorbed cellulases [E_f] were found to be directly proportional to the initial cellulases concentration [E_0]. The results also indicated that the cellulases adsorbed on the surface of Avicel PH 101 homogenously and 96% adsorption is monolayer type. Similar results were observed by other researchers, as well, for adsorption of cellulases on Avicel [25,26].

Langmuir adsorption isotherm for adsorption of cellulases on Protobind 1000 is shown in Figure 6. This plot is made by using the ratio of specific adsorbed cellulases to non-adsorbed cellulases (Y-axis) versus the specific adsorbed cellulases (along X-axis). The plot gave a straight line with a slope of (K_a) and an intercept of (K_a E_a). The curve line obtained from the plotted data-points follow a model equation as $E_e/E_f = 0.015\ (E_e) + 0.007$. The slope obtained from the Figure 6 was positive while in the Scatchard regression Equation 3 showed a negative slope. Thermo Fisher Scientific explained in the 'theory of binding data analyses' that a positive slope is due to the strong interaction between adsorbate and adsorbent.

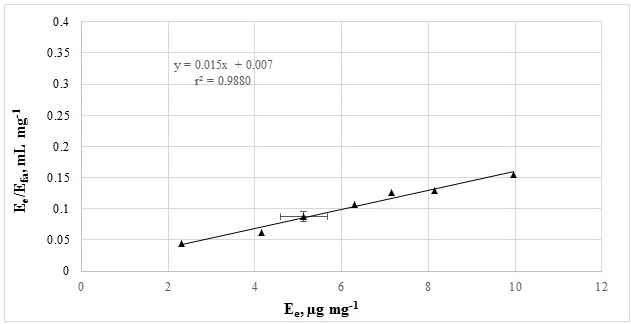

Figure 6: Langmuir isotherm for adsorption on Protobind 1000.

Similar reasoning for positive slope in adsorption analysis was also given by other researchers [27-29]. Strong interaction between Protobind 1000 and cellulases was observed when desorption of cellulases was studied [30]. The plotted data showed a perfect positive correlation between $E_e/[E_f]$ Vs E_e. The r^2 value is 0.9880 (approximately 0.99) which indicated that 99% of cellulases adsorbed on the Protobind 1000 follow Langmuir isotherm Scatchard type, as represented by Equation 3.

Freundlich Adsorption Isotherm

Freundlich adsorption isotherm of cellulases on Avicel is shown in Figure 7, it was obtained by plotting natural logarithm of specific adsorbed cellulases (ln E_e) against natural logarithm of cellulases present in the bulk of solution ln [E_f]. The model equation obtained after plotting the experimental data was: ln E_e = 0.292 (ln [E_f]) + 0.360. The obtained model equation is comparable with equation of straight line, y = m x + c where, m represents slope of the line and c represents intercept on Y axis. Plotting the graph a straight line with value of slope equal to 0.292 and ln K_f as Y-axis intercept was 0.360. The K_f value obtained was 1.43. According to Freundlich isotherm this intercept was expected. The slope is called heterogeneity factor and ranges between 0 and 1. A system is considered to be more heterogeneous when slope is closer to 0.

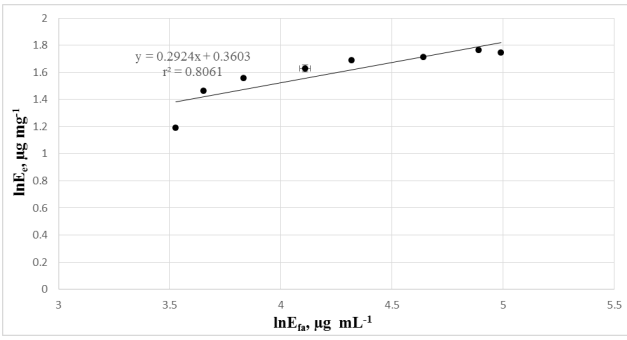

Figure 7: Freundlich isotherm for Avicel PH 101.

Freundlich isotherm is used for adsorption of heterogeneous surfaces or surface supporting sites of varied affinities [31]. Since r^2 value is equal to 0.8061 which means that Avicel surface was not having different affinities and we cannot expect any multilayer adsorption. The [E_e] value predicted was in around 3.0% error with respect to the corresponding experimental value.

Figure 8 showed Freundlich adsorption isotherm for adsorption of cellulases on Protobind 1000. The plot was obtained by plotting of lnE_e (y-axis) vs ln [E_f] was a straight line with r^2 = 0.2813 and its intercept giving lnK_f. The K_f calculated for wheat straw lignin (Protobind 1000) was 8.21 × 10^{-6} µg mg^{-1} while K_f for Avicel PH 101 was 1.43 µg.mg^{-1} which provided an unrealistically small value of adsorption capacity of lignin. Reading the value of K_f with r^2 value showed not to use Freundlich isotherm as representative for adsorption on Protobind 1000.

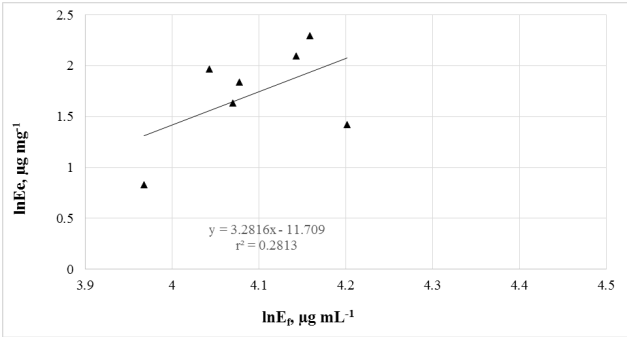

Figure 8: Freundlich isotherm for Protobind 1000.

The correlation factor obtained for Freundlich adsorption isotherms from Avicel and Protobind were 0.8679 and 0.2813 respectively. By removing one outlier at ln [E_f] = 4.2 µg mg^{-1} (Figure 8), the shape of the trend line obtained was: ln E_e = 6.683 (ln [E_f]) - 25.46, with r^2 became equal to 0.8120 with remaining 6 data points. The r^2 = 0.8120 (for 6 data points) was still not better than r2 = 0.9880 (for 7 data point for Langmuir isotherms as shown in Figure 6). Hence Freundlich isotherm was not a good representative of cellulases adsorption on the studied substrates. Therefore, it can be stated that during adsorption, cellulases did not make multilayers adsorption on Avicel as well as on Protobind.

The Experiment determined that adsorption of cellulases on microcrystalline cellulose can be explained by Langmuir model [32,33]. In this work, Langmuir adsorption isotherm (Scatchard type linear regression as given with Equation 3) appear to be a good selection for the representation of adsorption of cellulases on Avicel PH 101 and Protobind 1000. Among other adsorption methods it stand out because of correlation coefficient for both substrates. Therefore, adsorption of cellulases on Avicel PH 101 and Protobind 1000 followed Langmuir's adsorption pattern. This adsorption pattern suggested that the adsorption of cellulases in monolayer and interaction between cellulases do not form multilayer of enzymes as suggested by some others [14,16].

Free energy of adsorption

Gibbs free energy can be used to evaluate the thermodynamics feasibility of the adsorption of cellulases on to Avicel PH 101 and Protobind 1000. The Gibbs free energy for enzymatic adsorption can be estimated from the van't Hoff equation

$\Delta G = -RT \ln K_T$ ..... Equation (7)

where ΔG is the Gibbs free energy change, K_T is the equilibrium constant, R is the gas constant and T is the absolute temperature. The equilibrium constant of adsorption cellulases is given as [30]:

$$K_T = \frac{[E_0] - [E_f]}{[E_f]}$$

where f and 0 are the equilibrium and initial concentrations of the cellulases in the solution.

The Gibbs free energy of adsorption of cellulases onto Avicel PH 101 showed an increasing trend from -1.755 kJ.mol^{-1} to 0.550 kJ.mol^{-1} with initial concentration increasing from100 μg.mL^{-1} to 262 μg.mL^{-1}. The negative sign of ΔG continued for $[E_0]$ ranging from100 μg.mL^{-1} to 220 μg.mL^{-1}. The negative value of ΔG indicated that the adsorption was spontaneous. The spontaneity of the adsorption process decreased as the $[E_0]$ increased. Further increase in $[E_0]$ from 250 μg.mL^{-1} to 262 μg.mL^{-1} provided a positive ΔG. Adsorption of cellulases onto Protobind 1000 was quite opposite. For initial concentration of 100 μg.mL^{-1}, ΔG was positive (0.288 kJ.mol^{-1}) further increase in $[E_0]$ ranging from150 μg.mL^{-1} to 262 μg.mL^{-1}, the value of ΔG was negative and constantly increasing from -0.681 kJ.mol^{-1} to -3.012 kJ.mol^{-1}. Typically, the ΔG for PHysical adsorption is in the range from 0 to −20 kJ.mol^{-1} and the ΔG for chemical adsorption (adsorption that results from chemical bond formation) in the range from −80 to −400 kJ.mol^{-1} [34]. Different results were reported for heat of adsorption for example experiment found no heat of adsorption for adsorption of cellulose to cellotriose while Boraston et al., reported it -22.21 kJ.mol^{-1} for Avicel [35]. Similarly, ΔG for cellulase adsorption on enzymatically prepared lignin from lodgepole pine wood was reported −29.9 kJ.mol^{-1} [13]. The different results for the change in free energy indicated that the adsorption of cellulase on substrates differs significantly depending on the type of substrates, the pretreatment applied to the lignocellulosic biomass and other operational conditions.

Conclusion

The amounts of cellulases distributed on substrate and in bulk solution is not directly proportional as given by Nernst model. The amount adsorbed increased and decreased with increase or decrease in initial cellulases taken as but not in the same proportion.

Langmuir adsorption isotherm represented adsorption of cellulases on Avicel PH 101 and Protobind 1000. The results indicated that cellulases adsorbed on the surface of substrates in monolayer. Cellulases do not interact with each other therefore they cannot form multilayers. Cellulases interact with substrates only and the interaction with Protobind 1000 (lignin) is stronger than that of Avicel PH 101 (cellulose).

Protobind showed almost twice more adsorption capacity than that of Avicel. The initial amount of cellulases required for the conversion of wheat straw to ethanol can be adjusted accordingly by the concentration and accessibility of cellulose and lignin contents in the wheat straw. Because if more lignin is available more cellulases will be consumed in adsorption on lignin. Lignin adsorbed twice the amount of cellulases as that of cellulose and adsorption of cellulases was faster on lignin that on cellulose.

The thermodynamic evaluation of the adsorption of cellulases Avicel showed that the concentration of cellulases (100 μg.mL^{-1} to 220 μg.mL^{-1}) were favourable for adsorption onto cellulose. The spontaneity of the adsorption process decreased as the initial enzyme concentration increased for cellulose. Though adsorption on lignin is not productive but it slow for 150 μg.mL^{-1} and less. ΔG is negative at 150 μg.mL^{-1} and above showing spontaneity of adsorption on lignin.

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