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Adsorption of Cellulose Enzymes on Lignocellulosic Materials and Influencing Factors: A Review

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Abstract

Adsorption of enzymes onto lignocellulosic substrates and their integration is a major concern in the production of bioethanol. Information of the adsorption of enzymes, adsorption characteristics and practical viewpoints are necessary for understanding of an adsorption system. Adsorption of enzymes depends on so many factors, instead of examining the role of all these, this study deals with the factors affecting the key adsorption process such as accessibility of enzymes to lignocellulosic substrate or substrate components and adsorption isotherms.

Extensive analysis of the published literature on enzymatic adsorption onto lignocellulosic materials from 1994 to 2016 has consolidated in this review. The observations have enlightened some of the conflicting results in literature, especially increase or decrease to enzymatic adsorption under the influence of temperature, pH, presence or removal of lignin and hemicellulose for adsorption, enzyme to substrate ratio, inhibition by end-product and synergy of the components of cellulases mixture. It was concluded that an optimum removal of lignin is more important for creating accessibility of enzymes to substrates than non-removal. Effect of pH along with varying temperature on natural substrates has more room to work. End-product inhibition may be controlled by pretreatment and optimum enzyme to substrate ratio. The synergic actions of cellulases are obstructed by high enzyme concentration or high substrate concentration. This review will help in design of enzymatic adsorption step for bioethanol production from lignocellulosic materials.

Keywords: Adsorption; Cellulose; Lignin; Pretreatment; Inhibition; Enzyme/substrate; Synergism; Bioethanol

Introduction

To start enzymatic hydrolysis of a lignocellulosic substrate, enzymes have to adsorb on the substrate and hydrolysis of substrate enzymes have to leave (desorb) the substrate because the attached site of substrate has been hydrolyzed to reducing sugar. Therefore, adsorption of enzymes is the basic step for conversion of cellulose to glucose for production of bioethanol from lignocellulosic biomass [1-3].

Understanding of mechanism of enzymatic adsorption and its controlling factors is important to improve production of sugars and hence enhance bioethanol production from lignocellulosic biomass. Adsorption is mainly a physical contact of its two components: substrate, and enzyme. The factors affecting both components and hence adsorption were studied [4-8]. The parameters studied were crystallinity index of substrate, degree of polymerization of substrate, accessibility of cellulases, and synergy of cellulases. For a substrate which is comprised of pure cellulose, mainly physical parameters such as pore structure of fibers, crystallinity and external surface area govern adsorption. For a natural lignocellulosic biomass, in addition to these physical parameters, the chemical composition also becomes important. Lignocellulosic biomass (i.e. wheat straw, corn stover etc.) are mainly composed of cellulose, lignin, hemicellulose which make the structure compact and the cellulose is protected by lignin. The parameters related to chemical properties are: i) lignin content, or the extent of lignin removed [9-12], ii) substrate particle size [13,14], and iii) substrate accessibility to cellulase [15]. According to some of the researchers removal of lignin was important because it increased conversion of lignocellulosic biomass [16-18], while for others removal of both the lignin and xylan helped to increase the conversion. Some other researchers proved that the ease of accessibility of cellulases to cellulose was the main parameter for lignocellulosic conversion [19,20]. Particle size reduction was apparently energy consuming and hence cost increasing process [21], and for others fiber size does not matter [22]. A typical enzymatic lignocellulosic conversion process goes through four phases: i) rapid adsorption of cellulases on accessible substrate, ii) initial rate of adsorption/hydrolysis/desorption, iii) intermediate phase where 70% of substrate is hydrolyzed, iv) a very slow but steady decrease of adsorption due to inaccessible substrate. A long time of process for complete conversion lignocellulosic material adds operating costs to the process [23,24]. Enzymatic conversion of lignocellulosic materials is a heterogeneous biochemical reaction. It includes the three important practices: i) the physical contact of the reactants (adsorption), i.e., cellulose accessibility and adsorption of enzymes onto cellulose, ii) the reactivity of the substrate/s and cellulases, e.g., cellulase activity and cellulose structure, and (iii) the adsorption/hydrolysis conditions, such as temperature, pH, etc. There are controversial reports (as given above) about enzymatic lignocellulosic conversion process such as the effect of temperature, pH, lignin content and accessibility of enzymes. The accessibility of cellulases is considered as the most influencing factor for the adsorption of cellulases on the lignocellulosic substrates [25-27].

In lignocellulosic materials, the rate of enzymatic hydrolysis and its yield was observed to be directly related to the amount of the adsorbed enzymes [6,27-31] little information were reported on substrate–enzyme interactions, which include adsorption of enzymes and the

accessibility of enzyme to lignin and cellulose in pretreated lignocellulosic materials [32]. The maximum capacity of substrates to adsorb enzymes were measured and reported in literature by conducting practical adsorption of enzymes (cellulases) onto lignocellulosic substrates. The techniques reported to measure the amount of adsorbed enzymes (cellulases) and hence, to evaluate accessibility of cellulases to substrates can be divided into two groups: indirect measurements, and direct measurements. Indirect measurements include determination of surface area by using: i) Water Retention Value (WRV) method [33], ii) Differential Scanning Calorimetry analysis, [34], NMR porosimetry [35,36], iii) Solute Exclusion Technique [37,38]. It has been established that, some of the techniques used to predict the available surface area of lignocellulosic materials are not mostly accurate (e.g., water retention value, mercury porosimetry etc.). The direct method include i) Staining method which measures the adsorption of dyes [36,39], ii) cellulase adsorption methods that measure the amount of cellulase adsorbed [40-42]. Thygesen et al. [43] used polarized light microscopy along with confocal microscopy to investigate the degradation mechanism of filter paper and pretreated wheat straw cellulosic fibers by a mixture of Cellullast and Novozym 188. A fluorescently labeled cellulase (endoglucanase GH45) was used to study the changes appearing at adsorption locations present in the wheat straw fibers by noticing the adsorption of the labelled cellulases. It was observed that as adsorption progressed, the fibers remaining in the tested sample became shorter and the number of vacant locations was reduced [43]. Recently, Luterbacher et al. [44] also used confocal fluorescence microscopy, monitored the adsorption of fluorescently labelled Cel7A 6 CBHI (cellulase) and degradation of bacterial microcrystalline cellulose (BMCC) by adsorption/hydrolysis of cellulases. It was concluded that the accessibility of cellulases was a key factor for the degradation of BMCC [44]. In order to correlate accessibility of substrates to the amount of cellulases adsorbed, the maximum enzyme adsorbed to a substrate became a requirement for efficient adsorption. The correlation between enzymes adsorbed and accessibility is given by adsorption isotherms.

Adsorption isotherms is a curve relating the equilibrium concentration of adsorbate/s (enzyme/s) on the surface of an adsorbent, to the concentration of the enzymes in the solution with which it is in contact at fixed temperature at equilibrium [2,3,45]. The adsorption capacity of adsorbent may be determined by the use of an adsorption isotherm. Some reports are available on cellulases adsorption on various lignocellulosic substrates, such as microcrystalline cellulose [40,46], corn stover [47], steam-exploded Douglas fir [48], pretreated hardwood [49], isolated lignin from softwood [50] and lignin preparations from lodgepole pine [51]. The information about adsorption of cellulases onto lignin is very rare. Some of the researches have shown that adsorption of cellulases enzymes onto microcrystalline cellulose can be represented by Langmuir model [45,52]. Others have suggested that Freundlich isotherm for adsorption of cellulases [53,54]. Few interesting reports used both Langmuir and Freundlich isotherms to represent adsorption of cellulases on their substrates [55-57]. Therefore, a thorough study on the adsorption of cellulases on cellulose and lignin was required.

A literature survey was conducted on the reviews published from 1994 to 2016 on the enzymatic immobilization, adsorption and hydrolysis [4,6,30,58-68]. The gaps identified in literature were that the extensive data was not available for the parameters such as substrates types, enzyme preparation method, enzyme/substrate ratios, substrate adsorption capacity, and ease of adsorption due to accessibility. Instead of examining all the parameters that affect adsorption of cellulases during hydrolysis of cellulose for production of ethanol from lignocellulosic materials, in this review a general approach based on recent literature data has been discussed. The objective of this investigation was to: i) consolidate some of the conflicting results on enzymatic adsorption of lignocelluloses and, ii) improve understanding of adsorption mechanism of cellulase enzymes onto lignocellulosic materials. Wherever it was fond necessary and the examples from adsorption of enzymes on lignocellulosic materials were not available, the examples from adsorptions of atoms, ions and molecules were given to make a point. This review paper will also give insights to settle the conflicts about adsorption pattern on lignocellulosic materials and how to improve mechanism of adsorption onto substrate fibers by examining influencing factors. The influencing factors discussed are temperature, pH, product inhibition, E/S ratio, and synergy of action of cellulases onto substrates.

Accessibility of Cellulases to Cellulose

The accessible surface area of the cellulose is one of the most important factors which shape the rate and extent of enzymatic adsorption of lignocellulosic substrates [1-3]. As the enzymatic adsorption of cellulose is a surface-subjugated phenomenon, and direct physical contact between the cellulase enzymes and substrate must occur. Converse et al. [69] studied adsorption of cellulases on cellulose obtained from a pretreated wood at 40°C and pH 5 and found that adsorption was dependent on surface area. It was also pointed out that the surface area provided for adsorption was not a function of substrate concentration rather it was a function of pretreatment conditions which means accessibility of cellulases to the substrate surface [69]. Some other researchers also suggested that the accessible surface area of the cellulose was one of the most important factors for adsorption of cellulases [25,68-71].

Cellulose accessibility was generally measured by the molecule adsorption methods, and a wide range of gross area values were reported in literatures. For example following Table 1 give surface area (accessibility) measurement of cellulose obtained from different type of cellulosic substrates while corresponding technique of measurement accessibility is also given.

Due to variations in experimental conditions (techniques used) such as adsorption time, vacuum time, vacuum pressure, sample preparation, varying quality of cellulose depending on batches and production location and sample origin [72,74], the values were found different. The surface area measured follow two techniques: i) external surface area, ii) external plus internal surface area. The external surface area of cellulose is related to the shape and particle size of cellulosic samples, therefore it was estimated by microscopy [4,72,75]. The internal surface area techniques falsely overestimate the accessible surface area [69]. To avoid the confusion in overestimation, labelled enzymes were used for assessing the cellulose accessibility to cellulases, it was found that the molecule used to probe into the substrate reached to the pores where average enzyme cannot reach. Enzymes were found adsorbed onto lignin as well [41,76]. The analysis of adsorption data reported lead to the conclusion that there was a linear dependency of the accessibility of crystalline and non-crystalline cellulose surface on the enzymes adsorbed [76] and the accuracy of measurement of the enzymes adsorbed depends on the technique used. The more surface was exposed the more adsorption there was. In fact, more surface exposure means more accessibility to enzymes.

Cellulose	Accessibility, m^2/g	Technique	Ads max, mg/g	Reference
Filter paper	9.76	Nonhydrolytic TGC Protein Adsorption	86.2	Hong et al. [40]
CF	0.158	Microscopy	-	Weimer et al. [72]
Sigmacell 20	0.301	Microscopy, ESA	-	Weimer et al. [72]
Avicel PH 105	2.38	Nonhydrolytic TGC Protein Adsorption	21	Hong et al. [40]
Avicel PH 101	-	Adsorption	84	Kumar and Wyman [31]
Avicel PH 101	-		34.9	Zheng et al. [73]
AC	0.606	Microscopy, ESA	-	Weimer et al. [72]
Avicel 101	20	BET, N2 adsorption	-	Marshall and Sixsmith [74]
BMCC	33.5	Nonhydrolytic TGC Protein Adsorption	295	Hong et al. [40]
RAC	41.9	Nonhydrolytic TGC Protein Adsorption	369	Hong et al. [40]

Table 1: The maximum cellulases adsorbed (Adsmax) and their accessibility on various cellulosic substrates.

Accessibility of Cellulases to Lignin

Lignin is a natural protective barrier that constraints water and enzyme accessibility to cellulose. The lignin samples used in all adsorption reported studies were prepared from pretreated from agricultural and forestry materials by extensive enzymatic hydrolysis or by a pretreatment technology. This materials were then exposed to enzymes for adsorption and the equilibrium behavior was determined. Table 2 showed maximum amount of cellulases adsorbed on lignin prepared from different techniques and different substrate. The reported adsorption capacity values of lignins varied from 1.91 to 133.6 mg enzymes/g lignin and increased with respect to the pretreatment types using ammonia fiber expansion (AFEX), dilute acid, steam explosion and lime or the case of CEL-SELP and CEL-EPLP. Zheng et al. [73] adsorbed cellulases on two substrates corn stover lignin and Rice straw lignin. While corn stover was pretreated by two different techniques (Dilute acid and Steam explosion). Steam exploded corn stover and rice straw had almost similar maximum cellulases adsorbed [73-80]. Kumar and Wyman [18] compared cellulases adsorbed on corn stover lignin prepared from five different techniques [31]. There is no match in the amount of cellulases adsorbed on dilute acid pretreated corn stover by the two authors (e.g., 2.34 27 mg/g and 90.7 mg/g).

Gharpuray et al. [80] worked on wheat straw, decreased its lignin contents from 11.53% to 1.33%, its surface area increased from 0.64 m^2/g to 1.8 m^2/g. In the next step they adsorbed cellulases QM 9414 on the delignified wheat starch and found that the relative rate of hydrolysis increased more than 10 times of the original wheat straw [80]. Similarly, Kong et al. [81] delignified Aspen Wood from 21.3% to 4.3% lignin contents by alkaline pretreatment and adsorbed Cellulase Cytolase TM 123 on Aspen wood and found that the adsorption of proteins increased which in turn increased sugar yield from 50.7% to 70.1% [81].

Lignin	Enzyme type	Ads max, mg/g	Reference
APCL	Accellerase 1000	2.34	Zheng et al. [73]
SPCL	Accellerase 1000	1.91	Zheng et al. [73]
SRPL	Accellerase 1000	2.3	Zheng et al. [73]
CEL-SELP	Celluclast 1.5	10.2	Tu et al. [54]
CEL-SELP	MSUBC	4.87	Tu et al. [54]
EL-SEHW	Cellulase GC 123	12.3	Nonaka et al. [78]
CEL-EPLP	Celluclast 1.5	2.73	Tu et al. [54]
LHW-Wood10.44	Cellic Ctec 2	37	Ko et al. [77]
LHW-Wood11.39	Cellic Ctec 2	44.8	Ko et al. [77]
LHW-Wood11.56	Cellic Ctec 2	44.5	Ko et al. [77]
CS-AFEX	Spezyme CP cellulase	99.7	Kumar and Wyman [31]
CS-ARP	Spezyme CP cellulase	113.8	Kumar and Wyman [31]
CS-Controlled pH	Spezyme CP cellulase	101.7	Kumar and Wyman [31]
CS-DA	Spezyme CP cellulase	90.7	Kumar and Wyman [31]
CS-Lime	Spezyme CP cellulase	133.6	Kumar and Wyman [31]
CS-SO2	Spezyme CP cellulase	124.8	Kumar and Wyman [31]
Eucalyptus-L	Cellulase ATCC2692	60	Ooshima et al. [79]
Eucalyptus-SEL	Cellulase ATCC2692	80	Nonaka et al. [78]

Table 2: The maximum cellulases adsorbed (Ads max) on lignin substrates. APCL: Dilute Acid Pretreated Corn Stover Lignin; SPCL: Steam Explosion Pretreated Corn Stover; SPRL: Steam Explosion Pretreated Rice Straw; CEL: Cellulolytic Enzyme Lignin; SEHW: Steam Exploded Hard Wood (Birch 90% + Maple 10%); Accellerase 1000, Cellulclast, GC123: Commercial Cellulase Preparation from *T. reesei* produced by Du pont, and Gencore respectively; CS: Corn Stover; LHW-Wood: Lignin prepared by Liquid Hot Water (LHW) Pretreatment.

Similarly, Chang and Holtzapple [82] delignified Switch grass (original 22.3% lignin), Poplar wood (original 26.1% lignin) and Bagasse (original 24.2% lignin) and delignified lignocellulosic materials to varying lignin concentration by applying lime pretreatment [82]. On the basis of their results plotted they predicted that if lignin contents could be reduced to 15% of the original lignin concentration (i.e., 3.34%, 3.91% and 3.63%) in the tested substrates and an adsorption mixture of CBH I and β-glucosidase can produce

100% sugar conversion in 72 hours of hydrolysis. Exam reported that the ethanol yields increased with increased delignification [83]. It also stated that with decrease of the lignin contents of furfral residues from 43.46% to 8.7% that ethanol yield increased up to 75.6% of theoretical yield [84]. Therefore, removal of lignin to an optimum level increased the adsorption of cellulases onto the substrate because lignin adsorb cellulases. Very rare studies were found to compare cellulases (enzymes) adsorbed onto same type of substrates and under same experimental conditions.

Accessibility of Cellulases to Lignocellulosic Substrates

Pretreatment of lignocellulosic materials modify the surface properties of a substrates and hence alters adsorption capacities of the substrate during hydrolysis. Cellulases showed a quick initial adsorption followed by increasing adsorption on the pretreated Douglas-fir (softwood) [48,85]. The adsorption capacity of the total proteins on the three cellulosic substrates was studied: i) These included Avicel PH101 (a microcrystalline cellulose), ii) SO2 impregnated, steam-exploded Douglas-fir (DF), and iii) the water-insoluble fraction of the DF substrate obtained from hot alkali peroxide (DFP). The DF and DFP substrates demonstrated similar amount of cellulases adsorbed but they differed from that of Avicel (95 mg/g) and the maximum protein adsorbed was much higher (171.3 mg/g, 162.4 mg/g, respectively). While the initial adsorption/hydrolysis rate for the DFP was almost 3 times of the DP. On the contrary, Mooney et al. [86] found that a delignified refiner mechanical pulp had almost the same adsorption capacity as a regular refiner mechanical pulp [86]. On the other hand, during the study of pretreated lodgepole pine, it was observed that the adsorption remain at a constant level [87]. Cellulase adsorption onto ethanol pretreated lignocellulosic substrate (EPLP) was 87.69 mg/g and on steam exploded lodgepole pine (SELP) substrate, it was 101.05 mg/g with the affinities to substrates were 3.48 mL/mg and 1.48 mL/mg respectively. The cellulase adsorption onto corresponding isolated lignin showed a much lower adsorption capacity (2.73–10.20 mg/g) and higher affinity (3.21–6.44 mL/mg). It provided explanation of phenomena why lignin in the lignocellulosic substrates made a negative effect on the adsorption/hydrolysis because lignin has higher affinity for cellulose [54]. It was never tried to determine what features could give similar or different behavior of adsorption onto substrates prepared from different pretreatment technologies.

Some researchers claim that deacetylation had a higher impact on cellulose accessibility than delignification [19,88-93]. In order to evaluate impact of acetylation Kumar and Wyman [31] decaylated corn stover and CBH I cellulases were adsorbed on it and found that initial glucose and xylose release was increased by 65% and 74% respectively with respect to the non-deacylated corn stover. While a mixture of CBH I and β-glucosidase was adsorbed on delignified corn stover an initial increase of 198% and 780% in glucose and xylose release was observed. Therefore, saying same thing in another way, it still means that it is the lignin whose removal increased access to cellulose. Chang and Holtzapple [16] concluded that extensive delignification was sufficient to obtain high conversion of substrates regardless of acetyl content and crystallinity. Complete lignin reduction invites an extra cost; therefore, it is not justified. These results indicate that a delignification treatment process should remove optimum level of lignin. The accessibility of substrate to adsorption/hydrolysis of proteins were studied [28,41,94] but not in combination, with the surface properties of pretreated lignocelluloses. The experiments reported in order to measure adsorption experiments were rarely extended to measure surface area. A correlation between enzyme adsorption characteristics of the natural existing substrate, pretreated substrate and the substrate surface area may be developed. In order to compare adsorption of cellulases to the pretreated substrates under same experimental conditions, no data is available in the literature.

Adsorption Isotherms

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. 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 [95]. The adsorption isotherms have history for being used in representing adsorption of gases 29 on liquids [96,97] and liquids on solid surfaces [98,99]. Klyozov [100] worked with cellulase preparations, among these 10 were highly purified cellulases suggested that there exist a strong co-relation between hydrolysis rate and the adsorption equilibrium constant of enzymes adsorbed [100]. Recently, some reports are available on cellulases adsorption on various lignocellulosic substrates, such as microcrystalline cellulose [40,46], corn stover [47], steam-exploded Douglas fir [48], pretreated hardwood [49], isolated lignin from softwood [50] and lignin preparations from lodgepole pine [51]. Peitersen et al. [45] determined that adsorption of cellulases onto microcrystalline cellulose can be represented by Langmuir model [45,101,102]. Some of the researchers have suggested Freundlich isotherm for adsorption of cellulases [51,53]. Few reports used both Langmuir and Freundlich isotherms to represent adsorption of cellulases [55-57]. 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 information about adsorption of cellulases onto lignin is very rare. A dedicated study on the adsorption of cellulases on cellulose and lignin under similar conditions was required.

Conditions of Adsorption

Effect of temperature

Temperature is one of the two principal adsorption environment parameters. Some results of influence of temperature on adsorption are presented in this section. Ooshima et al. [103] adsorbed cellulases on Avicel at 5°C and 50°C [103]. The amount of cellulases adsorbed was decreased with increase in temperature. Kim et al. [104] also adsorbed purified components (CBHs and EGs) of cellulases on Avicel PH 101 and CMC for the temperature range of 25°C to 45°C [104]. The amount of cellulase components adsorbed decreased with increasing temperature. Kyriacou et al. [105] studied adsorption of EG I, EG II, EG III and CBH I (components of cellulases) on Solka floc (purified wood cellulose) at temperature 5°C, 30°C and 50°C [105]. It was observed that increasing the temperature from 5°C to 50°C had a negligible effect on adsorption of CBH I. For EG I, increase in adsorption by 3 times of that of CBH I between temperature 30°C to 50°C. For EG II and EG III, the effect temperature was almost the same but less than other two components. Over all there was an increase in cellulases adsorbed with increase in temperature. Kim et al. [102]

adsorbed enzymes obtained from *T. viride* on to Sigmacell 20 and Sigmacell 50 and observed that with the increase in temperature, the adsorption capacity of Sigmacell (cellulose) was decreased [52]. As it was expected for Langmuir adsorption isotherms. The van der Waals forces between the proteins and cellulose were considered responsible for the adsorption, and leading towards an exothermic and enthalpy-controlled adsorption process. The effect of temperature on adsorption to lignin was inverse. It was an endothermic process which could be result of hydrophobic interactions between lignin and enzymes involved in the adsorption process [54,106,107]. The findings agreed with that of other researchers in stating that there was an entropy-dominant adsorption process [108,109]. Therefore, hydrophobic interaction between enzymes and lignin and the nature of the structure of lignin have impact on the adsorption capacities of lignin. Increasing temperature would also effect on protein structures and the hydrophobic regions of proteins would be more exposed to surroundings [110], and as a result, the adsorption capacity of lignin increased. Andreaus et al. [111] reported that hours thermal pretreatment of proteins (without a substrate) could only reduce activity of proteins slightly [111]. On the contrary, Baker et al. [112] investigated the effect of temperature on all the components of an enzyme-mixture and reported that activity of the components reached to a maximum with increase in temperature from 55°C to 60°C [112]. Tomme et al. [113] adsorbed CBHI and CBHII from *T. reesei* on Avicel from changing temperature from 5°C to 50°C and the difference in the amount adsorbed in both the extreme temperature was less than 10% [113]. Lee [99] adsorbed cellulases on Solka floc SW-40 and the profile of the adsorbed cellulase at 4°C were closely parallels to that at 50°C. Some reported only a small effect on the adsorption by changing the temperature from 4 to 50°C [73]. There were conflicting reports on the effect of temperature on the adsorption of cellulases some group of researchers said it increased with increasing temperature and other said it decreased with it and there are some who claim that temperature has insignificant effect on adsorption. Therefore, a detailed study to determine the effect of temperature on enzymatic adsorption onto cellulose, lignin and lignocellulosic materials should be conducted.

Effect of pH

pH may cause surface charge leading to altered surface hydrophobicity. The change in surface charge may affect electrostatic interactions and hence, adsorption between components of a substrate and cellulases. The cellulases QM 9414 were adsorbed on Avicel PH 102, Solka Floc SW 20 (hammer milled spruce pulp-40 mesh), Sweco 270 (Ballmilled spruce pulp-270 mesh) at pH 3.8, pH 4.3, pH 4.8 and pH 5.0 at 30°C and observed that the adsorption of enzyme was largely independent of pH [45]. It was noted that the studies used a narrow range of pH. It adsorbed cellulases (CTec2) on four (4) different lignin residues of acid pretreated Lodgepole pine. It was observed that as the pH increased from 4.8 to 5.5, the adsorption of cellulase to lignin was decreased. The decreased adsorption of cellulase to lignin was attributed to enhanced electrostatic interactions at raised pH through the increased negative charges of cellulase enzymes. This study contradicts the well-established concept that the optimal pH is 4.8–8 5 for enzymatic hydrolysis using *Trichoderma reesi* cellulose [113]. Kyriacou et al. [105] adsorbed fractionated *T. reesei* cellulases (EG I, EG II, EG III, CBH I) onto Solka floc BW-40 and observed that the maximum adsorption at 5°C and 50°C was similar even though the pH increased from pH 3 to pH 7 with the exception of EG I at 50°C. Changes in adsorption were attributed to structural reconformation of enzymes due to charge distribution. The sharp increase in maximum cellulases (EG I) adsorbed at 50°C between pH 5.0 and 7.0 remained impenetrable [105]. Other groups of researches have reported a decrease in the degree of adsorption with increasing pH [114-116]. Tolan and Foody [116] studied adsorption of cellulases on cellulosic substrate and concluded that the curves of activity of enzymes vs pH were bell shaped and maximum was between pH 1 to pH 3. Moloney et al. [117] examined the effects of pH on the adsorption of Talaromyces cellulase by using Avicel and filter paper as the substrates. The maximum adsorption (around 50%) of Talaromyces cellulases on Avicel occured at pH 4.2 While 35% of adsorption was observed at the filter-paper. Therefore, adsorption characteristics of cellulases were influenced by the nature of the substrate [117]. Criquet [118] investigated effect of pH on cellulases activity using two types of buffer currently used in studies of cellulolytic enzymes [118]. The use of Na-acetate buffer showed a single and maximum peak at pH 6.0. The cellulase activity of the enzyme solution showed two optimum values at pH 4.5 and 6.0. From literature survey, it was observed that cellulase activity measured using the citrate buffer was lower than with the acetate buffer [119-121]. Vasquez et al. [122] prepared substrate Cellulignin by acid treatment of sugarcane bagasse and used the substrate to evaluate effect of some factors including pH on adsorption. The pH values used were 5, 5.5, 6 and it was found that the effect of pH was negligible [122]. A big spectrum of pH range was rarely used and the effect of pH along with temperature needs to be addressed. We are suggesting that the activity of cellulases should be measured on a global buffer say acetate buffer or citrate buffer which may reflects the activities of various cellulase on one scale. It would not be surprising to find contradictory results on adsorption of cellulases in the literature, given that the experimental conditions, substrates, type of buffer used were not similar.

Effect of product inhibition

A lignocellulosic substrate is inhibited by nonspecific (nonproductive) adsorption of cellulase tonon-cellulosic components (e.g. lignin, hemicellulose) [123-125] and also by the end product formed which reduces effectiveness of enzymes (cellulases). Additives were applied to address inhibition adsorbed lignin [126-130]. The use of additives at the suggested dosage was fond expensive and may cause potential problems. Holdzapple adsorbed cellulases on a pretreated poplar wood and found that sugars (glucose, δ-gluconolactone, and cellobiose) and solvents (ethanol, butanol, and acetone) produced during hydrolysis decrease adsorption and hence hydrolysis [131]. Zhang et al. [132] pretreated swollen cellulose and it was adsorbed/hydrolyzed by two Thermomonospora fusca endoglucanase, EG II and EG V at (3- to 4-fold) slower rate than untreated swollen cellulose [132]. Xiao et al. [133] attempted to determine the inhibition effect of end products (glucose) on two (2) types of substrates (Avicel and Acetic acid pretreated soft wood) during ethanol production by using a mixture of cellulases and β-glucosidase [133]. The increased glucose resulted in a dramatic decreased in the adsorption/hydrolysis which was reflect by a decrease in activities of both enzymes. Bezerra and Dias (2005) adsorbed crude cellulase and purified exoglucanase Cel7A on Avicel and carboxymethyl cellulose and let the hydrolysis complete. End products found were cellobiose and ethanol. Integrated Michaelis–Menten equations were used to determine inhibition. The calculated inhibition constants showed that cellobiose inhibited much more than ethanol using exoglucanase (1.6 × 1015 mM, 0.035 mM) and with the crude enzyme (151.9 mM, 0.05 mM) as well. Among the cellulose degrading enzymes, beta-glucosidases are essential for efficient

hydrolysis of cellulosic biomass as they relieve the inhibition of the cellobiohydrolases and endoglucanases by reducing cellobiose accumulation [134]. Zhao et al. [135] through their adsorption/hydrolysis studies suggested the use of glucose tolerant non-cellulolytic β-glucosidase enzyme which will decrease inhibition caused by cellobiose and tolerate glucose [135]. Qing et al. [136] studied adsorption/hydrolysis of cellulases on Avicel PH 101 and that xylooligomers were more powerful inhibitors than glucose and cellobiose [136]. Cellulose accessibility and degree of adsorption of cellulases onto cellulosic substrates are controlling factors for conversion of cellulose and yields of production of glucose and bioethanol [19,137]. Based on the above, the use of a pretreated substrate, β-glucosidase should be used to impair substrate accessibility by inhibition.

Effect of E/S

In adsorption studies a very crucial parameter is an enzyme/substrate (E/S) ratio. According to survey there exist a linear correlation between adsorption/hydrolysis rate and enzyme to substrate ratio. Bernardez et al. [49] adsorbed cellulases (obtained from Clostridium thermocellum strain ATCC 27405) on Avicel 105 and on pretreated mixed wood (90% Brich wood, 10% Maple) and studied the effect of enzyme to substrate ratio on adsorption., At low E/S ratios, the results of experiments showed a linear trend of adsorbed enzyme with respect to the initial enzyme concentration. At higher E/S ratios the adsorbed enzyme leveled off due to the saturation of the cellulosic substrate with enzyme. For PTW 220, as a lignin substrate the cellulases adsorbed in relation to increasing concentrations of pretreated mixed wood of 0 to 0.5 g /L and an enzyme activity of 95 to 100 U/L. Saturation was achieved around 0.1 g/L of cellulases concentration. Comparison of the results indicated that, 30-fold fewer enzymes were required to saturate Avicel than for PTW220 [49]. This comparison was indicative of the profoundly greater capacity of lignin to adsorb cellulase. Sattler et al. [138] studied the effect of enzyme (cellulases) concentration on adsorption/hydrolysis of Sigmacell 50 and pretreated poplar. The substrate concentration was 20 g/L while enzyme concentration varied from 5 FPU to 100 FPU/g [138]. It was observed that increase in cellulase loading increased adsorption for all enzyme concentrations. Moldes et al. [139] used delignified Eucalyptus globulus wood chips to study effect of enzyme to substrate ratio on adsorption and found that for low concentration 13 FPU/g 39% adsorption/hydrolysis was achieved when it was increased to 28 FPU/g 53.5% of adsorption/hydrolysis was observed [139]. The subjected acid treated spruce wood chips were to adsorption by the amounts of Celluclast 2 L added were 4%, 8%, 16%, and 24% w/w DM, corresponding to cellulase activities of 5, 10, 21, and 32 FPU/g cellulose, respectively. The whole slurry from the pretreatment stage, diluted to different dry weights of solid material (2%,16%,5%,7.5%,and 10%), was used as substrate. It was observed that maximum conversion of substrate was achieved with cellulase activities from 4 to 7 g/L for % substrate concentration. For 5% substrate concentration, the time to reach maximum adsorption/hydrolysis increased and this concentration was found optimal. A low concentration of substrate formed byproducts and high concentration was not suitable for downstream process (i.e. fermentation). At 7.5% substrate, the inhibition reduced the productivity whereas the final ethanol yield varied randomly. And the results of 10% substrate showed no fermentation at all. This is contrary to observations in previous separate hydrolysis and fermentation experiments at concentrations comparable to the SSF with 10% dry material [140]. The inhibitors formed in the pretreatment of substrate were likely to limit the substrate concentration unless substrate were washed. Vasquez et al. [122] prepared substrate Cellulignin G by acid treatment of sugarcane bagasse and used this substrate to evaluate enzyme/substrate ratio for enzyme loading of 5.0, 17.5 and 30.0 FPU/g Cellulignin as substrate with concentration of 2.0%, 6.0% and 10.0%. The conditions of hydrolysis that yielded the highest glucose concentration, 58.40 g/L, were: temperature 47°C, enzyme loading 25.6 FPU/g Cellulignin G, and 10.0% substrate. Enzymes accessed to Cellulignin G and converted it to glucose, the highest value, 67.0%, was obtained when enzyme loading 24.4 FPU/g Cellulignin G, and solid percentage 2.0% [122]. Medve et al. [46] adsorbed CBH I and CBH II from *T. reesei* onto Avicel at various E/S ratios. It was observed that at E/S > 0.2, the adsorption of CBH I was decreased by up to 23% in the presence of equimolar amount of CBH II. The adsorption of CBH II was decreased by up to 45 % (at E/S = 2.56 μmol/g) by competition from CBH I. At low saturation (E/S < 0.2 μmol/g) the results indicated that the presence of the other enzyme has very little effect in this concentration range [46]. Van conducted adsorption/hydrolysis of ball milled Spruce wood pulps with varying concentrations of cellulases from (*T. viride*) and found that rate of adsorption/hydrolysis increased with initial substrate concentration until an optimum is reached whereupon it decreased. Overall rate of adsorption hydrolysis was dependent on concentration of endoglucanase at optimum substrate concentration. The decrease in hydrolysis was due to the inaccessibility of enzymes to adsorb onto the substrates [62]. Howell and Struck [141] studied adsorption/hydrolysis of cellulases QM9123 from *T. viride* on Solka Floc (BW200) and reported a decrease of adsorption/hydrolysis rate at high substrate concentration [141]. For a fixed enzyme concentration when substrate concentration were increased the accessibility of enzymes 30 to adsorb decreased and hence the hydrolysis [142,143].

Wang et al. [144] investigated the adsorption of enzymes (cellulases from *T. reesei*), affectivity of the enzymes and concentration of substrate (Avicel PH 101). The enzyme concentration was increased equivalently with the substrate concentration and it was observed that the glucose yield decreased with the increased substrate concentration. The mechanism causing the glucose yield decreased at high substrate loading in enzymatic adsorption/hydrolysis of cellulose is yet to explore [144]. Similar results were reported by Triwahyuni et al. [145] and they also achieved maximum ethanol yield by adding substrate slowly and gradually (instead of filling all substrate in the stat). The efficiency of enzymatic digestibility and fermentation is significantly reduced at high concentrations of pretreated biomass, as mixing becomes difficult with increased viscosity [145]. For adsorption reactions, the rate of adsorption increased with initial substrate concentration until an optimum is reached whereupon it decreased. The increase in enzyme (cellulase) loading increased adsorption for all enzyme concentrations till the optimum value. For a fixed enzyme concentration when substrate concentration were increased the accessibility of enzymes to adsorb decreased and hence the adsorption. For a substrate to use an optimum E/S ratio should be determined under the given conditions and such a data bank is not available.

Effect of enzymes synergy

Synergism of cellulase systems is a phenomenon when collective activity of the components of system exhibit higher activity than the sum of the activities of individual cellulase components. There are four (4) reported forms of synergism in literature: i) between endoglucanases and exoglucanases (endo-exo), ii) between exoglucanases processing cellulose from the reducing andnon-

reducing ends (exo-exo), iii) between exoglucanases and β-glucosidases that remove cellobiose (and cellodextrins), and iv) between catalytic domains and carbohydrate-binding modules. Henrissat et al. (1985) studied the action of cellobiohydrolases (CBH I and CBH II) and endoglucanases (EG I and EG II) on various substrates and found that synergy between endoglucanases and CBH II follow the pattern of endo-exo cooperation [34]. The endoglucanase (Endo I, 11,111, IV, V, and VI) were adsorbed on Avicel along with CBH II by Beldman et al. [146]. The results showed that initial endoglucanase to exoglucanases ratio were dependent upon the adsorption of these enzymes to conduct hydrolysis of Avicel. Endo I, Endo III, and Endo V, adsorbed strongly on Avicel, proved the maximal synergistic effect with Exo III (adsorbed strongly on Avicel as well). The initial endoglucanase-to-exoglucanase ratio was much lower than these were used in incubations where Endo II, Endo IV, and Endo VI were used. The endo-exo synergy examined by a number of researchers and it was observed that: i) The degree of synergy (DS) was increases significantly with increasing degree of polymerization of substrate [147], as accessibility of enzymes increased [34,147], and as enzymes loading increased [147-149]. It is an endo-exo mechanism degrades the amorphous region of cellulose [150]. Tomme et al. [113] found that CBH I and CBH II adsorbed strongly on Avicel and equilibrium reached very slow (more than 40 min) and the synergistic effects were dependent on the ratio of the enzymes [113]. In order to obtain maximally synergistic effects, both enzymes were suggested to be added simultaneously and in non-saturating concentrations. Ikeo et al. [151] investigated the mechanism of the exo-exo synergism between CBH I and CBH II by comparing the characteristics of enzyme adsorption and desorption to the cellulose under agitated and static conditions [151]. Under both conditions CBH I and CBH II adsorbed 100% and 80% respectively. In adsorption study of cellulases to cellulose between different cellulase fractions, more adsorption was observed when both CBHs were present. Under agitation CBH I and CBH II adsorbed quickly and get deactivated which indicated that CBHs was deactivated by shear stress from agitation. Under static conditions at the end of hydrolysis 10-20% of CBH I and CBH II were found inactive. CBHs from family can occasionally transform to have some endo-character (never demonstrated for family CBHs), for the altered character a mechanism for endo-exo synergism was proposed also to be responsible for synergism between different CBHs [152-154]. For hydrolysis of lignocellulosic substrate the problem is enzyme inhibiting end-product (cellobiose). To overcome end-product inhibition the addition of β-glucosidase is needed and β-glucosidases work efficiently on cellulolytic residues in a synergistic manner and produce glucose. Activity of β-glucosidase can be increased by using a thermo-tolerant CBH I [155]. Anderson et al. [156] reported synergistic effect by a mixture of Cel 45A, Cel 6A (an endoglucanse and an exoglucanase respectively from Humicola insolens) and β-glucosidase from Penicillium brasilianum on amorphous cellulose [156]. Some reports has shown a competition in adsorption to cellulose between different cellulase fractions. On the crystalline region of cellulose, these enzymes inhibit each other due to the competition between them for binding sites on cellulose. The investigated the adsorption characteristics of families Ce1 7A and Ce1 6A, and attempted to elucidate the mode of action of between catalytic domains and carbohydrate-binding modules in synergy [157]. The adsorption affinity of the cellulase binding domain (CBD) for Ce1 7A are larger than of Ce1 6A, hence, CBD of Ce17A binds more rapidly and tightly to Avicel than that of Ce16A. Warner et al. [158] studied substrate binding of a cellulose binding module (CBM) and catalyticdomain (CD) from *Ruminococcus flavefaciens* (a glycoside hydrolase family 44 xyloglucanase/endoglucanase), both were quantified when binding various β-1,4-linked glucans and xylans when separated and attached. It was found that the CBM was adsorbed on cello-oligosaccharides (xylotetraose, xylopentaose, or xylohexaose). The CD appeared to adsorb carboxymethylcellulose (CMC) and onto xylan very weakly, while the CBM and the CD/CBM adsorbed much more strongly. A synergistic effect was observed for the adsorption of cellopentaose and cellohexaose with the CD/CBM as compared to the separated CD and CBM alone [158]. Synergism is hindered at high enzyme concentrations due to saturation of the adsorption sties, therefore, an increase in synergistic action with increased enzyme/substrate ratio was not found. The mechanistic cause of the observed synergism is still unknown. The exo-exo, endo-exo, endo-β glucosidase and intra molecular synergies were found, however, synergism between endoglucanases has not been clearly demonstrated till now. A reason could be that natural lignocellulosic substrates are not used commonly due to their heterogeneous nature and the performance of individual endoglucanases were not observed on natural substrates.

Conclusion

A number of studies have emphasized on the importance of cellulose accessibility during adsorption and most of the studies have used a small number of samples or pure cellulosic substrates, which do not represent the natural heterogeneous, lignocellulosic materials.

External surface area plays small role in adsorption and similarly, not all internal area is good for adsorption. The only area is accessible which is wider than the size of an enzyme. The accessibility (available surface area) is determined by practical adsorption of enzymes (cellulases) on the substrates. The maximum adsorbed amounts on the substrates were used to determine accessibility of cellulases with the help of adsorption isotherm. The relationship between the accessibility and cellulose conversion is sometimes measured yet it was contradictory and in many cases inadequate.

In order to achieve efficient adsorption onto lignocellulosic substrates an optimum level of lignin removal is required. It appeared that accessible surface area of cellulose in pretreated substrate require lower enzyme levels to attain high conversions. The amount of enzymes required for a known level of lignin removed from lignocellulose substrates under given conditions need to be worked on.

There are conflicting reports about the effect of temperature therefore, for a test substrate the optimum temperature under the given condition is recommended to determine. Similarly, a narrow slit of the effect of pH was studied, and the effect of pH along with the temperature was rarely studied.

Regardless of significant differences in the origin, structure and chemical composition of the substrates and the pretreatment process used, the optimum E/S is required for efficient adsorption of pretreated lignocellulosic substrates. A data bank should be developed for E/S ratios for components of lignocellulosic materials and lignocellulosic materials as well.

The end-product inhibition can be controlled by using modified glucose tolerant β-glucosidase in an optimum amount. The acceptable glucose (end product) level for each lignocellulosic material (which can be used for bioethanol production) should be determined.

Enzymes perform their action in synergy and synergism is obstructed at high enzyme concentrations due to saturation of the adsorption sties. An optimum concentration of enzyme components to

convert a corresponding lignocellulosic material to reducing sugars should be determined. Working at low E/S is suggested to have more synergy among enzymes. Such strategic information is difficult to find in published literature, therefore, a data bank should be developed.

References

1. Lee Y, Fan LT (1983) Kinetic studies of the enzymatic hydrolysis of cellulose: Analysis of extended hydrolysis times. Biotechnology Bioengineering 26: 939-966.
2. Holtzapple MT, Caram HS, Humphrey AE (1984) The HCH-I model of enzymatic cellulose hydrolysis. Biotechnology and Bioengineering 26: 775-780.
3. Converse AO, Matsuno R, Tanaka M, Taniguchi M (1988) A model for enzyme adsorption and hydrolysis of microcrystalline cellulose with slow deactivation of the adsorbed enzyme. Biotechnol Bioeng 32: 38-45.
4. Henrissat B (1994) Cellulases and their interaction with cellulose. Cellulose 1: 169-196.
5. Teeri TT (1997) Crystalline cellulose degradation: new insight into the function of cellobiohydrolases. Trends in Biotechnology 15: 160-167.
6. Zhang Y-HP, Lynd LR (2004) Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems. Biotechnol Bioeng 88: 797–824.
7. Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, et al. (2007) Biomass recalcitrance: engineering plants and enzymes for biofuels production. Science 315: 804-807.
8. Zhao X, Zhang L, Liu D (2012) Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. Biofuels, Bioproducts and Biorefining 6: 465-482.
9. Studer MH, DeMartini JD, Davis MF, Sykes RW, Davison B, et al. (2011) Lignin content in natural Populus variants affects sugar release. Proc Natl Acad Sci U S A 8: 6300-6305.
10. Yu Z, Jameel H, Chang H, Park S (2011) The effect of delignification of forest biomass on enzymatic hydrolysis. Bioresource Technology 102: 9083-9089.
11. Zhu W, Houtman CJ, Zhu JY, Gleisner R, Chen KF (2012) Quantitative predictions of bioconversion of aspen by dilute acid and SPORL pretreatments using a unified combined hydrolysis factor (CHF). Process Biochem 47: 785-791.
12. Wang ZJ, Zhu JY, Gleisner R, Chen KF (2012) Ethanol production form poplar wood the rough enzymatic saccharification and fermentation by dilute acid and SPORL pretreatments. Fuel 95: 606-614.
13. Dasari RK, Berson RE (2007) The effect of particle size on hydrolysis reaction rates and rheological properties in cellulosic slurries. Appl Biochem Biotechnol 137: 289-299.
14. Zhu J, Wang G, Pan X, Gleisner R (2009) Specific surface to evaluate the efficiencies of milling and pretreatment of wood for enzymatic saccharification. Chemical Engineering Science 64: 474-485.
15. Leu S, Zhu JY (2013) Substrate-Related Factors Affecting Enzymatic Saccharification of Lignocelluloses: Our Recent Understanding. Bioenergy Research 6: 405-415.
16. Chang VS, Holtzapple MT (2000) Fundamental factors affecting biomass enzymatic reactivity. Appl Biochem Biotechnol 84: 5-37.
17. Kim TH, Kim JS, Sunwoo C, Lee Y (2003) Pretreatment of corn stover by aqueous ammonia. Bioresour Technol 90: 39-47.
18. Kumar R, Wyman CE (2009) Access of cellulase to cellulose and lignin for poplar solids produced by leading pretreatment technologies. Biotechnol Prog 25: 807-819.
19. Jeoh T, Ishizawa MF, Davis ME, Himmel WS, Adney DK (2007) Cellulase digestibility of pretreated biomass is limited by cellulose accessibility. Biotechnol Bioeng 98: 112–122.
20. Ishizawa CI, JeoH T, Adney WS, Himmel ME, Johnson DK, et al. (2009) Can delignification decrease cellulose digestibility in acid preteated corn stover?. Cellulose 16: 677-686.
21. Zhu W, Zhu JY, Gleisner R, Pan XJ (2010) On energy consumption for size-reduction and yield from subsequent enzymatic saccharification of pretreated lodgepole pine. Bioresour Technol 101: 2782-2792.
22. Del Rio LF, Chandra RP, Saddler JN (2012) Fibre size does not appear to influence the ease of enzymatic hydrolysis of organosolv-pretreated softwoods. Bioresour Technol 107: 235- 242.
23. Gregg D, Saddler JN (1996) Factor affecting cellulose hydrolysis and the potential of enzyme recycle to enhance the efficiency of an integrated wood to ethanol process. Biotechnol Bioeng 51: 375-383.
24. Shen J, Agblevor FA (2008) Optimization of enzyme loading and hydrolytic time in the hydrolysis of mixtures of cotton gin waste and recycled paper sludge for the maximum profit rate. Biochemical Engineering Journal 41: 241-250.
25. Sinitsyn AP, Gusakov AV, Vlasen EY (1991) Effect of structural and physicochemical features of cellulosic substrates on the efficiency of enzymatic hydrolysis. Applied Biochemistry and Biotechnology 30: 43-59.
26. Chandra PR, Esteghlalian AR, Saddler JN (2008) Assessing substrate accessibility to enzymatic hydrolysis by cellulases. In Characteristics of lignocellulosic materials.
27. Jeoh T, Wilson DB, Walker LP (2002) Cooperative and competitive binding in synergistic mixtures of Thermobifida fuscacellulases Ce15A, Ce16B, and Ce19A. Biotechnol Prog 18: 760-769.
28. Karlsson J, Medve J, Tjerneld F (1999) Hydrolysis of steam-pretreated lignocellulose. Applied Biochemistry and Biotechnology 82: 243-258.
29. Kotiranta P, Schsson J, Siika-Aho M, Medve J, Viikari L, et al. (1999) Adsorption and activity of Trichoderma reesei cellobiohydrolase I, endoglucanase II, and the corresponding core proteins on steam pretreated willow. Applied Biochemistry Biotechnology 81: 81-90.
30. Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS (2002) Microbial cellulose utilization: Fundamentals and biotechnology. Microbiol Mol Biol Rev 66: 506-577.
31. Kumar R, Wyman CE (2009) Does change in accessibility with conversion depend on both the substrate and pretreatment technology?. Bioresour Technol 100: 4193-4202.
32. Kumar R, Wyman CE (2008) Cellulase Adsorption and Relationship to Features of Corn Stover Solids Produced by Leading Pretreatments. Biotechnol Bioeng 103: 252-267.
33. Luo X, Zhu JY (2011) Effects of drying-induced fiber hornification on enzymatic saccharification of lignocelluloses. Enzyme Microb Technol 48: 92-99.
34. Schlumberger ED (2013) Application of differential scanning calorimetry to core analysis. International Symposium of the Society of Core Analysts held in Napa Valley, California, USA.
35. Felby C, Thygesen LG, Kristensen JB, Jørgensen H, Elder T (2008) Cellulose-water interactions during enzymatic hydrolysis as studied by time domain NMR. Cellulose 15: 703-710.
36. Meng X, Foston M, Leisen J, DeMartini J, Wyman CE, et al. (2013) Determination of porosity of lignocellulosic biomass before and after pretreatment by using Simons' stain and NMR techniques. Bioresour Technol 144: 467-476.
37. Hui L, Liu Z, Ni Y (2009) Characterization of high-yield pulp (HYP) by the solute exclusion technique. Bioresour Technol 100: 6630-6634.
38. Wang QQ , He Z, Zhu Z, Zhang ZP, Ni Y, et al. (2011) Evaluations of cellulose accessibilities of lignocelluloses by solute exclusion and protein adsorption techniques. Biotechnol Bioeng 109: 381-389.
39. Meng X, Wells T, Sun Q, Huang F, Ragauskas AJ (2015) Insights into the effect of dilute acid, hot water or alkaline pretreatment on the cellulose accessible surface area and the overall porosity of Populus. Green Chemistry 17: 4239-4246.
40. Hong J, Ye X, Zhang YH (2007) Quantitative determination of cellulose accessibility to cellulase based on adsorption of a nonhydrolytic fusion protein containing CBM and GFP with its applications. Langmuir 23: 12535-12540.
41. Zhu Z, Sathitsuksanoh N, Vinzant T, Schell DJ, McMillan JD, et al. (2009) Comparative study of corn stover pretreated by dilute acid and cellulose

solvent-based lignocellulose fractionation: enzymatic hydrolysis, supramolecular structure, and substrate accessibility. Biotechnology and Bioengineering 103: 715-724.

42. Wang QQ, He Z, Zhu Z, Zhang YH, Ni Y, et al. (2012) Evaluations of cellulose accessibilities of lignocelluloses by solute exclusion and protein adsorption techniques. Biotechnol Bioeng 109: 381-389.
43. Thygesen LG, Hidayat BJ, Johansen KS, Felby C (2010) Role of supramolecular cellulose structures in enzymatic hydrolysis of plant cell walls. J Ind Microbiol Biotechnol 38: 975-83.
44. Luterbacher JS, Walker LP, Moran-Mirabal JM (2013) Observing and modeling BMCC degradation by commercial cellulase cocktails with fluorescently labeled Trichoderma reseii Cel7A through confocal microscopy. Biotechnol Bioeng 110: 108-117.
45. Peitersen N, Medeiros J, Mandels M (1977) Adsorption of Trichoderma cellulase on cellulose. Biotechnology and Bioengineering 19: 1091-1094.
46. Medve J, Stahlberg J, Tjerneld F (1994) Adsorption and synergism of cellobiohydrolase I and II of Trichoderma reesei during hydrolysis of microcrystalline cellulose. Biotechnol Bioeng 44: 1064-1073.
47. Xu F, Ding H, Osborn D, Tejirian A, Brown K, et al. (2008) Partition of enzymes between the solvent and insoluble substrate during the hydrolysis of lignocellulose by cellulases. Journal of Molecular Catalysis B Enzymatic 51: 42-48.
48. Lu Y, Yang, B, Gregg D, Saddler JN, Mansfield SD (2002) Cellulase adsorption and an evaluation of enzyme recycle during hydrolysis of steam exploded softwood residues. Appl Biochem Biotechnol 98: 641-654.
49. Bernardez TD, Lyford K, Hogsett DA, Lynd LR (1993) Adsorption of CIostridium thermocellum cellulases onto pretreated mixed hardwood, Avicel, and Lignin. Biotechnol Bioeng 42: 899-907.
50. Palonen H (2004) Role of lignin in enzymatic hydrolysis of lignocellulose. Doctoral Dissertation. University of Helsinki, Helsinki, Finland.
51. Tu M, Pan X, Saddler JN (2009) Adsorption of cellulase on cellulolytic enzyme lignin from lodgepole pine. Journal of Agricultural Food Chemistry 57: 7771-7778.
52. Kim DW, Yang JH, Jeong YK (1988) Adsorption of cellulase from Trichoderma viride on microcrystalline cellulose. Applied Microbiology and Biotechnology 28: 148-154.
53. Sun Y, Lin CY, Liu MH, Liu YF (2011) Equilibrium adsorption behavior and kinetic characterics of oxymatrine on spherical cellulose adsorbent. Bioresources 6: 631-640.
54. Tu MB, Zhang X, Paice M, McFarlane P, Saddler JN (2009) Effect of surfactants on separate hydrolysis fermentation and simultaneous saccharification fermentation of pretreated lodgepole pine. Biotechnol Prog 25: 1122-9.
55. Kanchagar AP (2003) Adsorption of purified cellulases on cotton fibres. Ph.D. Dissertation. University of Georgia, Athens, Georgia.
56. Vadi M, Abbasi M, Zakeri M, Yazdi BJ (2010) Application of the Freundlich, Langmuir Temkin and Harkins-Jura adsorption isotherms for some amino acids and amino acids complexation with manganese ion(II) on carbon nanotube. Journal of Physical and Theoretical Chemistry 7: 95-104.
57. Singh J, Kaur M (2014) Partial purification of cellulase enzyme from Fusarioum oxysporium by using egg white matrix. British Biotechnology Journal 4: 108-115.
58. Mansfield SD, Mooney C, Saddler JN (1999) Substrate and enzyme characteristics that limit cellulose hydrolysis. Biotechnol Prog 15: 804-816.
59. Zhao X, Zhang L, Liu D (2011) Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. Biofpr.
60. Yang B, Dai Z, Ding S, Wyman CE (2011) Enzymatic hydrolysis of cellulosic biomass. Biofuels 2: 421-450.
61. Arantes V, Saddler JN (2011) Cellulose accessibility limits the effectiveness of minimum cellulase loading on the efficient hydrolysis of pretreated lignocellulosic substrates. Biotechnology for Biofuels 4: 3.
62. Van dyk JS, Pletschke (2012) A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes —Factors affecting enzymes, conversion and synergy. Biotechnol Adv 30: 1458-1480.
63. Modenbach AA, Nokes SE (2013) Enzymatic hydrolysis of biomass at high-solids loadings e A review. Biomass and Bioenergy 56: 526-544.
64. Ecard AD, Muthukumarappan K, Gibbon W (2013) A Review of the Role of Amphiphiles in Biomass to Ethanol Conversion. Applied Sciences 3: 396-419.
65. Leu S, Zhu JY (2013) Substrate-related factors affecting enzymatic saccharification of lignocelluloses: our recent understanding. Bioenergy Research 6: 405-415.
66. Jesionowski T, Zdarta J, Krajewska B (2014) Enzyme immobilization by adsorption: a review. Adsorption 20: 801-821.
67. Mohamad NR, Marzuki NHC, Buang NA, Huyop F, Wahab RA (2015) Review: agriculture and environmental biotechnology. An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes. Biotechnology and Biotechnological Equipments 29: 205-220.
68. Karimi K, Taherzadeh MJ (2016) A critical review on analysis in pretreatment of lignocelluloses: Degree of polymerization, adsorption/desorption, and accessibility. Bioresource Technology 203: 348–356.
69. Converse AO, Ooshima H, Burns SD (1990) Kinetics of enzymatic hydrolysis of lignocellulosic materials based on surface area of cellulose accessible to enzyme and enzyme adsorption on lignin and cellulose. Applied Biochemistry and Biotechnology 24: 67.
70. Thompson DN, Chen HC, Grethlein HE (1992) Comparison of pretreatment methods on the basis of available surface area. Bioresource Technology 39: 155-163.
71. Varnai L, Viikari K, Marjamaa M, Siika-aho (2011) Adsorption of mono component enzymes in enzyme mixture analyzed quantitatively during hydrolysis of lignocellulose substrates. Bioresour Technol 102: 1220-1227.
72. Weimer PJ, Lopez-Guisa JM, French AD (1990) Effect of cellulose fine structure on kinetics of its digestion by mixed ruminal microorganisms in vitro. Applied Environment and Microbiology 56: 2421-2429.
73. Zheng Y, Zhang S, Miao S, Zhiguo S, Wang P (2013) Temperature sensitivity of cellulase adsorption on lignin and its impact on enzymatic hydrolysis of lignocellulosic biomass. J Biotechnol 166: 135-143.
74. Marshall K, Sixsmith D (1974) Some physical characteristics of microcrystalline cellulose 1. Powders for pharmaceutical use. Drug Development Communications 1: 57-71.
75. Lehtio J, Sugiyama J, Gustavsson M, Fransson L, Linder M, et al. (2003) The binding specificity andaffinity determinants of family 1 and family 3 cellulose binding modules. Proc Natl Acad Sci USA 100: 484-489.
76. Kawakubo T, Karita S, Araki Y, Watanabe S, Oyadomari M, et al. (2010) Analysis of exposed cellulose surfaces inprettreated wood biomass using carbohydrate-binding module (CBM)-cyan fluorescent protein (CFP). Biotechnol Bioeng 105: 499-508.
77. Ko JK, Ximenes E, Kim Y, Ladisch MR (2015) Adsorption of Enzyme onto Lignins of Liquid Hot Water Pretreated Hardwoods. Biotechnology and Bioengineering 112: 447-456.
78. Nonaka H, Kobayashi A, Funaoka M (2013) Lignin isolated from steam-exploded eucalyptus wood chips by phase separation and its affinity to Trichoderma reesei cellulase. Bioresour Technol 140: 431-434.
79. Ooshima H, Burns DS, Converse AO (1990) Adsorption of cellulase from Trichoderma reesei on cellulose and lignacious residue in wood pretreated by dilute sulfuric-acid with explosive decompression. Biotechnology and Bioengineering 36: 446-452.
80. Gharpuray MM, Lee Y, Fan LT (1983) Structural modification of lignocellulosics by pretreatments to enhance enzymatic hydrolysis. Biotechnology and Bioengineering 25: 157-172.
81. Kong R, Engler CR, Soltes EJ (1992) Effects of cell-wall acetate, xylan backbone, and lignin on enzymatic-hydrolysis of aspen wood. Applied Biochemistry Biotechnology 34: 23-35.

82. Chang VS, Holtzapple MT (2005) Fundamental factors affecting biomass. Appl Biochem Biotechnol 82: 10-27.
83. Mohammad J, Keikhosro K (2008) Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. International Journal of Molecular Science 9: 1621-1651.
84. Bu L, Tang Y, Xing Y, Zghang W, Shang X, et al. (2014) Comparison of hydrophilic variation and bioethanol production of furfural residues after delignification pretreatment. Biosci Biotechnol Biochem 78: 1435-1443.
85. Boussaid A, Saddler JN (1999) Adsorption and activity profiles of cellulases during the hydrolysis of two Douglas fir pulps. Enzyme Microbial Technology 15: 138–143.
86. Mooney CA, Mansfield SD, Touhy MG, Saddler JN (1998) The effect of initial pore volume and lignin content on the enzymatic hydrolysis of softwoods. Bioresource Technology 64: 113–119.
87. Tu MB, Chandra RP, Saddler JN (2007) Recycling cellulases during the hydrolysis of steam exploded and ethanol pretreated lodgepole pine. Biotechnol Prog 23: 1130–1137.
88. Wood TM, McCrae SI (1986) The effect of acetyl groups on the hydrolysis of ryegrass cell walls by xylanase and cellulase from Trichoderma koningii. Phytochemistry 25: 1053–1055.
89. Grohmann K, Mitchell DJ, Himmel ME, Dale BE, Schroeder HA (1989) The role of ester groups in resistance of plant cell wall polysaccharides to enzymic hydrolysis. Applied Biochemistry Biotechnology 20: 45.
90. Suh JH, Choi YJ (1996) Synergism among endo-xylanase, b-xylosidase, and acetyl xylan esterase from Bacillus stearothermophilus. Journal Microbiol Biotechnol 6: 173–178.
91. Anand L, Vithayathil PJ (1996) Xylan-degrading enzymes from the thermophilic fungus Humicola lanuginosa (Griffon and Maublanc) Bunce: Action pattern of xylanase and [beta]-glucosidase on xylans, xylooligomers and arabinoxylooligomers. Journal of Fermentation and Bioengineering 81: 511–517.
92. Samios E, Dart RK, Dawkins JV (1997) Preparation, characterization and biodegradation studies on cellulose acetates with varying degrees of substitution. Polymer 38: 3045–3054.
93. Silveira FQP, Ximenes FA, Cacais AOG, Milagres AMF, Medeiros CL (1999) Hydrolysis of xylans by enzyme systems from solid cultures of Trichoderma harzianum strains. Braz J Med Biol Res 32: 947-952.
94. Piccolo C, Wiman M, Bezzo F, Liden G (2010) Enzyme adsorption on SO2 catalyzed steam-pretreated wheat and spruce material. Enzymes and Microbial Technology 46: 159-169.
95. Dhir B, Kumar R (2010) Adsorption of heavy metals by Salvinia biomass and agricultural residues. Int J Environ Res 4: 427-432.
96. Dabarowski A (2001) Adsorption-from theory to practice. Advances in colloid and interface science 93: 135-224.
97. Wylie RG (1952) On the hysteresis of adsorption on solid surfaces. Austrailian J Scientific Research, Series A, Physical sciences 5: 188-302.
98. Berti C, Ulbig P, Schulz S (2000) Correlations and prediction of adsorption from liquid mixtures on solids by using G-Models. Adsorption 6: 79-91.
99. Lee S (1999) Molecular adsorption at solid/liquid interfaces using self-assembled monolayer films. Ph.D. Thesis. Department of Chemical Engineering. Massachusetts Institute of Technology.
100. Klyozov AA (1990) Trends in biochemistry and enzymology of cellulose degradation. Biochemistry 29: 10577-10585.
101. Kim DW, Yoon YH, Jeong YK, Lee JK, Jang YH (1995) Effect of adsorption of endoglucanase on the degradation of microcrystalline cellulose. Bulletin of the Korean Chemical Society 16: 720-726.
102. Kim DW, Jang YH, Jeong YK (1998) Adsorption kinetics and behaviour of two cellobiohydrolases from Trichoderma reesei on microcrystalline cellulose. Biotechnology and Applied Biochemistry 27: 97-102.
103. Ooshima H, Sakata M, Harano Y (1983) Adsorption of cellulase from Trichoderma varied on cellulose. Biotechnology and Bioengineering 25: 3103-3114.
104. Kim DW, Kim TS, Jeong YK, Lee JK (1992) Adsorption kinetics and behaviors of cellulase components on microcrystalline cellulose. J Ferm Bioeng 73: 461-466.
105. Kyriacou A, Neufeld RJ, MacKenzie CR (1988) Effect of physical parameters on the adsorption characteristics of fractionated Trichoderma reesei cellulase components. Enzyme Microbiology Technology 10: 675-681.
106. Boija E, Johansson G (2006) Interactions between model membranes and lignin-related compounds studied by immobilized liposome chromatography. Biochim Biophys Acta 1758: 620-626.
107. Qin C, Clarke K, Li K (2014) Interactive forces between lignin and cellulase as determined by atomic force microscopy. Biotechnology for Biofuels 7: 65.
108. Sammond DW, Yarbrough JM, Mansfield E, Bomble YJ, Hobdey SE, et al. (2014) Predicting enzyme adsorption to lignin films by calculating enzyme surface hydrophobicity. 289: 20960-20969.
109. Fritz C, Ferrer A, Salas C, Jameel H, Rojas OJ (2015) Interactions between cellulolytic enzymes with native, autohydrolysis, and technical lignins and the effect of a polysorbate amphiphile in reducing nonproductive binding. Biomicromolecules 16: 3878−3888.
110. Bonomo LA, Minim JSR, Coimbra RCI, Fontan LH, Mendes da Silva VPR (2006) Hydrophobic interaction adsorption of why proteins: effect of temperature and salt concentration and thermodynamic analysis. J Chromatogr B Analyt Technol Biomed Life Sci 844: 6-14.
111. Andreaus J, Azevedo H, Cavaco-Paulo A (1999) Effects of temperature on the cellulose binding ability of cellulase enzymes. Journal of Molecular Catalysis B: Enzymatic 7: 233-239.
112. Baker JO, Tatsunoto K, Grohmann K., Woodward J, Wichert JM, et al. (1992) Thermal denaturation of Trichoderma reesei cellulases studied by differential scanning calorimetry and tryptophan fluorescence. Applied Biochemistry and Biotechnology 34: 217-231.
113. Tomme P, Heriban V, Claeyssens M (1990) Adsorption of two cellobiohydrolases from Trichoderma reesei to Avicel: evidence for exo-exo synergism and possible loose complex formation. Biotechnol Lett 12: 525-530.
114. Reese ET (1982) Elution of cellulase from cellulose. Process Biochemistry 17: 2-6.
115. Manning K, Wood DA (1983) Production and regulation of extracellular endocellulase by Agaricus bisporus. Journal of General Microbiology 129: 1839-1847.
116. Tolan JS, Foody B (1999) Cellulase from submerged fermentation. Advances in Biochemical Engineering and Biotechnology 65: 41-67.
117. Moloney A, Considine PJ, Coughlan MP (1983) Cellulose hydrolysis by the cellulases produced by Talaromyces emersonii when grown on different inducing substrates. Biotechnol Bioeng 25: 1169-1173.
118. Criquet S (2002) Measurement and characterization of cellulase activity in sclerophyllous forest litter. Journal of microbiological methods 50: 165-173.
119. Deng SP, Tabatabai MA (1994) Cellulase activity of soils. Soil Biology and Biochemistry 26: 1347- 1354.
120. Sachslehner A, Nidetzky B, Kulbe KD, Haltrich D (1998) Induction of Mannanase, Xylanase, and Endoglucanase Activities in Sclerotium rolfsii. Applied Environmental Microbiology 64: 594-600.
121. Kaya F, Heitmann JA, Joye TW (2000) Influence of lignin and its degradation products on enzymatic hydrolysis of xylan. J Biotechnol 80: 241-247.
122. Vasquez MP, Silva NP (2007) Enzymatic hydrolysis optimization to ethanol production by simultaneous saccharification and fermentation. Appl Biochem Biotechnol 137-140: 141-153.
123. Ximenes E, Kim Y, Mosier N, Dien B, Ladisch M (2010) Inhibition of cellulases by phenols. Enzyme Microbiology Technology 46: 170-176.
124. Qing Q, Yang B, Wyman CE (2010) Xylooligomers are strong inhibitors of cellulose hydrolysis by enzymes. Bioresour Technol 101: 9624-9630.
125. Duarte GC, Moreira LRS, Jaramillo PMD, Filho EXF (2012) Biomass-derived inhibitors of holocellulases. Bioenergy Research 5: 768-777.

126. Pan XJ, Xie D, Gilkes N, Gregg DJ, Saddler JN (2005) Strategies to enhance the enzymatic hydrolysis of pretreated softwood with high residual lignin content. Appl Biochem Biotechnol 121: 1069-1079.

127. Yang B, Wyman CE (2004) Effect of xylan and lignin removal by batch and flow through pretreatment on the enzymatic digestibility of corn stover cellulose. Biotechnology and Bioengineering 86: 88-95.

128. Borjesson J, Peterson R, Tjerneld F (2007) Enhanced enzymatic conversion of softwood lignocellulose by poly (ethylene glycol) addition. Enzyme Microbial Technology 40: 754-762.

129. Zheng Y, Pan Z, Zhang R, Wang D, Jenkins B (2008) Non-ionic surfactants and non-catalytic protein treatment on enzymatic hydrolysis of pretreated creeping wild ryegrass. Appl Biochem Biotechnol 146: 231-248.

130. Liu H, Zhu JY (2010) Eliminating inhibition of enzymatic hydrolysis by lignosulfonate in unwashed sulfite-pretreated aspen using metal salts. Bioresour Technol 101: 9120-9127.

131. Holtzapple MT, Humphrey AE, Taylor JD (1989) Energy requirements for the size reduction of poplar and aspen wood. Biotechnol Bioeng 33: 207–210.

132. Zhang S, Wolfgang DE, Wilson DB (1999) Substrate heterogeneity causes the nonlinear kinetics of insoluble cellulose hydrolysis. Biotechnol Bioeng 66: 35-41.

133. Xiao ZZ, Zhang X, Gregg DJ, Saddler JN (2004) Effects of sugar inhibition on cellulases and beta-glucosidase during enzymatic hydrolysis of softwood substrates. Appl Biochemistry and Biotechnology 113: 1115-1126.

134. Bezerra RMF, Dias AA (2005) Enzymatic kinetic of cellulose hydrolysis Inhibition by ethanol and cellobiose. Appl Biochem Biotechnol 126: 49-59.

135. Zhao L, Pang Q, Xie J, Pei J, Wang F, et al. (2013) Enzymatic properties of Thermo- anaerobacterium thermos-saccharolyticum β-glucosidase fused to Clostridium cellulovorans cellulose binding domain and its application in hydrolysis of microcrystalline cellulose. BMC Technol 13: 101.

136. Qing Q, Yang B, Wyman CE (2010) Xylooligomers are strong inhibitors of cellulose hydrolysis by enzymes. Bioresour Technol 101: 9624-30.

137. Tanaka M, Nakamura H, Taniguchi M, Morita T, Matsuno R, et al. (1986) Elucidation of Adsorption Processes of Cellulases during Hydrolysis of Crystalline Cellulose. Applied Microbiology and Biotechnology 23: 263-268.

138. Sattler W, Esterbauer H, Glatter O, Steiner W (1989) The effect enzyme concentration on the rate of the hydrolysis of cellulose. Biotechnol Bioeng 33: 1221-1234.

139. Moldes AB, Alonso JL, Parajo JC (1999) Cogeneration of Cellobiose and Glucose from Pretreated Wood and Bioconversion to Lactic Acid: A Kinetic Study. J Biosci Bioeng 87: 787-792.

140. Stenberg K, Bollok M, Reczey K, Galbe M, Zacchi G (2000) Effect of substrate and cellulase concentration on simultaneous saccharification and fermentation of steam-pretreated softwood for ethanol production. Biotechnology and Bioengineering 68: 204-210.

141. Howell JA, Stuck JD (1975) Kinetics of Solka Floc cellulose hydrolysis by Trichoderma viride cellulase. Biotechnology and Bioengineering 17: 873-893.

142. Liaw ET, Penner MH (1990) Substrate-velocity relationships for the Trichoderma viride cellulase-catalyzed hydrolysis of cellulose. Appl Environ Microbiol 56: 2311-2318.

143. Huang XL, Penner MH (1991) Apparent Substrate Inhibition of the Trichoderma reesei Cellulase System. J Agric Food Chem 39: 2096-2100.

144. Wang W, Kang L, Wei H, Arora R (2011) Study on the decreased sugar yield in enzymatic hydrolysis of cellulosic substrate at high solid loading. Appl Biochem Biotechnol 164: 1139-1149.

145. Triwahyuni E, Muryanto SY, Abimanyu H (2015) The effect of substrate loading on simultaneous saccharification and fermentation process for bioethanol production from oil palm empty fruit bunches. Energy Procedia 68: 138-146.

146. Beldman G, Voragen AGJ, Rombouts FM, Searlevanleeuwen MF, Pilnik W (1987) Adsorption and kinetic behavior of purified endoglucanases and exoglucanases from Trichoderma viride. Biotechnology and Bioengineering 30: 251-257.

147. Zhang YH, Lynd LR (2006) A functionally based model for hydrolysis of cellulose by fungal cellulase. Biotechnol Bioeng 94: 888-898.

148. Woodward J, Lima M, Lee NE (1988) The role of cellulase concentration in determining the degree of synergism in the hydrolysis of microcrystalline cellulose. Biochemistry Journal 255: 895-899.

149. Nidetzky B, Steiner W, Hayn M, Claeyssens M (1994) Cellulose hydrolysis by the cellulases from Trichoderma reesei: a new model for synergistic interactions. Biochem J 298: 705-710.

150. Jalak J, Kurasin M, Teugjas H, Valjamae (2012) Endo-exo synergism in cellulose hydrolysis revisited. J Biol Chem 287: 28802-28815.

151. IKeo M, Ueno Y, Taneda D (2010) Mechanism of Exo-Exo synergism between CBH 1 and CBH 2 during the hydrolysis of cellulose. Biological Conversions and Processes for Renewable Energy Feedstocks. 12th AICHE Annual Meeting, Pittsburg, PA.

152. Varrot A, Hastrup S, Schulein M (1999) Crystal structure of the catalytic core domain of the family 6 cellobiohydrolase II, Cel6A, from Humicola insolens, at 1.92 angstrom resolution. Biochemistry Journal 337: 297-304.

153. Boisset C, Fraschini C, Schülein M, Henrissat B, Chanzy H (2000) Imaging the Enzymatic Digestion of Bacterial Cellulose Ribbons Reveals the Endo Character of the Cellobiohydrolase Cel6A from Humicola insolens and Its Mode of Synergy with Cellobiohydrolase Cel7A. Appl Environ Microbiol 66: 1444-1452.

154. Kurasin M, Valjamae P (2011) Processivity of cellobiohydrolases is limited by the substrate. J Biol Chem 286: 169-177.

155. Murray P, Aro N, Collins C, Grassick A, Penttila M, et al. (2004) Expression in Trichoderma reesei and characterisation of a thermostable family 3 beta-glucosidase from the moderately thermophilic fungus Talaromyces emersonii. Protein Expr Purif 38: 248-257.

156. Anderson N, Johansen KS, Michelson M, Stenby EH, Krogh KBRM, et al. (2008) Hydrolysis of cellulose using mono-component enzymes shows synergy during hydrolysis of phosphoric acid swollen cellulose (PASC), but competition on Avicel. Enzyme Microbiol Technololgy 42: 362-70.

157. Kim DW, Hong YG (2000) Ionic strength effect on adsorption of cellobiohydrolases I and II on microcrystalline cellulose. Biotechnology Letters 22: 1337-1342.

158. Warner CD, Unal GC, Pohl NLB, Ford C, Reilli PJ (2013) Substrate binding by the catalytic domain and carbohydrate binding module of Ruminococcus flavefaciens FD-1 Xyloglucanase/ Endoglucanase. Industrial and Engineering Chemistry Research, Special issue.