

REVIEW

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Interaction of enzymes with lignocellulosic materials: causes, mechanism and influencing factors

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Abstract

For the production of biofuel (bioethanol), enzymatic adsorption onto a lignocellulosic biomass surface is a prior condition for the enzymatic hydrolysis process to occur. Lignocellulosic substances are mainly composed of cellulose, hemicellulose and lignin. The polysaccharide matrix (cellulose and hemicellulose) is capable of producing bioethanol. Therefore, lignin is removed or its concentration is reduced from the adsorption substrates by pretreatments. Selected enzymes are used for the production of reducing sugars from cellulosic materials, which in turn are converted to bioethanol. Adsorption of enzymes onto the substrate surface is a complicated process. A large number of research have been performed on the adsorption process, but little has been done to understand the mechanism of adsorption process. This article reviews the mechanisms of adsorption of enzymes onto the biomass surfaces. A conceptual adsorption mechanism is presented which will fill the gaps in literature and help researchers and industry to use adsorption more efficiently. The process of enzymatic adsorption starts with the reciprocal interplay of enzymes and substrates and ends with the establishment of molecular and cellular binding. The kinetics of an enzymatic reaction is almost the same as that of a characteristic chemical catalytic reaction. The influencing factors discussed in detail are: surface characteristics of the participating materials, the environmental factors, such as the associated flow conditions, temperature, concentration, etc. Pretreatment of lignocellulosic materials and optimum range of shear force and temperature for getting better results of adsorption are recommended.

Keywords: Biofuel, Bioethanol, Adsorption, Mechanism, Shear force

Introduction

Plant dry matter is called lignocellulosic material or biomass because this material consists of cellulose, which is closely associated with lignin. Lignocellulosic materials are abundant on earth with their annual production around 1.815 billion tons (Dahmen et al. 2019). Agricultural residues and forestry wastes represent more than 90% of lignocellulosic material in the world (Saini et al. 2015), where lignocellulosic biomass contains up to 75% of polysaccharides (Marriot et al. 2016). Polysaccharides are long chains of polymeric carbohydrates where monosaccharide units are bound with each other by glycosidic

linkages. Lignocellulosic materials are receiving huge attention as a renewable and economical alternative to fossil resources for the production of various value-added products such as biofuel, hydrogel, and specialty chemicals.

The basic steps involved in the production of biofuels from biomass include: pre-treatment, saccharification, fermentation, and distillation. Pretreatment is designed to break down the complex carbohydrates into cellulose, hemicellulose and lignin so that the cellulose and hemicellulose can be hydrolyzed by enzymatic catalysis. Lignin consists of phenols, which is not fermentable; therefore, lignin is separated and it is used to provide process heat and electricity for the industry. Distillation is the product purification step. The core unit operation in the hydrolysis process is the saccharification which

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starts with the desorption of enzymes. The attachment of enzymes or cells to the surfaces of biomaterials (live or dead) is adsorption. Adsorption is a key phenomenon in modern industry such as: (1) catalysis, (2) clarification of sugar (3) pollutant separation (air purification, hard water softening, wastewater treatment, etc.), (4) biomaterials implantation, (5) and in medicine. In addition to these, adsorption indicators are gaining popularity in both research laboratory and industry. During enzymatic adsorption, the interaction of enzymes could be specific and non-specific to adsorb or to oppose the detachment from the biomaterial surface (Vladkova 2010; Dhowre et al. 2015; Navarro-Sanchez et al. 2019). Poor adsorption results in less hydrolysis and hence less production of biofuel. Similarly, poor adsorption results in unsuccessful implantation of biomaterials. The interactions at the molecular level that regulate enzymatic adsorption to biomass are not completely understood (Taegtmeyer 2012; Jalak and Våljamäe 2014; Donaldson and Vaidya 2017; Bonnin et al. 2019). The comparative influences of specific and nonspecific adsorption mechanism of enzymes are dependent on the surface characteristics of the biomass, the provided flow rate and the environmental conditions.

The combined production of biofuel by USA and Brazil is 49.6% and 38.3% of the total international production, respectively (Coyle 2007). There is a forecast by International Energy Agency (IEA), which indicates biofuel production is expected to increase 165 billion liters by 2023. In this huge market, the share of bioethanol is going to be 119 billion liters (Voegelé 2019). Table 1 compares the annual growth of biofuel production against its required growth rate for the top three largest bioethanol producing countries.

It would be interesting to note that the annual growth in demand is more than the required growth in production of biofuel. Canada is also in the production and use of biofuel because of an estimation that the use of biofuel can reduce greenhouse gas (GHG) emissions by approximately 30–96% as that of gasoline,

depending on the type of biomass and the biomass conversion technology used (Fulton et al. 2004; Khattab and Watanbe 2019). In fact the use of ethanol in Canada has increased from 3140 million liters in 2018 to 3250 million liters in 2019. Refineries produced 1755 million liters in 2018 and 1830 million liters in 2019. Obviously, Canada follows the same trend of less production than demand as that of large ethanol producers. If biofuel is produced by grains (corn, wheat, etc.), it creates food scarcity for people. If it is produced from corn stover, animal food supply is in trouble. Similarly, use of wood will risk tree conservation. If Canada uses waste lignocellulosic materials (agricultural waste, forestry waste) for the production of bioethanol, the cost of domestic ethanol will reduce. For example, Canada is producing 37.5 million tons of wheat straw, which in turn can produce 13.125 billion liters of ethanol (Baig 2016a). The annual allowable cut (AAC) for Canadian forest growth is 250 million m³, which can produce 52,000 million liters of biofuel (National Forestry Database 2017). Only 25% of wheat straw or 10% of AAC can produce Canadian total ethanol demand. Therefore, Canada can use indigenous lignocellulosic waste materials to produce bioethanol without being a burden on grain foods supplies, animal feed cycle or on tree conservation. Lignocellulosic feed stock materials need pretreatment to enhance adsorption of enzymes to the lignocellulosic substrates; the more the specific adsorption, the more is production of bioethanol through hydrolysis. One of the reasons for the less growth rate in actual production of biofuel is due to less adsorption or less specific adsorption or non-specific adsorption, which can be increased by the greater understanding of the mechanism of adsorption. Another aspect of enzymatic adsorption to a biomaterial (lignocellulosic biomass) surface is that it is a sequential process where initial interactions are weak, reversible, non-productive and instantaneous; with the passage of time, the interplay leads to establishment of productive molecular interactions. The factors involved in the process of enzymatic adsorption, techniques and principles are reconsidered in this study. Knowing the physical structures and chemical compositions of enzymes/proteins and biomass/biomaterials, a conceptual mechanism is suggested which will help researchers to understand the adsorption process more deeply and increase the biofuel production. In the last few years, substantial work has been reported for the process of adsorption of enzymes to lignocellulosic biomass surfaces; however, many questions remain unanswered to win confidence of biofuel producing industry. Some of the questions are addressed in the coming sections.

Table 1 Forecast of production growth and growth required by 2030

Country	Production	
	Annual growth (%)	Growth required (%)
USA	1	6
Brazil	3.5	6
China	16	17

Adapted from IEA (2019)

Factors influencing enzymatic adsorption (bacterial adhesion)

Adsorption of enzymes on lignocellulosic materials is a delicate and intricate process, which is influenced by the characteristics of enzymes and adsorbent substrate, environmental dynamics, such as the connected flowing states. A detailed discussion on the factors influencing enzymatic adsorption is given in the review of Baig et al. (2016b). Some additional aspects and research results are interpreted towards an advanced insight for the application of adsorption. The surface characteristics such as topography, roughness, composition of substrates, and the chemical interactions of enzymes with the substrates are essential to develop understanding of the adsorption process to apply strategically.

Constituents of various biomass materials

Lignocellulosic materials are the common raw materials used for the production of biofuel. Cellulose, hemicellulose and lignin are the three main components present in lignocellulosic biomasses. Depending on their source, type, and kind of biomass the contribution of these components varies in their composition as given in Table 2.

Hardwood is obtained from alder, balsa, beech, hickory, mahogany, maple, oak, teak, and walnut trees while cedar, Douglas fir, juniper, pine, redwood, spruce, and yew provide softwood. Comprehension of the structures of the biomass components is important to appreciate the adsorption process; therefore, these are discussed here.

Cellulose is a polymer, its average molecular weight is approximately 100,000. A single unit of glucose is shown

in Fig. 1. The glucose units are in 6-membered rings having 5 carbons and one oxygen, called pyranoses. The glucose units are joined with the C-1 of one pyranose ring and the C-4 of the next ring by an acetal linkage.

In the glucose ring all the 'H' atoms are in axial positions while the OH groups are in the equatorial positions. The equatorial positions of the hydroxyl groups develop hydrogen bonding with other groups. These hydrogen bonds hold the glucose chains together and form crystalline regions. The glucose chains are long and encompass numerous crystalline regions, with patches of disordered regions in between. The insolubility of cellulose in most of the solvents is due to the strong inter-chain hydrogen bonds in the crystalline areas. They also impart non-thermoplasticity to cellulose. At the disordered regions, the glucose chains are at a distance and are available to interact with other molecules. There are six conceivable structural arrangements of glucose chains to be held in

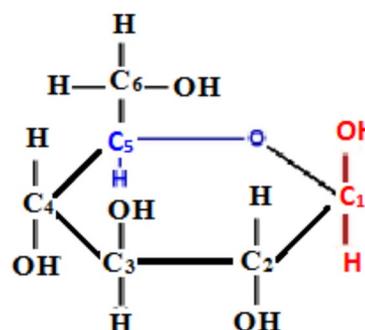


Fig. 1 Chemical structure of a glucose unit (Adapted from Klemm et al. (2005), Netrabukkana (1996))

Table 2 Composition of various biomass substances in percentage

Substrates	Cellulose	Hemicellulose	Lignin	References
Hardwood	40–50	20–40	15–30	Reyes-Rivera et al. (2015)
Softwood	45–50	25–35	20–25	Reyes-Rivera et al. (2015)
Nut shells	30–40	25–30	30–35	Queirós et al. (2019), Lourenco and Pereira (2018)
Corn cobs	31–40	30–40	15–25	Rofiqah et al. (2019)
Corn stovers	30–41	25–35	15–25	Wang et al. (2019)
Coastal Bermuda grass	31–35	26–30	14–25	Canizo et al. (2014)
Pineapple leaf	25–30	30–45	15–20	Mansor et al. (2019)
Cotton seed hairs	80.95	5–20	0.0	Kumar et al. (2009)
Wheat straw	35–40	20–30	16–25	Isikgor and Becer (2015)
Barley straw	30–35	20–30	18–25	Lara-Serrano et al. (2019)
News paper	40–55	25–40	18–30	Kumar et al. (2009)
Waste papers from chemical pulps	30–80	5–15	1–20	Ioelovich (2014)
Primary municipal wastewater solids	8–15	NA	24–29	Sun and Cheng (2002)
Solid cattle manure	2–5	2–4	2–6	Jung et al. (2015)

the elementary fibrils depending on the polymerization conditions (O'Sullivan 1997). Native cellulose is a heterogeneous mixture of not less than two structural arrangements (O'Sullivan 1997). The center of the elementary fibrils (micro-fibrils) of cellulose is very crystalline (Reza et al. 2019), and insoluble in water (Li et al. 2018). The crystalline order of cellulose is hard to disrupt. By the action of enzymes, cellulose was broken down to cellobiose (a glucose dimer) which was further degraded to glucose. Reaction of cellulose may occur by disrupting the linkages holding the units together, or it may be any of the usual reactions of hydroxyl groups. In most reactions, the distribution of hydroxyl groups along the cellulose chain provides excellent binding (adsorption) sites for enzymes (Lindman et al. 2017).

Hemicellulose ($C_{31}H_{34}O_{11}$)_n is a mixture of polysaccharides and its mean molecular weight is around 30,000. Hemicellulose is constituted with sugars (arabinose, glucose, mannose, and xylose) and uronic acids (methylglucuronic acid, and galacturonic acids). Chemical structure of hemicellulose contains D-glucosidic units that are attached to each other by β -1,3 and β -1,4 linkages as shown in Fig. 2.

Hemicellulose is a branched polysaccharide which fastens to the surface of each cellulosic micro-fibril (Hopkins and Huner 2017). In biomass, cellulose is generally 40–50% and hemicellulose is 20–40% of the material by weight (Brigham et al. 1996; Pauly and Keegstra 2008; Saini et al. 2015; Zoghli and Paes 2019). Hemicellulose has five-carbon sugars while cellulose has 6-carbon sugars.

Lignin is an amorphous compound, which has phenyl propanes [six-carbon ring attached with a three-carbon chain]. A polymeric form is shown in Fig. 3.

The methoxyl ($-OCH_3$) groups may be attached to the six member carbon rings. Three conformational isomers of lignin named as I, II and III are obtained due to attached 0, 1, 2 methoxyl groups, respectively. The conformation-I is present in grasses (Bykov 2008), conformation-II is in conifers wood and conformation-III is instituted in hardwoods. Lignin strengthens the cell structures by holding the fibers of polysaccharides together. Lignin contains methoxyl, hydroxyl (phenolic or alcoholic), carbonyl and carboxyl groups, in varying amounts, depending on origin and the lignin separation process applied (Gosselink et al. 2004). A brief availability of these function groups is given below:

Methoxyl groups: the methoxyl groups are 0.92 and 0.94 per one phenyl propane unit in softwoods and hardwoods, respectively (Bykov 2008; Davis et al. 2016; Lu et al. 2017).

Hydroxyl groups: there are three types of hydroxyl groups: (i) the straight chain hydroxyl groups are bound with T-carbon; (ii) secondary hydroxyl groups are bound with α -carbon, and phenolic groups are bound with 4th carbon C atom of aromatic rings. Lignins have almost 0.2 primary hydroxyl groups for each phenyl propane monomer, 0.84 secondary hydroxyl groups for each phenyl propane monomer, 0.30–0.35 phenolic hydroxyl groups for every phenyl propane monomer.

Carboxyl groups: there are 0.05 COOH-groups per 1 phenyl propane units of lignin. The carboxyl groups are generated through delignification due to oxidation of carbonyl, and hydroxyl groups. The alkaline lignin removal gives rise to carboxyl groups from 0.15 to 0.16 for each phenyl propane monomer. The increased concentration of carboxyl groups result in increased hydrophobicity of lignin material. The '-COOH' connects to

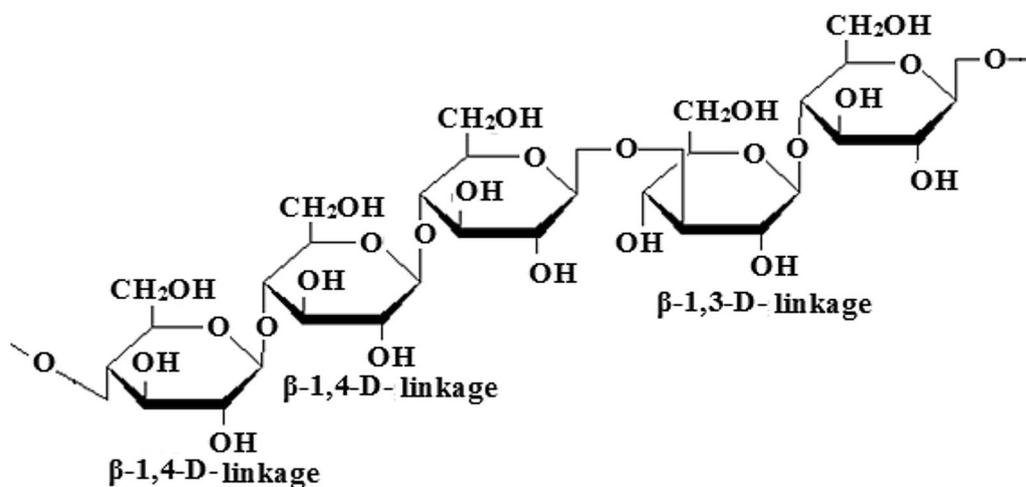
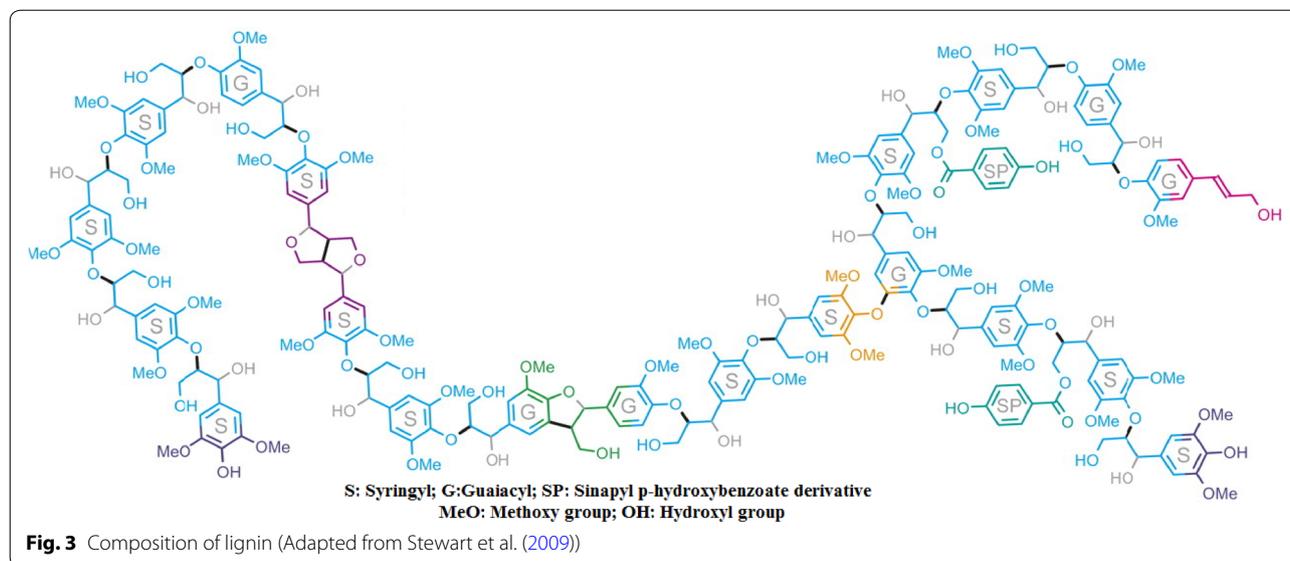


Fig. 2 A conformation of hemicellulose (Adapted from Klemm et al. (2005))



the functional groups of other molecules by hydrogen bonding.

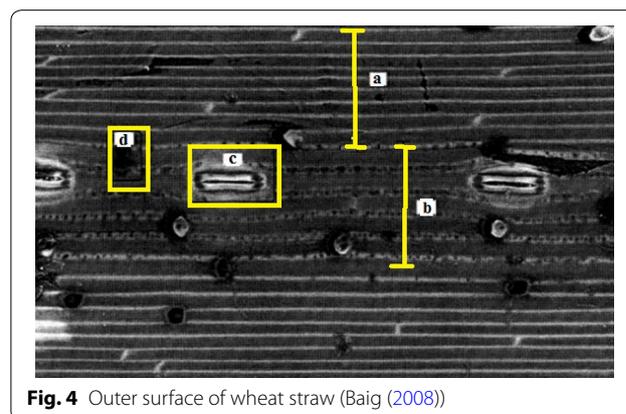
Carbonyl groups: there are 0.21 carbonyl groups for each phenyl propane monomer. There are 4 carbonyl groups attached with T-C atom in the form of aldehydes (0.04) and the rest of carbonyl groups, i.e., 0.17 are ketone. Hardwood lignin is easier to break down. Hardwood lignin can connect with other molecules through ether linkages more than softwood because the monomer in hardwood lignin is sinapyl alcohol. Because of the high reactivity of lignin towards enzymes, the presence of lignin is one of the major problems in bioethanol production (Katsimpouras et al. 2017).

Physical structure of biomass

From within the available biomass sources, the interest to use the grasses as energy crops has been increasing. The second largest family of monocotyledons is grasses (grass) having more than 700 genera and about 10,500 species including cereal crops such as wheat, barley, oats, rye and rice. The cereal crops are composed of nodes and internodes. Considering that wheat straw is the largest agricultural waste in Canada (37.52 million tons annually), it is discussed here. The wheat straw consists of internodes and nodes. Nodes are the hard points. The internodes are concentric rings, giving a void or cavity at the center. The outer layer (epidermis) is a dense layer of cellulose which has a silicon coating. Underneath the epidermis, there is a loose layer made of parenchyma and vascular bundle (Liu et al. 2005). Parenchyma are soft plant tissues made up of thin cells that form the greater part of leaves, stem pith, roots and fruit pulp. Vascular tissues are fluid-carrying vessels, e.g., sap-carrying

vessels in plants. The vascular bundles are means of transportation in the wheat plants. The framework of the vascular bundles is made of cellulose. The primary wall is the outer layer containing a lignified thickening secondary wall, which contains cellulose (Liu et al. 2005). Figure 4 presents the outer surface of wheat straw.

The surface morphologies of wheat straw, indicate two subsets of fiber structure: (i) one with the fibrils of about 5- μm diameter (part a), and (ii) the second with the diameter of about 10 μm (part b), the part c and the part d represent natural holes in surface for ventilation and metabolism (Baig 2008). Figure 5 shows vascular bundles with their longitudinal views. The annular or spiral form structure in vessel is quite bright. The other bright holes show protoxylem tracheid cells that have dense lignified thickening in the surrounding walls. The other part is lignin, which exist all around the cellulosic cells, between the cells and in vascular bundles. It provides bigger



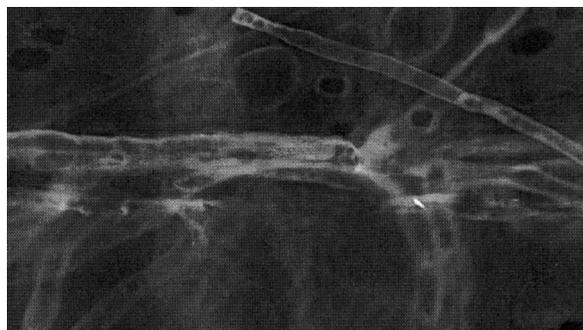


Fig. 5 Inner surface of wheat straw (Baig (2008))

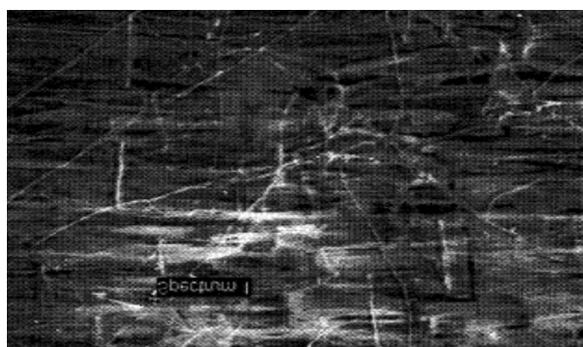


Fig. 6 Inner surface of wheat straw after soaking in distilled water (Baig (2008))

portion of sites for interaction due to its large number of phenolic groups, hydroxyl groups, methoxide groups and carboxylic groups (Baig 2008).

If enzymes are allowed to attach to the surface most probably the interaction with enzymes will make nonproductive bonding with enzymes. Therefore, the pretreatment of biomass to remove lignin is suggested. Figure 6 shows the appearance of wheat straw after soaking in distilled water and drying.

Enzymatic reactions normally occur in aqueous medium. Figure 6 shows how substrate fibers become more noticeable after soaking in aqueous medium, which helps in enzyme adsorption.

Cellulases

Cellulases are a mixture of enzymes which contain cellobiohydrolase I and II (CBHs), endoglucanase I and II (EGs) and β -glucosidase (though in small quantity). Fungi and bacteria produce extracellular cellulases, which degrade a number of wood and plants (Mandels and Weber 1969). A number of *Trichoderma* species such as *T. reesei*, *T. viride*, *T. kongii* and other fungal species such as *Penicillium funiculosum*, *A. wentii* produce extracellular cellulases. *Cellulomonas* and *Clostridium thermocellum* are bacteria that produce extracellular species able to degrade cellulose. Table 3 represents the main components of cellulases and their compositions in some available commercial enzyme complexes.

The modular structure of enzymes was deduced from the studies of *T. reesei* and *Cellulomonas fimi*. The modular structure has features: (i) carbohydrate-binding domain (CBD); (ii) catalytic domain (CD) or core; (iii) linker which join CBD and CD (Srisodusk et al. 1993). Some researchers (Hefford et al. 1992; Ramalingam et al. 1992; Wilson, 1992; Park et al. 1993) have confirmed this structure. The focus of the researches on cellulase adsorption onto crystalline cellulose was on the CBD–cellulose relative actions; CBDs adsorb more to cellulosic surfaces (Palonen et al. 1999). The α - and β -structures of cellulose tend to have hydrophilic and hydrophobic interactions. From the crystalline regions of 36 parallel cellulose chains only 38% of surface area have hydrophobic tendency (Nimlos et al. 2007). The CBDs of family 1 and family 3 cellulases adsorbed on the hydrophobic regions cellulose produced from algae (Lehtio et al. 2003). The interaction of enzymes with carbohydrates occurred due to aromatic amino acid residues and the surface charges. The hydrogen bonding or van der Waals attractive forces may develop interactions between enzymes

Table 3 Main components of cellulase enzymes

Enzyme	Lutzen et al. (1983)	Rosgaard et al. (2007)	Kang (2011)	Hilden and Johansson (2004)
	Celluclast	Cellulases	Spezyme CP	NS 50013
Cel 7B, endoglucanase I	10	5–10	12	10
Cel 5A, endoglucanase II	10	1–10	9	10
Cel 7A, cellobiohydrolase I	55	40–60	50	60
Cel 6A, cellobiohydrolase II	10	12–20	14	15
β -Glucosidase	1–2	N.D.	<2	2

and carbohydrates (Rutledge and Wetmore, 2010; Wilson et al. 2014). There are contradicting results reported about the effect of pH on cellulase adsorption. The pH has a reticent influence on the adsorption of *T. reesei* Cel7A onto BMCC because the binding onto catalytic domain was remained unaffected by pH (Reinikainen et al. 1995). Some others reported that for high substrate concentration, substrate recognition is not an issue, hence, the CBDs adsorption becomes slightly influential (Le Costaouec et al. 2013; Varnai et al. 2010; Kwon et al. 2019).

Interactions between enzyme and material surfaces at the molecular level

Three types of interactions are involved in adsorption of enzyme onto lignin: (i) hydrophobic interactions (Ying et al. 2018; Tokunaga et al. 2019); (ii) electrostatic interactions (Nakagame et al. 2011; Scott et al. 2016), and (iii) hydrogen-bonding (Rahikainen et al. 2013; Liu et al. 2016). The CBDs of *T. reesei* cellulases, Cel7A and Cel5A were adsorbed onto lignin in significantly high amount (Yarbrough, et al. 2015; Liu et al. 2016) due to hydrophobic interaction. Some hydrophobic groups such as long chain carboxylic acid, phenolic and aliphatic hydroxyl groups were found in isolated lignin samples (Berlin et al. 2005). Lignin model compounds were deprotonated during adsorption process between pH 6.2 to pH 11.3 (Ragnar et al. 2000). Generally, the hydrolysis of lignocellulosic materials is carried out at pH 5, when carboxylic acid groups present in lignin are deprotonated. Nakagame et al. (2011) reported that at pH 4.8 the isolated lignin showed a negative charge, and *T. reesei* enzymes (Cel6A and Cel5A) were positively charged, resulting in better adsorption. At elevated pH values the repulsive electrostatic forces between enzymes and lignin were increased, and hence adsorption onto ligneous substrate was decreased (Lou et al. 2013); enzyme reactions normally occur in aqueous medium. This figure shows how substrate fibers became more pronounced by soaking in aqueous medium, which helps in enzyme adsorption). This finding has a practical value from adsorption and desorption point of view (Baig 2016a).

The hydrophobic and electrostatic forces provide non-covalent interactions between enzymes and lignin surface while hydrogen bonding and dipolar interactions contribute a little (Kulkarni et al. 2015; Lundell 2018). The enzymatic cellulose degradation is hindered during prolonged reaction on biomass surfaces due to the inaccessibility of enzymes to the cellulose caused by the slow and regular accumulation of lignin (Djajadi et al. 2018). The lignin-rich components of biomass adsorbs enzymes more than non-lignin components, this type of adsorption is non-productive because it ceases the hydrolysis of

feedstock (Nitsos et al. 2019; Yang et al. 2019). Adsorption onto lignin is non-productive, and hence disadvantageous for process economics.

The enzyme–cellulose substrate interactions at crystalline region of cellulose were vigorously studied. It was discovered that the enzymes adsorb onto cellulosic surfaces yielding productive hydrolysis (Baig 2016a; Podgorbunskikh et al. 2019). The penetrating action of cellulose-binding modules of cellulases depends on their size, concentration, and binding equilibrium. There are 12 types of fundamental active amino acids residues present in enzymes. The surface amino acid residues on cellulases are charged residues. They have amide and hydroxyl functionalities. They probably interact with the C1 and C4 of the glucose unit to get adsorbed on the cellulose substrate. The adsorption connection is strengthened by the hydrogen bonding between cellulose and cellulase through hydroxyl groups on cellulose. To aid substrate binding, enzyme modification is another thought where protein stability at higher temperatures indicates a crucial role. Based on the structural information, a conceptual mechanism is given below which needs further research for validation.

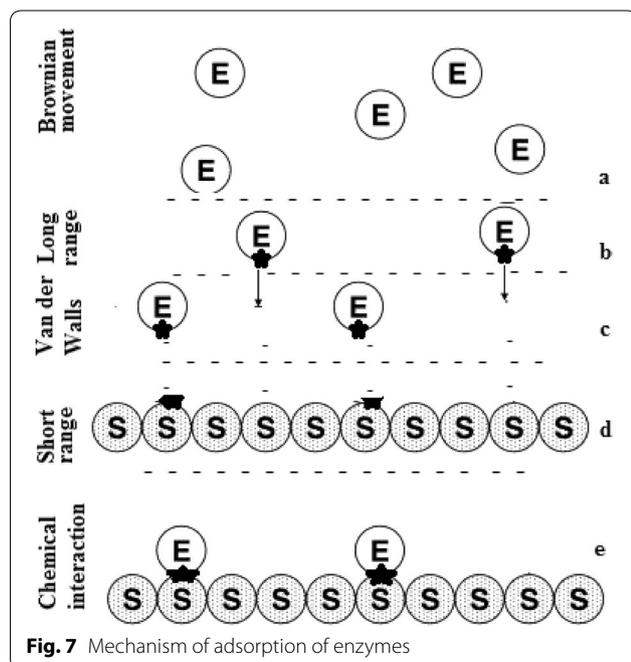
Adsorption mechanism of enzymes

Enzymatic adsorption is initiated by movement of enzyme to the substrate surface in reaction solution under the influence of physical forces. The influencing physical forces are Brownian motion (Romanczuk et al. 2012; Lee et al. 2014; Yanagishima et al. 2014; Zhao and Mason 2018; Zhang and Hess 2019), van der Waals attraction forces (Hanefeld et al. 2009; Reis et al. 2018), and gravitational forces (Arca-Ramos et al. 2018). In addition to these, the effect of surface electrostatic charge (Feller et al. 2010; Filali et al. 2019) and hydrophobic interactions (Faccio 2018) is also present. Movement of enzymes is also controlled by concentration, diffusible or surface-bound chemical factors such as amino acids, sugars, and oligopeptides. These chemical factors regulate cellular adsorption components and prepare enzyme–surface and cell–surface interactions (Hoarau et al. 2017).

Enzymes are transported from the bulk of the solution to the enzyme–substrate interface by the long-range attractive interactions. The long-range interactions between enzymes and surfaces are reciprocal forces depending on the distance in between and free energy available. The long-range interactions comes into play when the distance is greater than 50 nm (Katsikogianni and Missirli 2004; Qin and Buehler 2014). As the enzyme drifts closer to substrate surface, Van der Waals forces start acting within the distance range of 10–20 nm (Hermann et al. 2017). Short-range interfaces actions are influential at a

distance of less than 5 nm between the active sites of enzymes and the substrate surface (Al-Haddad et al. 2013). This is a preliminary attachment which helps to develop interplay between the enzymes and substrate more. This first attachment of enzyme to surfaces is the preliminary adhesion, which makes the molecular or cellular phase of adhesion possible. Further to this towards adsorption, the main interactions are molecular-specific among surface-participating structures of enzymes and biomass substrate surfaces. This indicates a steadier adsorption of enzymes to the substrate surface. In brief, once the enzyme reaches the substrate surface, the adsorption is defined by chemical interactions. The chemical interactions may be due to attraction or repulsion of the functional groups taking part in the adsorption process. The interactions are led by the chemistries of the enzyme and substrate surfaces and the reaction medium, which is normally aqueous. Adsorption mechanism is depicted in a conceptual Fig. 7.

In Fig. 7, E enzymes are in the bulk of aqueous solution (adsorption medium). The gravitational forces and Brownian movement keep them moving as in part 'a'. They enzymes come in the range of long-range forces and they start moving towards substrate as in part 'b'. In the same time van der Waals forces start acting on the enzymes. Now enzymes are more directed toward substrates. They reach very close to the region where short-range attraction pulls them towards the substrate as in part 'c'. At this stage, enzymes are under the



influence of all three forces (long range, van der Waals and short range) as the enzymes and substrate are very close to each other, the chemical interaction clamps enzymes with substrates as in part 'e'.

Procedures to evaluate enzyme–substrate interactions

There are simple, low-cost, easy systems to evaluate enzymatic adsorption onto lignocellulosic surfaces (Tamer, et al. 2016; Zadrta et al. 2018; Reis et al. 2018). The procedure adapted may be as follows: a ready-to-use surface is allowed to come across with the enzymes suspension for a pre-established time interval. Subsequently, the non-adsorbed enzymes are removed by centrifugation and the adsorbed enzymes on the surface are calculated. The non-adsorbed or softly adsorbed enzymes are separated by centrifugation and this step may be used to evaluate an overall strength of adhesion (Baig 2016). Various techniques can be used to examine the adsorbed enzyme:

1. Microscopy to determine the morphology of adsorbed enzymes and calculate the numbers. Light microscopy used to examine the specimen must properly illuminate to view the enlarged image of specimen.
2. Scanning electron microscope (SEM). SEM uses a beam of electron to give details of the examined surface. The magnification range is around $2\times$ to $250,000\times$.
3. Scanning confocal microscopy. It is a laser scanning technique which develops pixel-by-pixel image by assembling the emitted photons from the fluorophores of the sample.
4. Atomic force microscopy. It is a scanning probe microscopy. It measures local properties of a sample such as height, friction and magnetism. It can perform in vacuum, gas and liquid environments.
5. Radiolabelling. 5-Cyano-2,3-ditoyl tetrazolium chloride.
6. Direct and indirect methods.
 - a. Spectrophotometry.
 - b. Coulter counter apparatus can be used to count and size enzymes.

Environment

The adsorption of enzymes onto biomass substrates depends on a number of factors such as associated flow conditions, temperature of medium, contact time of enzymes (incubation time), concentration of enzymes, concentration of substrate, the presence of contaminants. Flow conditions strongly influence the adsorption

process by governing the number of attached enzymes (Bekard et al. 2011; Lippok et al. 2016). It is commonly conceived that lower shear rates or low agitation give higher adsorption. As soon as enzymes were exposed to an adsorbent material, the enzymes develop interactions due their surface features. The binding interactions became ineffective with increases in shear rates from 50 to 300 s⁻¹.

Shear forces

The shear forces are created by agitation, shaking or mixing. Therefore, the flow conditions for process should be selected carefully. Table 4 shows that at 100 rpm the cellulose in spruce wood was 20% converted to glucose. While keeping all other conditions (substrate loading, enzymes loading, temperature, contact time, etc.) constant and rpm were increased to 300, the conversion was increased to 31%, a further increase in rpm to 600 resulted in 37% conversion. Varnai et al. (2011) has reported similar results on adsorption of cellulases enzymes. Shaking, agitation, varying rpm all have an influence on the number of adsorbed enzymes, i.e., increased (Thomas et al. 2010; Palmqvist et al. 2011; Kadic et al. 2014). It is important to remind that enzymes those are adsorbed will only convert the material to product and those who are not adsorb will not be able to cause any conversion. Therefore, conversion (hydrolysis) is taken as an indirect measure of adsorption.

While keeping all other factors constant the substrate loading was decreased to 7%, there was almost no change in adsorption on enzymes to the substrate surface. Similar results are available in literature when there was no change in adsorption (Kadic et al. 2014; Ingesson et al. 2001) or a little change in adsorption was observed due to change in flow conditions (Kadic et al. 2014; Bhagia

et al. 2019). The cellulases and biochar obtained by low-temperature hydrothermal carbonization (LTHTC) of biomass were intensively shaken on a shaker at 300 rpm and 500 rpm for 7 h for adsorption. Higher adsorption efficiency and residual enzyme activity were achieved when the carrier and the enzyme were shaken at lower agitation speed. Higher agitation speeds obviously shortened the time of contact between the enzyme and the carrier; therefore, adsorption efficiency and residual enzyme activity decreased (Mercon et al. 1997). The literature survey led to the following hypotheses: (i) layers of enzymes were formed which hinder the access of enzymes to the substrate; (ii) the enzyme configuration has been modified by vicinity. It is commonly believed that advanced shear rates gives better disengagement forces that decrease the number of adsorbed enzymes (Katsikogianni and Missirlis 2004) with decrease in boundary layer and thinned the biofilm (Qureshi et al. 2005).

Once in contact with substrate, the enzymes develop a relationship with the substrate surface. To further explain it consider the adsorption of a ligand molecule onto a receptor protein, the number of adhesive bonds formed would depend on the charge densities of the ligand and the receptor (Robert et al. 2011; Ding et al. 2019). A specific force is always required to disintegrate a bond. The shear stress required to separate enzymes from the substrate can be estimated by the amount of bonds linking enzymes and surface (Engler et al. 2009; Kim et al. 2016). The adsorption of cells, *S. aureus* to collagen (proteins present in skin) was increased with increase in the shear rates between 50 s⁻¹ up to 300 s⁻¹ and dropped when shear rates were increased above 500 s⁻¹ (Mohamed et al. 2000; Ribeiro et al. 2012). During adsorption, the quantity of bonds of the attached enzyme and substrate can be

Table 4 Mixing to facilitate enzymatic adsorption

Substrate	Enzyme	Mixing	References
Spruce 13% loading	Cellulases	100 rpm, 48 h, 20% conversion	Kadic et al. (2014)
Spruce 13% loading	Cellulases	300 rpm, 48 h, 31% conversion	Kadic et al. (2014)
Spruce 13% loading	Cellulases	600 rpm, 48 h, 37% conversion	Kadic et al. (2014)
Giant reed, 13% loading	Cellulases	100 rpm, 48 h, 43% conversion	Kadic et al. (2014)
Giant reed 13% loading	Cellulases	300 rpm, 48 h, 53% conversion	Kadic et al. (2014)
Spruce 7% loading	Cellulases	100 rpm, 48 h, 31% conversion	Kadic et al. (2014)
Spruce 7% loading	Cellulases	100 rpm, 48 h, 32% conversion	Kadic et al. (2014)
Giant reed 7% loading	Cellulases	300 rpm, 48 h, 56% conversion	Kadic et al. (2014)
Giant reed 7% loading	Cellulases	300 rpm, 48 h, 56% conversion	Kadic et al. (2014)
Cellulose 7.5% loading	Cellulases	25 rpm, 72 h, 61.3% conversion	Ingesson et al. (2001)
Cellulose 7.5% loading	Cellulases	150 rpm, 72 h, 68.4% conversion	Ingesson et al. (2001)
Cellulose 2.5% loading	Cellulases	25 rpm, 72 h, 78.8% conversion	Ingesson et al. (2001)
Cellulose 2.5% loading	Cellulases	150 rpm, 72 h, 82.2% conversion	Ingesson et al. (2001)

changed (rise or decline). The enzyme will detach as the number of bonds decreased below a critical value (Sridhar et al. 2018). Walstra (2001) studied the adsorption of an enzyme of 40 kilodalton (kDa) molecular weight with the surface coverage of 2 mg m^{-2} . The sheer stress of 1 Pa provided deformation energy of about 10^{-25} J for each molecule while a sheer stress of about $5 \times 10^5 \text{ Pa}$ is required to denature an enzyme (Bekard et al. 2011). As the working medium is water, which means a 1000 times higher shear rate of 5×10^{50} (i.e., $5 \times 10^8 \text{ Pa s}^{-1}$) would be required to denature a small globular enzyme. Jaspe and Hagen (2006) predicted that a sheer stress of 10^7 s^{-1} could unfold cytochrome enzyme having free energy 42 kJ mol^{-1} . Walstra (2001) used another technique (surface tension) to estimate attraction at air–liquid interface and found that the free energies applied were about 400 kJ mol^{-1} to the adsorbed enzyme, which is 10^{-18} J for every molecule. Therefore, it can be expected that enzymes may unfold at interfaces under high sheer stresses. The force required to detach enzymes increases as the contact time reached the largest value which indicates that the supplementary interconnections are developed gradually after the first temporary attachment of an enzyme to the substrate.

Temperature

The reported results about the influence of temperature on the adsorption of cellulases are controversial. Some of the investigators proposed that the adsorption of cellulases onto the biomass material was an exothermic

process which was controlled by enthalpy. It was also observed that the concentration of cellulases adsorbed was reduced as the temperature was augmented (Medve et al. 1994; Kim et al. 2000; Ooshima et al. 1983). Conversely, Hoshino et al. (1992) and Creagh et al. (1996) proposed that the adsorption of enzymes onto MCC was an endothermic reaction and it was controlled by entropy, means increased with temperature. Tu et al. (2009) investigated reaction of cellulases on a soft wood (lodgepole pine, *Pinus contorta*). The soft wood is composed of 45–50% cellulose, 30–35% hemicellulose, 20–25% lignin, and 4–5% extractives). At 4 °C, 25 °C and 45 °C. The lignin obtained by steam exploding lodgepole pine (L-SELP), and lignin obtained by ethanol-pretreated lodgepole pine (L-EPLP) was used for the adsorption experiments. A maximum amount of cellulase was adsorbed onto lignin at 45 °C than at 4 and 25 °C, implying that the adsorption of cellulases onto L-SELP and L-EPLP was an endothermic process (Tu et al. 2009; Zheng et al. 2013a). This is true for the adsorption of cellulases onto lignin, because there exists a hydrophobic interplay between lignin and enzymes (Tu et al. 2007). The driving force for adsorption under the influence of hydrophobic interactions is entropy (Tu et al. 2009; Wang et al. 2010). The adsorption process, which is entropy-driven, was established by experimental data obtained from titration micro-calorimetry (Creagh et al. 1996). It means that lignin adsorbs more cellulases and with increase in temperature the adsorption on lignin increased. These results also implied that delignification

Table 5 Effect of temperature on adsorption

Adsorbate	Adsorbent	Temperature studied	Effect	References
BsEXLX1	Cellulose	25 °C, 45 °C only	Decreased with increasing temp	Duan et al. (2018)
Cellulase	Wheat straw	25–70 °C	Increased till 50 °C	Baig et al. (2016c, d)
Cellulase ATCC 26921	Treated, spent coffee residues	30–50 °C	Increased till 40 °C	Buntic et al. (2016)
Cellulases	H ₂ SO ₄ treated corncob	4–50 °C	Increased with increasing temp	Du et al. (2014)
EGs <i>Aspergillus fumigatus</i>	NaOH treated bagasse, corn husk, coconut coir, rice bran, rice straw, saw dust, wheat bran, wheat straw	4–40 °C	Decreased with increasing temp	Das et al. (2013)
Accellerase 1000	Commercial pure cellulose	4 °C, 50 °C only	Decreased with increasing temp	Zheng et al. (2013b)
Celluclast	AL, HL, OL, LS, SL, PL, BWX, GM,	4, 45 °C only	Increased with increasing temp	Pareek et al. (2013)
Accellerase 1000	ACS-L, SECS-L, SERS-L	4 °C, 50 °C only	Increased with increasing temp	Zheng et al. (2013a)
Purified endoglucanase (CMCase)	Commercial pure cellulose	10–40 °C	Decreased with increasing temp	Das et al. (2012)
Penicillium notatum NCIM NO-923	Commercial pure cellulose	10–40 °C	Decreased with increasing temp	Das et al. (2012)
CBHs, EGs/	Steam exploded spruce lignin	4, 30 °C only	Almost the same adsorption	Piccolo et al. (2010)
CBHs, EGs/	Commercial pure cellulose	4, 30 °C only	Almost the same adsorption	Piccolo et al. (2010)
CBHs, EGs/	Pretreated WS	4, 30 °C only	Almost the same adsorption	Piccolo et al. (2010)
<i>Aspergillus niger</i> cellulase	Activated carbon powder	20–60 °C	Increased with increase in temp	Daoud et al. (2010)

BsEXLX1: *Bacillus subtilis* expansion; CMCase: carboxy methyl cellulase

of lignocellulosic substrates for cellulases desorption was necessary. Through a literature survey (Table 5) it was observed that effect of temperature is a complex phenomenon because on the one hand it influences adsorption, desorption and on the other hand, it affects activity of the cellulases.

Other activities of endoglucanase and exoglucanase enzymes after adsorption reported that 29% of the initially added cellulases enzymes were free in solution at 45 °C, while 9% of cellulases were free at 55 °C, after 4 h of contact time. Additionally, enzymes were strongly bound on spruce (enzymatically purified) lignin at high temperature, which presented a sudden decrease in the concentration of adsorbed enzyme at 55 °C than that of at 45 °C (Rahikainen et al. 2013). The increase in adsorption of enzymes onto lignin and increased denaturation of enzymes were due to negative effect of temperature on the adsorption (Rahikainen et al. 2013; Borjesson et al. 2007; Viikari et al. 2007). Bonomo et al. (2006) investigated adsorption of bovine serum albumin (BSA) and β -lactoglobulin (β -lg) onto a hydrophobic adsorbent (Streamline Phenyl, packed in a column HR 5/5) to evaluate stability of enzymes in the temperature range of 10–40 °C and suggested that over 30 °C a great conformational change occurs. Cellulases from *T. reesei* (Celluclast 1.5 L, Spezyme CP), cellulases from *Penicillium* sp. (MSUBC) onto CEL from SELP and EPLP showed that the activity of Celluclast 1.5 L increased from 25 to 45 °C and enzymes were denatured from 55 to 75 °C (Tu et al. 2009). Baig (2016) observed a decrease in desorption of enzymes, and those desorbed were not active any

more. It was concluded that cellulase enzymes degraded on at temperature 60 and above. Degradation of enzymes means conformational changes and loss of capability to perform their function (activity). It is important to know what is the focus of study whether the researcher wants to determine adsorption only or researcher needs to know about biodegradability of the biomaterial which includes the processes of adsorption, hydrolysis and desorption.

Time of contact

The adsorption profile of cellulases onto microcrystalline cellulose (Avicel) was established by Singh et al. (1991) who reported that most of the enzymes were adsorbed onto Avicel and pulp within the first 10 min of contact at 30 °C. Steiner et al. (1988) reported that 50% of the maximally adsorbed enzyme was adsorbed within 1–2 min of contact with microcrystalline cellulose; however, adsorption equilibrium was established less than 30 min. Pulp exhibited higher enzyme adsorption than Avicel. The greater part of cellulase (around 18%) was adsorbed onto xylan substrate within 10–20 min of contact. Jager et al. (2010) reported that cellulase adsorption on α -cellulose was rapid, cellobiohydrolase CBH I reached equilibrium in 20 min and endoglucanase EG I in 30 min. Pareek et al. (2013) adsorbed cellulases on spruce lignin (SP) and black cotton wood lignin (BCWL) and found that around 60% of the enzymes were adsorbed within first 30 min of contact time, while adsorption took more than 2 h to reach to the same value for BCWL. To establish a dynamic

Table 6 Adsorption equilibrium for various adsorption systems

Enzyme	Substrate	Equilibrium time	References
Cellulase	Lignin from corn stover	60 min 4 °C	Yuan et al. (2018)
CBH I EG I	Cellulose	40 min 45 °C	Jager et al. (2010)
Cellulases	Cellulose (Avicel PH 101)	20 min 25 °C	Baig et al. (2016e)
Cellulases	Lignin (Protobind)	40 min 25 °C	Baig et al. (2016e)
Cellulase	Avicel	10 min 4 °C, 50 °C	Machado et al. (2015)
Cellulase	Organosolv bagasse, lignin	120 min 4 °C, 50 °C	Machado et al. (2015)
β -Glucosidase	Cellulose (Avicel), hydrothermal bagasse	No adsorption 4 °C, 50 °C	Machado et al. (2015)
<i>Fusarium oxysporum</i> cellulase	Egg white matrix	80 min 20 °C, 27 °C, 37 °C	Singh and Kaur (2014)
Cellulase CG220	Pretreated corncob	90 min 10 °C, 20 °C, 30 °C	Du et al. (2014)
<i>Lyophilized</i> cellulase	Cellulose	60 min 25 °C	Maurer et al. (2012)
<i>Aspergillus niger</i> cellulase	Activated carbon	40 min 20 °C, 50 °C	Daoud et al. (2010)
CBH I, CBH II	Microcrystalline cellulose	60 min	Kim et al. (2000)
Cellulases QM 9414	Microcrystalline cellulose	15 min 10 °C, 20 °C, 30 °C	Sethi et al. (1998)
CBH I, CBH II	Microcrystalline cellulose	90 min 4 °C, 25 °C, 40 °C	Medve et al. (1994)
<i>Talaromyces emersonii</i> cellulases	Cellulose	15 min 5 °C	Beldman et al. (1987)
<i>Talaromyces emersonii</i> cellulases	Cellulose	15 min 50 °C, more adsorption than 5 °C	Beldman et al. (1987)

adsorption equilibrium, a sufficient time of incubation is required. Table 6 shows that adsorption equilibrium time varies with the type of enzymes, type of biomass substrates and the environmental conditions.

Most of researchers investigated adsorption of metal ions, dyes and enzymes on various lignocellulosic substrates at varying temperatures. Their findings were that by increasing temperature, equilibrium constant value was increased, but the time to reach equilibrium remains the same (Medve et al. 1994; Sethi et al. 1998; Kim et al. 2000; Daoud et al. 2010; Zheng et al. 2013a; 2014; Du et al. 2014; Sukumaran et al. 2017; Houston et al. 2019). Some of the researchers have shown adsorption plot at one temperature and used the same equilibrium time for all other temperatures of studies (Sethi et al. 1998; Houston et al. 2019). Baig (2016a) submitted his observations about adsorption by stating at the start, adsorption progress with the time when the enzymes are placed with the substrate of interest. As the contact time increased, the cellulases adsorbed more and adsorption achieved its maximum almost at 20 min after of contact time, then it seemed to level off, i.e., adsorption equilibrium is achieved. Therefore, the time used for adsorption studies was set to 30 min. In the study of enzymatic adsorption/hydrolysis, it was a tradition that the enzyme contents in the supernatant were measured as the free cellulases $[P_f]$ and the adsorbed cellulases $[P_a]$ were calculated from the difference between the initial cellulases concentration and the free cellulases (Stuart and Ristroph 1984; Tu et al.

2009) which was carried out. In this set of experiments, it was found that on increasing the initial cellulases concentration $[P_0]$ from 100 to 183 $\mu\text{g mL}^{-1}$, adsorption increased from 68.45 to 109.3 $\mu\text{g mL}^{-1}$ for 100 mg of Avicel. Further increase in $[P_0]$ from 183 to 262 $\mu\text{g mL}^{-1}$ cannot bring any significant change in $[P_a]$ (i.e., from 109.3 to 116.2 $\mu\text{g mL}^{-1}$). The value of maximum cellulases adsorbed was similar to that determined by other researches on various lignocellulosic substrates (Singh et al. 1991; Nidetzky and Steiner 1993). The supernatant obtained after adsorption of 183 $\mu\text{g mL}^{-1}$, at 25 °C, pH 5 for a contact time of 60 min showed that no reducing sugars were formed. So, no noticeable hydrolysis took place during any of the adsorption experiments. Microcrystalline cellulase (Avicel PH-101) was 7% hydrolyzed in 24 h and 72% of it was hydrolyzed within initial 5 h of contact (Andersen et al. 2008). Therefore, for the study of adsorption only or the study adsorption for hydrolysis, contact time is an important factor. Contact time may vary depending on the enzyme–substrate system and the environmental condition applied to the enzyme–substrate system.

Concentration

Before reaching the optimum value, the increase in enzyme concentration increase the adsorption, for a fixed amount of substrate. Some enzyme and substrate pairs are given in Table 7 with the effect of increasing substrate concentration. The agricultural residues (corn stover,

Table 7 Effect of change in substrate concentration on adsorption process

Enzymes	Substrates	Comments	References
Cellic CTec2	Sorghum	Increased with increase in conc	Nghiem and Toht (2019)
<i>T. longibrachiatum</i> LC-M4	Sorghum	Increased with increase in conc	Dong et al. (2019)
Cellulase <i>T. reesei</i>	Cellulose	Increased with increase in conc	Tervasmäki et al. (2017)
Celluclast 1.5 L, Cellic CTec2 and Cellic CTec3	SO ₂ -pretreated poplar and lodgepole pine	Increased with increase in conc	Mok (2015)
Cellulase <i>T. reesei</i>	Cellulose (Avicel PH 101)	Increased with increase in conc	Baig et al. (2016e)
Cellulase <i>T. reesei</i>	Lignin (Protobind)	Increased with increase in conc	Baig et al. (2016e)
Accellerase 1000 <i>T. reesei</i>	Cellulose APC SPC SPR	Increased with increase in conc	Zheng et al. (2013a)
CBH I EG 2	Cellulose	Increased with increase in conc	Shi et al. (2016)
Spezyme-CP	Cellulose	Increased with increase in conc	Kumar and Wyman (2008)
GC-220	Cellulose	Increased with increase in conc	Kumar and Wyman (2008)
Cellulase	Avicel	Decreased with increase in conc	Eckard et al. (2013)
Cellulases	Pretreated forest wood	Increased with increase in conc	Matsakas et al. (2018)
Celluclast 1.5 L <i>T. reesei</i>	Corn stover and corn fiber	Increased with increase in conc	Arantes and Saddler (2011)
Cellic CTec 2	High CrI cellulose	Increased with increase in conc	Li et al. (2018)

APC: dilute acid-pretreated corn stover; SPC: steam explosion-pretreated corn stover; CrI: Crystallinity Index SPR: steam explosion pretreated rice straw; Cellic CTec2: cellulase and hemicellulase complex; *T. reesei*: *Trichoderma reesei*; *T. longibrachiatum*: *Trichoderma longibrachiatum*

corn fiber, rice husk, wheat straw, etc.) needs considerably lower protein loadings to achieve optimum adsorption than the forestry residues (poplar, Douglas fir and lodgepole pine). The adsorption efficiency depends in the resource and chemical constitution of a feedstock, and the employed pretreatment technologies. Pretreatment technologies are important, because efficiency of enzymatic adsorption depends on the accessibility of the enzymes to the cellulosic substrate.

Baig (2016a) conducted a vigorous study on the adsorption of cellulases on cellulose (Avicel PH 101) at room temperature, at various contact times, for eight initial cellulases concentration and found that adsorption increased with the increase in enzyme concentration before reaching an optimum value. Further increase in enzyme concentration makes no difference; in fact, the time to reach adsorption equilibrium was increased. In case adsorption is being conducted for biofuel production, the rate-limiting step during hydrolysis is not the catalytic cleavage of the cellulose chains, rather the accessibility of the enzymes to the cellulose. In case of biomedical implants an optimum adsorption of proteins is acceptable to develop protein layers for binding between implant and body.

Adsorption on lignin takes longer than that of cellulose. One cause of the delayed interaction could be the repulsion between amino acids tyrosine and histamine from cellulases and the carbonyl from lignin may allow cellulases to rearrange in order to adsorb onto lignin. Therefore, involvement of a number of positive and negative functional groups makes adsorption on lignin complex phenomena take a longer time. Detection of reducing sugars from the contact of cellulases with Protobind 1000 was not evaluated since lignin does not contain any polymeric sugars.

Discussion

Enzymes are very useful catalysts for degradation of biomass through adsorption, hydrolysis reactions. Like all catalysts, enzymes increase the speed of reactions by providing an alternative reaction pathway of lower activation energy. Enzymes take part in the reaction and provide an alternative reaction pathway. Enzymes are highly selective; therefore, catalyze specific reactions by altering the rate of adsorption, not the position of the adsorption equilibrium. This specificity requires the right selection of enzymes for substrates of given lignocellulosic materials under the provided environmental conditions, because every enzyme has certain advantages and disadvantages over the adsorption to a substrate. The universally used enzymes are cellulases for cellulose, xylenes, xylosidase and esterase for hemicellulose, and heme peroxidases, manganese peroxidase and laccases for

degradation of lignin. These enzymes adsorbed well on lignocellulosic biomass, which is a first stage for the production of biofuel. The cost of enzymes and the ease of distribution of enzymes over the accessible substrate area are important steps in the adsorption process. A very careful monitoring of enzymatic adsorption literature leads to a point that the adsorption is faster at the beginning and slower in the later stages. Imagine the adsorption process. When enzymes are adsorbed on the surface, after adsorption they hydrolysed the surface as the result three things happen: (i) product is formed, (ii) enzyme is freed for another adsorption, (iii) substrate offers more surface area that it was in the start of adsorption. It gives rise to questions such as, is it because enzymes are not productive anymore? Useful surface area is exhausted. Alternatively, the surface area is not a relevant parameter for adsorption and hence for hydrolysis. The probable reason is that the remaining crystalline cellulose has a structure that is more compact and it appears to be the influencing aspects, which finally regulate the efficiency of adsorption (Khodaverdi et al. 2012). In conducting tests for the evaluation of the adsorption process where amount of the enzymes adsorbed and that remaining in the solution should be precisely measured. The probability of a range of adsorption for each enzyme could be minimized by giving sufficient time to develop an equilibrium between adsorption and desorption process as detailed in the section 'Contact time'. In all the methodologies used, the postulations of the principal mechanisms of the enzymes moving toward the substrate surface, the adsorption kinetics, and the development of central site for adsorption, the assumption for the intermolecular linkage, and the number of particular receptors for the interacting ligands add to the nature of such researches. The accessibility of enzymes to cellulose and hemicellulose substrates is around 90% through the inside pores rather than the outer surface (Dutta and Chakraborty 2016).

Adsorption onto the outer surface has small contribution in the overall adsorption. Sometime lignocellulosic materials are subjected to pre-treatments. The pre-treatments improve a number of features of substrate, which help adsorption. Pretreatment produce improved solid surfaces that can enhance adsorption and hence produce more sugars during enzyme hydrolysis. It forestalls the degradation of sugars during the process. It reduces or ends the formation of inhibitors for succeeding fermentation process. Pretreatment makes recovery of lignin easy, which can be converted into value added by-products, and minimizes heat and power requirements to be cost effective. The vast array of biomass types is given in the section 'Constituents of various biomass materials' which excludes the possibility of applying one pretreatment

methodology or all various lignocellulosic materials. From the cost point of view, Eggeman and Erlander (2005) stated that one type of material might not be a feasible process for another lignocellulosic biomass type. Enzymatic adsorption is a type of heterogeneous catalysis. In catalysis, a catalyst postulates a substitute reaction pathway. The pathway offered is of lower activation energy. Hence, there are more molecules available to overcome the activation energy barrier with the result of increased reaction rate. In fact the increase in the temperature does not change the activation energy rather changes (increase) the amount of energy the molecules have. Imagine the Boltzmann distribution diagrams to conceptualize the number of molecules with a specific amount of energy. The area under the curve in the distribution with energy more than the activation energy required indicates the number of molecules that can react to give a product. Adding a catalyst or increasing the temperature of reaction will increase the rate of reaction. For a normal reaction, it has been observed that a ten-degree centigrade rise in temperature will increase the activity of most enzymes by 50 to 100%. Enzymatic catalysis is complicated because the catalyst and reactants are almost at the same temperature. Increasing the temperature of the catalyst means increasing the temperature throughout the reaction system. Increase in temperature brings no changes to the chemical structure of cellulose substrate, but it will affect the cellulases and their activities. Baig (2016a) observed that the increase in temperature from 25 °C to 40 °C could not bring any significant change on the reaction. Increasing the temperature from 25 to 50 °C was associated with almost 10% decrease in reaction rates. Some other researchers have observed similar behavior in enzymatic catalytic reaction (Tomme et al. 1990; Lee 1999). This increase in temperature brings no changes in the chemical structure of cellulose substrate and the increased temperature creates conformational changes in cellulases, such as folding, consequently, cellulases cannot remain adsorbed due to these conformational changes on the commercial cellulose (Avicel PH 101) and the adsorption is decreased. Therefore, it can be concluded that the increase in temperature affects the adsorption one way or the other. The catalysis of lignocellulosic material by an enzyme would be at a predetermined, specific range and optimized temperature.

The kinetics of catalytic reactions is almost the same as that of characteristic chemical reactions. The frequency of contact of the reactants molecules determines the rate of reactions. There is a pre-activation step in enzymatic catalytic reactions, which involves an induction period. For example, the contact time required for methane and carbon mono oxide is less than 1000 s while it is around

10,000 s for the reaction of aromatic and carbon dioxide (Yang et al. 2017). The contact time can be controlled by flowrate in case of a continuous flow reactor or an optimum mixing speed. A limited increase in temperature may help because conversion and consequently contact will increase. Unfortunately, higher temperatures may also lead to undesired results such as: (i) decrease in adsorption or (ii) loss of activity of enzymes or (iii) desorption, i.e., separation of adsorbed enzymes. In addition to other environmental factors, the efficiency of the reactions catalyzed by enzyme is influenced by: (i) amount of enzyme, and (ii) the amount of substrate. For a fixed enzyme amount, the adsorption increases with increasing amount of lignocellulosic substrate. This is valid up to a certain amount of substrate, any further increase for substrate no significant change in adsorption would occur. The enzyme–substrate complex formed has to be dissociated, and the active sites of the enzymes have to be freed to start the adsorption recycle again with the new substrate. At the higher substrate loading and at constant temperature and pH, the rate of adsorption is directly related to the amount of enzyme applied.

Concluding remarks

A huge number of research efforts have been exerted towards understanding the mechanisms of adsorption of enzymes on the substrate of a biomass. However, enzymatic adsorption is not a straightforward process. In fact, the adsorption is affected by many parameters such as characteristics of the substrate material (biomass) and enzymes, adsorption environment. The experimental evaluation of the governing factors is an extremely tedious job. The proposed mechanism of the transport of enzymes to the substrate may help to understand adsorption process.

Most of the studies have a variety of mixing dynamics. A slow mixing would provide chances of more adsorption on enzymes on lignocellulosic material. The application of higher shear force to increase contact not only decreases the chances of developing interaction between enzymes and lignocellulosic materials, but also decreases the activity of enzymes.

Increase in temperature increase the reaction rates, however in the case of biomaterials and enzymes the temperature effect is very delicate. In order to develop a successful adsorption equilibrium between adsorbing and desorbing enzymes, some factors such as shear forces, temperatures, concentration of enzymes and surface properties of the lignocellulosic materials need to be evaluated. A successful equilibrium means a desired (successful) yield of the product. The kinetics of enzymatic reactions is almost the same as that of characteristic chemical catalytic reactions.

Pretreatment of lignocellulosic materials often leads to surface heterogeneity and increased roughness. The best treatment is the one, which can provide more accessibility to enzymes with minimum loss of substrate materials.

Abbreviations

A. wentii: *Aspergillus wentii*; ATP: Adenosine triphosphate (ATP); BAC: Bioaffinity chromatography; BCWL: Black cotton wood lignin; β -Glucosidase: Beta-glucosidase; BsEXLX: *Bacillus subtilis* expansion (BsEXLX1); BSA: Bovine serum albumin; β -lg: β -Lactoglobulin; C-1: First carbon; C-4: Fourth carbon; EGs: Endoglucanases; EG I: Endoglucanase I; EG II: Endoglucanase II; CBHs: Cellobiohydrolases; CBH I: Cellobiohydrolase I; CBH II: Cellobiohydrolase II; CBD: Carbohydrate-binding domain; CD: Catalytic domain; CBM: Cellulose-binding modules; Cel 7B: Family 7 endoglucanase I, glycoside hydrolase; Cel 5A: Family 5 endocellulase, glycoside hydrolase; Cel 6A: Cellobiohydrolase II; Cel 7A: Cellobiohydrolase I; CEL: Cellulolytic enzyme lignin; CMCase: Carboxy methyl cellulase; CTC: 5-Cyano-2,3-ditoly tetrazolium chloride; GHG: Greenhouse gas; CrI: Crystallinity Index; IEA: International Energy Agency; kDa: Kilodalton; L-EPLP: Lignin derived from ethanol-pretreated lodgepole pine; L-SELP: Lignin derived from steam exploded lodgepole pine; LTHTC: Low-temperature hydrothermal carbonization; Rpm: Revolutions per minute; SEM: Scanning electron microscopy; SP: Spruce lignin; *T. reesei*: *Trichoderma reesei*; *T. viride*: *Trichoderma viride*; *T. kongii*: *Trichoderma kongii*; *T. longibrachiatum*: *Trichoderma longibrachiatum*.

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Authors' contributions

KSB conceptualize the idea, designed the work, conducted analysis, inter-related data and results for this work, and wrote this paper. Therefore, the author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work. The author read and approved the final manuscript.

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The data that support the findings of this study are available from the corresponding author (KSB), upon reasonable request.

Ethics approval and consent to participate

This research does not contain any studies on human participants or animals performed by any of the authors.

Consent for publication

I, Khurram Shahzad Baig (KSB), the corresponding author, hereby declare that that it is my study and I developed the manuscript titled 'Interaction of enzymes with lignocellulosic materials, causes, mechanism and influencing factors'.

Competing interests

The authors declare that they have no competing interests.

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