Substrate-Related Factors Affecting Cellulosome-Induced Hydrolysis for Lignocellulose Valorization

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Abstract: Cellulosomes are an extracellular supramolecular multienzyme complex that can efficiently degrade cellulose and hemicelluloses in plant cell walls. The structural and unique subunit arrangement of cellulosomes can promote its adhesion to the insoluble substrates, thus providing individual microbial cells with a direct competence in the utilization of cellulosic biomass. Significant progress has been achieved in revealing the structures and functions of cellulosomes, but a knowledge gap still exists in understanding the interaction between cellulosome and lignocellulosic substrate for those derived from biorefinery pretreatment of agricultural crops. The cellulosomic saccharification of lignocellulose is affected by various substrate-related physical and chemical factors, including native (untreated) wood lignin content, the extent of lignin and xylan removal by pretreatment, lignin structure, substrate size, and of course substrate pore surface area or substrate accessibility to cellulose. Herein, we summarize the cellulosome structure, substrate-related factors, and regulatory mechanisms in the host cells. We discuss the latest advances in specific strategies of cellulosome-induced hydrolysis, which can function in the reaction kinetics and the overall progress of biorefineries based on lignocellulosic feedstocks.

Keywords: cellulosome; lignocellulose; substrate-related factors; enzymatic hydrolysis; bioproducts; reaction kinetics

1. Introduction

Bioproducts, including biofuels and value-added chemicals derived from renewable resources, provide sustainable alternatives for petroleum-based products which contribute to climate change and energy crisis [1,2]. Among the variety of renewable resources, lignocellulosic biomass is the most abundant and economical carbon source on the earth. The development of bioproducts converted from lignocellulosic biomass should ultimately be essential for sustainable development without threatening food supplies and human survival [3]. However, as a natural protective barrier, the structure of plant cell wall is a recalcitrant network composed of cellulose, hemicellulose, and lignin, which is extremely difficult to degrade into fermentable sugars. Therefore, cellulose degradation and sugar release are becoming the typically rate-limiting factor for lignocellulosic biomass utilization [4]. Various efforts
have been paid to gain access and deconstruct fermentable sugars in lignocellulosic biomass. In the existing biorefinery process, commercial exogenous cellulases are employed to hydrolyze lignocellulosic biomass synergistically, whereas the large amount of cellulase consumption would almost counteract its benefit of using low-cost feedstock [5]. Combining microbial enzyme generation, saccharification with fermentation in one-step, the consolidated bioprocessing (CBP) has been accepted as an economically feasible strategy for bioprocess conversion from lignocellulosic biomass [6]. Although some aerobic fungi such as Trichoderma reesei, Aspergillus niger, and T. koningii exhibit potential cellulase extracellular secretion by the common natural habitats of these microorganisms, the requirement of continuous oxygen supply and nutrient competition with other co-cultured microorganisms has limited the possibilities of CBP with fungi [7]. Recently, cellulosomes—the multienzyme complexes produced by certain anaerobic cellulolytic bacteria have gained considerable attention, owing to their specifically design to overcome the natural recalcitrant network consisted by plant cell wall polysaccharides [8,9]. It has been reported that the polycellulosomes are as large as 100 MDa in nature, and the cellulosomes range in mass is 650,000 Da–2.5 MDa [10]. Therefore, as one of the most efficient naturally occurring biocatalysts to degrade lignocellulosic biomass, cellulosomes are potential substitutes for reducing enzyme loading in industrial scale biorefineries.

The supermolecular cellulosome complexes were first described in the cellulolytic thermophilic Clostridium thermocellum in the early 1980s [11]. Generally, cellulosomes consist of non-enzymatic scaffolding proteins associated with a variety of enzymatic subunits that play a decisive role to degrade cellulose and hemicellulose. The architectures and components of the multienzyme systems are various with different bacteria [12]. The main functions of cellulosomes include: (i) improvement of substrate uptake; (ii) tighten the specific interaction with certain substrates; and (iii) synergistic activity and processivity of cellulases [13]. Interestingly, cellulosomes have been verified to degrade not only crystalline cellulose, but also non-crystalline hemicelluloses, or even chitin and pectin [14]. The major producers of cellulosomes can be classified into several genera, i.e., Clostridium, Ruminococcus, Acetivibrio, Bacteroides, and Pseudobacteroides belong to both mesophile and thermophile [5,8,10,15] (Figure 1), but no cellulosome has been identified in microorganism that can grow above 65 °C and in the Archaea. These microorganisms exist in various environmental niches, such as sewage sludge, soil, animal guts, rumen, and wood chip piles. The different microbial sources are constantly observed by characterization and comparison of the cellulosomal enzyme properties.

Figure 1. Phylogenetic tree based on 16S rRNA sequencing of the anaerobic cellulosome-producing bacteria.
In recent times, numerous attempts have been described to improve cellulosomal catalysis by maximizing enzyme activities and/or creating the synergy between cellulosomal hydrolysis and the consequent fermentation [16,17]. Most of the efforts focus on engineering an ideal microorganism for CBP application, although the interaction between cellulosomes and lignocellulosic substrates remains to be clarified. This article presents a review of recent advances involved in properties of cellulosomes with respect of the composition and structural characterization. Moreover, the substrate-related physical and chemical factors affecting cellulose adsorptions and catalytic activities are discussed in detail. We also describe the enzyme diversity and regulatory mechanisms of cellulosomes, and their latest achievements and limitations in potential CBP of lignocellulosic biomass to bioproducts.

2. Cellulosome Composition and Assembly

The mechanisms of cellulosome assemblies are one of the greatest interests to reveal the structure–function relationship. Efficient degradation of lignocellulosic biomass by cellulosomes requires appropriate composition of enzymes and optimal cellulosome structures. The estimated molecular mass of individual cellulosome produced by different microorganism ranges from $2 \times 10^6$ to $6 \times 10^6$ [18]. The cellulosome consists of two major components, namely (i) non-enzymatic scaffoldins including enzyme-binding sites named cohesins and carbohydrate-binding module (CBM); (ii) catalytic enzymes with dockerins interacting with cohesins in scaffoldins (Type I interaction) or surface layer homology domain (Type II interaction) [9,10]. Figure 2 shows the assembly of each component of cellulosomes on the cell surface and their possible interactions with lignocellulosic substrate derived after different types of pretreatment processes. These micro-structures can either suspended freely in the liquid (Figure 2a), connect to intermediate scaffoldins (Figure 2b), or bind on the bacteria cell wall (Figure 2c). In this review, we summarize the cellulosome-related factors in Section 2 and the substrate-related factors in Section 3.

![Figure 2. Cellulosomal assemblies and the hypothetical interactions with pretreated substrates. (a) free enzymes; (b) cell-free scaffoldins; and (c) on cell wall. Lignin-O: sulfite treated; Lignin-A: dissolved; and Lignin-C: condensed lignin.](image)

2.1. Scaffoldin

The structure of scaffoldin forms the backbone of the enzymatic subunits, which is assembled by the dockerins. The scaffolding proteins contain one or more cohesin domains (Coh) and binding to substrates via CBM [19]. There are mainly three types of scaffoldin, i.e., primary, anchoring, and adaptor scaffoldins. Among them, the primary scaffoldin is the most common one and contains numerous Cohs that interact with dockerin-containing enzymes [20,21]. Although the mechanism by which a single primary scaffoldin can attach to the cell surface remains unknown, it is deduced that the scaffoldin should play a regulatory role during the assembly of cellulosome by using different substrates [10,21].
2.2. Cohesin–Dockerin Interaction

The cohesin–dockerin interaction can be considered as a mechanism of plug-and-socket in which the cohesin socket is plugged by the dockerin [22]. The various sequences of cohesin and dockerin are associated with the signature sequences of the cellulosomal enzymes [23]. In other words, the heterogenous nature of cellulosomes caused by the interactive variability of cohesin–dockerin pairs with different expressions in cohesin repeats, enzyme connections to the scaffoldins and species-specific variations [24–26]. The cohesin–dockerin interaction is known as one of the strongest protein–protein interactions in nature, even approaching the strength between high-affinity antigen and antibody (Ka~10^{11} M^{-1}) [27,28]. There are three types of cohesin–dockerin interaction have been reported according to sequence homologies of the cohesins and their binding partners, i.e., Type I, II, and III interactions [29,30]. Type I interactions are located between dockerin-containing enzymatic subunits and anchoring scaffoldins. Type II interactions are usually located between anchoring scaffoldins and enzyme-binding primary scaffoldins [9]. In addition, Type III interactions do not interact with either Type I or Type II domains [29]. Type I and Type II interactions are observed in Clostridium spp., while Type III interactions exist in ruminococcal cellulosomes [31].

2.3. CBMs (CBDs)

The cellulosomal CBM also called cellulose binding domain (CBD), belonging to carbohydrate-binding module family 3, is present on scaffoldins that bind the cellulosome tightly to the cellulosic substrate by disrupting its crystal surface at the solid–liquid interface [32,33]. Besides cellulose, some CBMs such as the CBM of Clostridium cellulovorans can also bind to chitin, which has similar crystalline structure to cellulose [34]. Although the CBM is a non-catalytic domain, it brings the cellulosomal enzymes close to its substrate, and therefore making the hydrolysis more efficient compared to free enzymes [35–37]. The CBM specific binding with substrate depends on the content and arrangement of amino acids [38]. For instance, cellulosomal CBM recognizes crystalline cellulose as reflected in homologous binding surface, which consists of mostly polar and aromatic side chains [39,40].

2.4. Cellulosomal Enzymes

The cellulosomal enzymes were first described in Clostridium thermocellum by cloning and expressing genomic libraries [41]. Cellulosomes usually exhibit better breakdown of substrates compared to free enzymes owing to their close proximity of the expressed enzymes, which act synergistically [42,43]. In general, the free enzymes depend on a CBM for guiding their catalytic domains to the substrates, whereas a dockerin domain located on the cellulosomal enzymes by which the enzymes are incorporated into the cellulosome complex. In this manner, cellulosomal enzymes contain the catalytic domains assembled by the duplicated dockerins linked to cohesins in scaffoldins via calcium dependent interactions [44,45].

So far, almost all cellulosome producers are characterized to produce large amount of glycoside hydrolase 48 (GH48) exoglucanase, which is crucial for enzymatic activity [46,47]. Intriguingly, not only cellulases but also hemicellulases [48–51] and other carbohydrate-active cellulosomal enzymes such as ligninases [52,53], pectinases [54,55], mannanases [56–58], and chitinases [59,60] were subsequently identified with the cellulosomes. These plant polysaccharide-degrading enzymes are highly complex and diverse, which makes it difficult to understand the mechanisms of protein assemblies and organizations. It has been reported that the complex cellulosomal architecture is responsible for minimizing the diffusion of certain carbohydrates and facilitate their uptakes by the cellulosomal enzymes for complete degradation [9,61]. The expressions and activities of cellulosomal catalytic subunits can be varied according to the substrate availability [62–64]. Cellulosomes with different compositions can be assembled on a microorganism with various enzyme complexes when grew on different carbon sources [65]. Therefore, understanding of the interactions between cellulosomes and
the utilized substrates is crucial to reveal the mechanisms of cellulosome expression and apply the potential CBP for bioproducts conversion.

3. Effects of Several Substrate-Related Factors on Cellulosome-Induced Hydrolysis

Despite the cellulosome complex being certainly viewed as an efficient natural system to break down the lignocellulosic biomass, there are several substrate-related factors that determine the reaction kinetics and yield of degradation of plant polysaccharide to its respective short chain monomers. These include: (i) carbon sources, i.e., types and sizes of lignin and hemicelluloses, as well as related complexes in the substrate; (ii) chemical compounds, i.e., inhibitors and/or promoters; and (iii) pretreatment effects, i.e., the extent of lignin and xylan removal, lignin structure, pore volume, or accessible surface area [66]. The interaction between cellulosome and substrate-related factors is shown in Figure 3. The key parameters involved in the hydrolysis system may include two major functions (i.e., substrate-cellulosome interaction and cellulolysis reaction), eight mechanisms (grey circles), and their corresponding biological factors (white circles). Since it is nearly impossible to obtain a homogenous substrate that containing particles in the same size, it is difficult to get a pure cellulosomal enzyme expressed in the same level. All these substrate-related factors are interconnected and any single alteration would affect the others. The performance of pretreatment can directly affect the product yields and kinetics of the downstream processes, i.e., substrate hydrolysis. Some mechanisms of delignification for different pretreatment techniques have been confirmed while many questions still remain unanswered, which is mostly due to high complexity of lignin structure [67,68].

The substrate-related factors are the most sensitive and representative parameters include the substrate accessibility to cellulase [69], enzyme-additive interactions [70], crystallinity [71], and others [66,72].

Figure 3. Interactions between cellulosome and substrate-related factors.

3.1. Effects of Different Carbon Sources

Extracellular carbon sources affect the assembly of the cellulosome by regulating its enzymes and structural compositions, thus ensuring the present of optimal factors to break down the available carbohydrates [73–75]. Although approximately 90% of native or treated cellulosic substrates can be degraded by the appropriate cellulosomes, there are still many challenges for effective hydrolysis of different carbon sources by the cellulosome enzyme complexes [76,77].

Several previous studies initially demonstrated alterations of cellulosome compositions upon cultivation of the cellulosome producers on different carbon sources. Han et al. [78] cultured C. cellulovorans ATCC 35296 anaerobically in medium containing 1% (w/v) of Avicel, xylan, pectin and mixed polysaccharides (Avicel/xylan/pectin (3:1:1, by wt)) as carbon substrates, respectively. As a result, the cellulosome population was observed heterogeneously, although the scaffolding protein CbpA, endoglucanase EngE, and cellbiohydrolase ExgS were relatively constant. The cellulase activity was promoted by cellulosome contained CbpA, EngE/EngK, ExgS/EngH, and EngL in cells grew on a...
mixture of carbon sources, while high xylanase activity was detected in cellulosomes derived from cellulose, pectin and mixed carbon, which had larger amounts of XynB, XynA, and unknown proteins (35–45 kDa). These results indicated that the ratio of cellulosomal subpopulations in \textit{C. cellulovorans} was controlled by its autogenous regulatory system that make up the cellulosomal population.

Similarly, the cellulosome assembly of \textit{C. thermocellum} strain DSM 1313 was examined for its response to available sole carbon sources, i.e., glucose, cellobiose, microcrystalline cellulose, alkaline-pretreated switchgrass, alkaline-pretreated corn stover, and dilute acid-pretreated corn stover [79]. Different catalytic and structural subunits (scaffoldins) were finally investigated in the different cellulosome samples. Cellulosomes derived from microcrystalline cellulose and glucose exhibited higher endoglucanase-to-exoglucanase ratios, as well as catalytic subunit-per-scaffoldin ratios compared to the lignocellulose-derived cellulosome types. The results verified glucose- and microcrystalline cellulose-derived cellulosomes were more efficient in their action on carbon sources than other cellulosome samples. Curiously, compared with the cellulosomes of strain \textit{C. cellulovorans} ATCC 35296 grew on carbon sources such as Avicel, xylan, AXP (Avicel–xylan–pectin, 3:1:1), and cellobiose, the enzyme compositions between Avicel and cellobiose culture were similar and that almost no repression of cellulase enzymes when cells grew on cellobiose [63].

On the other hand, in order to understand the synergistic relationship between cellulosomes and noncellulosomal (hemi)cellulolytic enzymes, changes in mRNA and protein expression were examined with cultures of \textit{C. cellulovorans} ATCC 35296 grew on cellobiose, cellulose, pectin, xylan, and corn fiber or mixtures, respectively [80]. Expression profiles of both the cellulosome and noncellulosomal enzymes were strongly affected by different carbon sources, whereas cellulosomal proteomes were more affected by the carbon source as compared to noncellulosomal enzymes. Furthermore, Fierobe et al. [81] compared hydrolysis effects of \textit{C. cellulolyticum} cellulosome and free enzyme systems on recalcitrant substrates and tractable substrates. For the recalcitrant cellulose–Avicel, the presence of a CBM on scaffoldin and enzyme proximity on the organization of cellulosome chimeras contributed almost equally to the elevated action on the recalcitrant substrate, whereas the cellulosome chimeras exhibited little or no advantage over free enzymes on the tractable substrate–bacterial cellulose.

Recently, cellulosomes displayed on the cell surface was compared between cells grew on soluble or recalcitrant insoluble substrates by using \textit{C. clariflavum} [82]. According to immunolabeling of four cellulosome components: ScaA, ScaB, ScaC, and the most prominent enzyme, GH48, the results explored that the cellulosome producer required closely attached cellulosomes on its surface to break down the highly recalcitrant substrates. How these specific variations occur in response to the available carbon sources? One possibility is that the substrate-induced enzyme expressions determine the amounts of the various cellulosomal enzymes during the cellulosome assembly [77,80,83–85]. The other may cause by the specific interactions between the dockerins and their cognate cohesins [10,65,86]. Since certain cohesins can bind to enzymes by the dockerins that are absent in other cohesins [10,15], the cellulosomal composition may vary with the enzyme expressions and the interactions of different cohesin–dockerin pairs. Moreover, Nataf et al. [87] revealed the cellulosomal regulatory mechanism at the genomic level. They suggested the cellulosomal genes were regulated via an extracellular sensing mechanism, in which alternative $\sigma$ factors (i.e., $\sigma^{11}$ or $\sigma^{16}$) were activated in response to the carbohydrates in the extracellular surroundings.

It is known that the accumulation of carbon monomers such as glucose, cellobiose, as well as some other end products of hydrolysis will inhibit cellulases and decrease glucose yields [88–90]. In contrast to aerobic cellulase, kinetics studies related to cellulosome are quite limited owing to the intricate structure and catalytic mechanism. Lin et al. [91] described the utilization of recombinant anchoring cellulosome from \textit{B. subtilis} W800N strain to degrade the \textit{Chlorella} lipid-deprived residues. The kinetics parameters of maximum reaction rate ($V_{\text{max}}$) and the Michaelis–Menten constant ($K_{\text{m}}$) values displayed a prevailing effect when the cellulosome obtained in the supernatant as compared to
the whole cells. Recently, a mathematical model was developed to estimate the inhibitory effect of glucose on cellulosome by using \textit{C. thermocellum} (Equation (1)) [92]

\begin{equation}
C = K - \frac{KA}{v}
\end{equation}

where \(C\) was the glucose concentration, \(K\) was the inhibition constant for glucose on cellulosome, \(v\) was the rate of the hydrolytic action of cellulosome, and constant \(A\) could be deduced from the slope of the straight line, which plotted based on \(C\) versus \(1/v\). It described the relationship between glucose concentration and saccharification rate at a specific glucose concentration or a specific time. Glucose accumulation in a long term is independent to the saccharification rate at a specific time. Hence, methods that can decrease the glucose-induced inhibition on cellulosome should be effective in enhancing cellulose saccharification by the anaerobic cellulosome-producing bacteria [93]. Attempts to eliminate side reactions such as ethanol and CO\(_2\) fermentation proved the utilization of certain adsorbents (i.e., activated carbon and biochar) could lower the inhibition of glucose and improve the adsorption of substrates onto cellulosome [92].

3.2. Effects of Different Chemical Compounds

The CBP efficiency can potentially be improved by optimizing cellulosome activity and/or creating the synergy between cellulosomic saccharification and the subsequently fermentation. Various compounds generated by the pretreatment process or derived from the fermentation by-products usually are inhibitory to cell growth and fermentation activity [94–96]. These chemical compounds exist in the substrates significantly affect the cellulosome-induced biorefineries based on lignocellulosic biomass. Amongst these, the cellulosome activity of wild-type strain was inhibited by ethanol concentrations above 2\% (\(v/v\)), whereas those evolved strains remained viable when ethanol concentrations increased up to 8\% (\(v/v\)). Compared with commercial enzymes, \textit{C. thermocellum} cellulosomes were generally able to tolerate higher ethanol concentrations [97]. In addition, in regard to the inhibitors released during the pretreatment of lignocellulosic biomass, typically, furfural and phenols are considered unfavorable side-products owing to their inhibitory effects on cell growth [98,99]. The \textit{C. thermocellum} cellulosomes demonstrated tolerance on certain concentrations of furfural (\(\leq 5\) mM), \(p\)-hydroxybenzoic acid (\(\leq 50\) mM), and catechol (\(\leq 1\) mM), respectively [97].

Parsiegla et al. [100] studied the chemical structure of the thiooligosaccharide methyl 4-S-\(\beta\)-celllobiosyl-4-thio-celllobioside (IG4), which performed as an inhibitor to the cellulosome of \textit{C. cellulolyticum}. The orientation of the inhibitor molecules Inh1 and Inh2 was consistent with a processive action towards the non-reducing ends from the reducing ends of the cellulose chains. Moreover, You et al. [101] assembled a cellulosome-microbe complex ex vivo on the \textit{Bacillus subtilis} surface, which displayed a mini-scaffoldin bound with three dockerin-containing cellulase components, i.e., endoglucanase Cel5, processive endoglucanase Cel9 and cellobiohydrolase Cel48. The hydrolysis performance indicated that high concentration cellodextrins in the boundary layer would inhibit cellulosome activity more strongly than short chain products because the \(\beta\)-glucosidase without a CBM usually works in the bulk phase [102]. Therefore, cellulosomes that expedite the cellulose bioconversion rate can help to construct CBP microorganisms with improved performance, which is expected to hydrolyze recalcitrant substrate efficiently under low secretory cellulase levels.

On the other hand, the organic acids almost occur as products or by-products in microbial fermentation [103,104], in which both pH changes and anion accumulations occur in the bioreactor. The change in pH will drastically alter cellulosome capability for cellulose digestion [105–107]. In order to determine the effects of organic acid anions on cellulosome-induced cellulose hydrolysis, the cellulosomal enzyme activities of \textit{C. thermocellum} JYT01 were investigated in the presence of formate, acetate, and lactate [97]. Interestingly, although these anions inhibited the cell growth, at the same time these acted as promoters to cellulosome activity at a concentration of formate, acetate, and lactate below 100, 200, and 50 mM, respectively, while negative effect was only observed beyond their critical
concentrations. As a result, the promoted Avicel hydrolysis was achieved by supplementing exogenous organic acid anions in a living-cell culture. It presumed that the active domains of certain cellulosome harbor a moiety for specific anion-binding, and -promoting substrate recognitions in the presence of cellulose–anion compounds [97,108].

3.3. Effects of Pretreatment

Lignocellulosic biomass with only exterior surface is not applicable for the microbial digestion, owing to its low accessible surface areas. Pretreatment is considered crucial for valorization of lignocellulosic biomass into value-added bioproducts. The direct physical contact between the cellulose producers and lignocellulosic surfaces are necessary to start the biocatalysis. Since pretreatment operations change several decisive factors concurrently, and it is hard to predict its effectiveness directly [66,109]. In fact, the effectiveness of pretreatment is usually evaluated by enzymatic hydrolysis or merely based on the yields of target products by fermentation [66]. Generally, the pretreatment process varies depending on the type of lignocellulosic biomass and there is no standalone method can be applied for all feedstock, because this varies with the type of natural biomass [110]. Currently, several available techniques have been developed to remove lignin from lignocellulosic biomass, i.e., acid pretreatment (such as organosolv or sulfite) and alkaline pretreatment (such as ammonia or NaOH). Table 1 compares the effect of different pretreatment on lignin structure and enzymatic hydrolysis. To the best of our knowledge, no special class of cellulases appear in cellulosomes because most of the cellulosomal enzymes belong to the same set of enzyme families as those of free cellulases. The understandings of free enzymes’ efficiency should provide a reliable foundation to evaluate the effects of pretreatment on cellulosomic catalysis.

| Changes after Pretreatment | Effect of Pretreatment | Enzyme Efficiency | Feedstocks | Reference |
|----------------------------|------------------------|-------------------|------------|-----------|
| Depletion of lignin content| Increase accessible surface area and porosity of substrate | Enhanced | Corn stover, sugarcane bagasse, *Eucalyptus globulus* | [111–114] |
| Formation of COOH | Reduce surface tension and increase electrostatic repulsion between lignin and enzymes | Enhanced | Aspen, corn stover, poplar, lodgepole pine | [115–118] |
| Sulphonation | Reduce surface tension and non-productive enzyme binding | Enhanced | Poplar, lodgepole pine, Norway spruce, black cottonwood | [118–120] |
| Alkoxylation of aliphatic side chains | Block lignin condensation | Enhanced | Beech | [121] |
| Reduced surface coverage by lignin | Increase porosity and surface area | Enhanced | Wheat straw, several wood and grass species *Eucalyptus globulus*, red maple, loblolly pine, mixed hardwood, aspen, bamboo | [122–124] |
| Formation of condensed units | Adsorb more enzymes due to hydrophobicity | Reduced | *Eucalyptus globulus*, red maple, loblolly pine, mixed hardwood, aspen, bamboo | [117,124–126] |
| Formation of phenolic OH | Hydrophobicity and hydrogen bonding | Reduced | Technical lignins, aspen, poplar, pine, bamboo, mixed hardwood, barley straw | [115,117,118,125] |
| Removal of aliphatic OH | Form more condense lignin | Reduced | *Eucalyptus globulus*, red maple, loblolly pine, mixed hardwood | [115,126] |
| Increased hydrophobicity | Adsorb more enzymes | Reduced | Poplar, lodgepole pine, bamboo | [118,125] |
| Formation of resinous products | Adsorb more enzymes | Reduced | Wheat straw | [127,128] |
Degradation of lignocellulosic biomass usually involves three steps: (i) enzyme adsorption to the substrate surface, (ii) hydrolysis of the substrate, and (iii) desorption of the cellulase into the liquid [129]. Similar to the microbial degradation, the lignocellulosic pore volume or accessible surface area for the cellulosomic enzymes is among the most affecting factors to the lignocellulose hydrolysis rate and yield. It means that once the diffusion of an enzyme molecule into a pore, the size of the enzymatic component should not be equal to the size of the pore owing to the wall confinement [130]. Moreover, when the pore size of lignocellulosic substrate is narrow, then β-glucosidase would not accompany other groups of cellulosomic cellulases into the pore. In other words, more spaces for synergistic actions between the different groups of cellulosomic cellulases are crucial for efficient hydrolysis of lignocellulosic biomass [66,130,131].

On the other hand, pretreatment is actually an important process to increase the surface area of substrate available for cellulosome. The total accessible surface area of lignocellulosic biomass is the sum of its external and internal surfaces, among which the external surface area depends on the size and shape of the material, while the internal surface area depends on its pore size and distribution [66,132]. Although higher than 90% of the sieved Avicel surface is accessible to free enzymes when it is in an average diameter of 100 µm [133], the large size of cellulosomes will prevent them from accessing many pores of the internal surface area. The presence of multiple enzymes on the cellulosomes can compensate for this limitation of cellulosomes to attack the binding sites in the pores [17,134]. Hence, the digestibility of lignocellulose for cellulosomes is significantly affected by the factor of accessible surface area, which will be gradually increased with the enzymatic hydrolysis caused by the removal of partial cellulose and hemicellulose. Besides the surface area, it is noticed that the cellulosomic hydrolysis rate also depends on the hydrolysis stages [79,135]. The rate of hydrolysis is normally rapid at the beginning stage and it becomes considerably slower during the latter stage, despite the availability of higher surface area. The slower hydrolysis rate should be a result of the higher crystallinity regions of the substrates, deactivation of the hydrolytic enzymes, and the increasing concentrations of the lignin [136,137].

In an attempt to increase the adsorption and hydrolysis rate of cellulosomal enzyme, Moraís et al. [138] observed the effects of reduced recalcitrance on wheat straw degradation by using native and designer cellulosomes, respectively. Actions of cellulosomes were estimated either directly following the size reduction by mechanical treatment or an additional pretreatment by sodium hypochlorite to reduce the lignin content in order to promote enzymatic hydrolysis. The result without chemical pretreatment demonstrated that there was no significant effect on lignin content of the wheat straw substrate when utilized both the native and designer cellulosomes. Thus, although microbial enzymes demonstrate ability to solubilize lignin and increase the cellulase access to cellulose [139,140], the cellulosomes hardly reduce cellulose content and/or decompose hemicellulose without prior pretreatment of the lignocellulosic substrate. However, it was reported that the chemical pretreatment of lignocellulose before enzymatic digestion usually generated lignocellulose-derived by-products such as phenolic compounds that would further inhibit the enzymatic saccharification [94,141]. To overcome this barrier, Davidi et al. [142] constructed a cellulosome with extra enzyme activities on lignin. The resultant chimera finally increased two-fold of the reducing sugars derived from wheat straw compared with the designed trivalent cellulosomes lacking the laccase, which can catalyze the oxidation of various phenolic and nonphenolic compounds [142,143].

4. Conclusions

Lignocellulosic biomass is a renewable resource with great potential to facilitate important bioproducts conversion by CBP in the context of biorefineries. However, the low rates and high costs of lignocellulose decomposition are the main barriers to commercialization of this biological conversion processes. To address these barriers, one significant area of heightened research activity is the study of naturally occurring cellulosomes produced by certain anaerobic bacteria. Numerous
cellulosome-related investigations have been confirmed at the molecular level such as the structures and functions of cellulosomes, as well as gene modifications to enhance the biocatalysis of the enzyme complex, although the understanding of interactions between cellulosomes and their lignocellulosic substrate are still limited.

In this review, the current understanding relating to several substrate-related physical and chemical factors affecting the activities of cellulosomes are summarized. Different carbon sources play significant impacts on the cellulosomal assembly by regulating the expression of enzyme activities and structural compositions. In addition, cellulosomic enzyme adsorption or desorption is an important biological parameter related to the degradation of the lignocellulosic substrates. Substrate accessibility is another crucial parameter of the lignocellulosic substrate, which is a desirable factor for all pretreatments. External surface area of the lignocellulosic biomass can be increased by the physical size reduction as well as changing of the particle shapes, while increase in the internal surface of the substrates should be followed by typical chemical or even biological pretreatments. Therefore, special attention should be paid to the pretreatment methods utilized for valorization of the lignocellulosic biomass into bioproducts prior to the cellulosomal catalysis.

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