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Review Article

ADDITIVES FOR CELLULASE ENHANCEMENT

Eugene M. Obeng[1], Chan Yi Wei[1], Siti Nurul Nadzirah Adam[1] and Clarence M. Ongkudon[1, 2*]

[1]Biotechnology Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
[2]Energy Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
*Corresponding author's email: clarence@ums.edu.my
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ABSTRACT

Cellulases have been vital for the saccharification of lignocellulosic biomass into reduced sugars to produce biofuels and other essential biochemicals. However, the sugar yields achievable for canonical cellulases (i.e. endoglucanases, exoglucanases and β-glucosidases) have not been convincing in support of the highly acclaimed prospects and end-uses heralded. The persistent pursuit of the biochemical industry to obtain high quantities of useful chemicals from lignocellulosic biomass has resulted in the supplementation of cellulose-degrading enzymes with other biological complementation. Also, chemical additives (e.g. salts, surfactants and chelating agents) have been employed to enhance the stability and improve the binding and overall functionality of cellulases to increase product titre. Herein, we report the roadmap of cellulase-additive supplementations and the associated yield performances.

Keywords: cellulases, hemicellulases, laccases, LPMOs, salts, surfactants

INTRODUCTION

Lignocellulose is an important cellulosic feedstock for producing bulk biochemicals and other value-added products due to its abundance, renewability and sustainability (Ramawat & Mérillon, 2015). The use of lignocellulosic biomass has received soaring attention within the last few decades because the ensuing products are believed to be a potential replacement of fossil fuels and fossil-based chemicals. For instance, unlike fossil fuels, (ligno) cellulosic biofuel putatively contribute no net increase in carbon

dioxide concentration in the atmosphere; thus, becoming beneficial in the quest to mitigate climate change and global warming (Balan, Bals, Chundawat, Marshall, & Dale, 2009; Vassilev, Baxter, Andersen, & Vassileva, 2010).

Lignocellulose has a complex structure consisting of inner cellulose (30 – 45%) wrapped by a sheath of hemicellulose (15 – 30%) and lignin (12 – 25%) (Parisutham, Kim, & Lee, 2014), but varies in composition based on the type of species, growth process, growing conditions, age and geographical source of the biomass (Magalhães da Silva, da Costa Lopes, Roseiro, & Bogel-Łukasik, 2013; Vassilev et al., 2010). The holocellulose (hemicellulose plus cellulose) content has been the main source of the substrate to produce vital biochemicals. The process requires a consortium of enzymes, called cellulases, to systematically breakdown the substrate into reduced sugars for specific essential applications (e.g. biofuels) (Chandel & Silvério da Silva, 2013). Specifically, endoglucanases, exoglucanases and β-glucosidases are the three basic cellulases required for cellulose depolymerization.

The cellulase industry has witnessed recommendable improvements in terms of multiplicities in cellulase sources. Different sources, for example, fungi, bacteria, protozoans and even plants and animals, have shown potential for cellulase production (Kim & Kim, 2012). However, the fraternity still faces challenges on stability (thermal and pH) and catalytic efficiency. Remarkably, several attempts have been pursued to enhance the activity of cellulase. Some of these attempts have focused on the pre-treatment of the biomass (Agrawal et al., 2015; Jia et al., 2015; Pandiyan et al., 2014), cellulase engineering (Bommarius, Sohn, Kang, Lee, & Realff, 2014; Ito, Ikeuchi, & Imamura, 2013; Kim, Chokhawala, Nadler, Blanch, & Clark, 2010; Lee, Chang, Jeng, Wang, & Liang, 2012), and the supplementation of the cellulose depolymerization process with additives (biological and non-biological).

Herein, we discuss the roadmap on the enhancement of cellulases for lignocellulose saccharification in the perspective of cellulase supplements and additives. More importantly, we discuss how some of these supplements/additives enhance the functionality of the three basic cellulases toward achieving high titer of reduced sugars to produce essential commodities. To improve comprehensibility, we commence the discussion with a brief description of the structure and nature of the key cellulases. A comprehensive review of these enzymes has been published by Bhat (2000).

CELLULASES

Cellulases are enzymes capable of hydrolysing the β-1,4-glycosidic linkages within the complex cellulose structure to yield reduced sugars such as glucose. The substrate of these enzymes, the cellulose, is a homopolymer which is composed of repeated units of D-glucose monomers linked together by β-1,4-glycosidic bonds. Cellulases

are carbohydrate-degrading enzymes – a type of glycoside hydrolases (GHs) – which commonly possess a carbohydrate-binding module (CBM) to ensure substrate targeting; a catalytic module (CM) to cleave the β-1,4-glycosidic bond; and other types of essential modules such as FN3-like modules (Davies, Gloster, & Henrissat, 2005; Garvey, Klose, Fischer, Lambertz, & Commandeur, 2013; Moraïs et al., 2012). The non-catalytic modules of the multi-modular structure of cellulases frequently assist in protein-protein and protein-carbohydrate interactions (Bommarius et al., 2014).

There are three (3) basic cellulases (namely: endoglucanases, exoglucanase and β-glucosidases) which have been identified to ensure the conversion of cellulose into the glucose monomers. These enzymes differ structurally and functionally, but their catalytic mechanism follow the classical acid-catalyst hydrolysis model, employing two critical glutamate residues which function as a proton donor and a nucleophile, respectively (Garvey et al., 2013; Isorna et al., 2007). The two amino acid residues facilitate the hydrolysis of glycosidic linkages through the retention and/or inversion of the anomeric carbons within the polysaccharide structure (Koshland, 1953).

Endoglucanases (E.C.3.2.1.4) are the most abundant GHs; they are structurally characterized with short loops having well-defined, open active site clefts and show affinity toward amorphous site along cellulose chains (Juturu & Wu, 2014; Wilson, 2015). In contrast to endoglucanases, exoglucanases have long loops that form a tunnel-like structure around the catalytic residue but have an affinity for crystalline sites within the cellulose matrix (Juturu & Wu, 2014). Functional elucidations have led to the discovery and classification of two distinct exoglucanases, namely: the reducing end (E.C.3.2.1.176) and non-reducing ends (E.C. 3.2.1.91) exoglucanases. The classification is based on the portion of the polysaccharide chain (i.e., reducing end or non-reducing end) each enzyme favourably attacks (Wahlström, Rahikainen, Kruus, & Suurnäkki, 2014). However, the two enzymes are complementary and processive. Based on performance, endoglucanases exhibit the most rapid dissociation with the greatest liquefaction that leads to cellulose depolymerization and viscosity reduction (Boyce & Walsh, 2015). The work of endo- and exoglucanases in cellulase cocktails is considered as the rate-determining step ensuring effective cellulose depolymerization (Chandel & Silvério da Silva, 2013). According to Luterbacher, Walker, & Moran-Mirabal (2013), the rate of limiting effect increases the surface area of the substrate and exposes a new binding site for successive enzymes to cleave. Notably, feedback inhibition – a condition where the product of the enzyme impedes the enzyme itself – has been a crucial challenge of endoglucanases and exoglucanases (Van Dyk & Pletschke, 2012); thus, the need to synergize their activity with β-glucosidases.

β-glucosidases (EC 3.2.1.21) have a rigid active site that resides in a large cavity (i.e., the active site pocket) known to permit the entry of disaccharides (Nam, Sung, & Hwang, 2010). Notably, some β-glucosidases are also able to break down soluble cellodextrins with a degree of polymerization ≤ 6 (González-Candelas, Aristoy, Polaina, & Flors, 1989; Zhang & Lynd, 2004). The cavity is surrounded by four hydrophobic loops of different conformations which facilitate substrate binding (Czjzek et al., 2000; Nam et al., 2010). The specificity of β-glucosidases is influenced by the loops, which are potentially stabilized by hydroxyl groups, either from substrate or water (Isorna et al., 2007; Nam et al., 2010). β-glucosidases are categorized into two sub-families, viz. sub-family A (e.g. plant and non-rumen prokaryotic sources) and sub-family B (fungal and rumen bacterial sources) (Park et al., 2011). Similar to endo- and exoglucanases, β-glucosidases also experience glucose feedback inhibition – a challenge that has led to the discovery of glucose tolerant derivatives (Das et al., 2015; Günata & Vallier, 1999; Rajasree, Mathew, Pandey, & Sukumaran, 2013; Riou, Salmon, Vallier, Günata, & Barre, 1998). *Trichoderma* and *Aspergillus* species have been key sources of primary cellulases (endo- and exocellulases) and β-glucosidases, respectively (Brijwani, Oberoi, & Vadlani, 2010; Gottschalk et al., 2010; Gutierrez-Correa, Portal, Moreno, & Tengerdy, 1999; Wang, Bay, Chew, & Geng, 2014).

Functional elucidations have theorized that endoglucanases begin the deconstruction process by hydrolyzing cellulose at amorphous sites into long-chain oligomers (e.g., cellodextrin); exoglucanases attack the long-chain oligomers at the crystalline regions from either reducing or non-reducing ends to yield short-chain oligomers (e.g. cellobiose, cellotriose, cellutetrose, etc.) and β-glucosidases complete the breakdown process by converting cellobiose to D-glucose (Juturu & Wu, 2014; Segato, Damásio, de Lucas, Squina, & Prade, 2014). The overall process involves a series of adsorption and desorption, and it is governed by synergism, cooperativity and substrate channelling (Wilson, 2009).

ADDITIVE EFFECT ON CELLULOSE HYDROLYSIS

The complex structure of lignocellulosic biomass, even after pretreatment, requires a multitude of enzymes in conjunction with the canonical cellulases for effective degradation (Chundawat, Beckham, Himmel, & Dale, 2011). For instance, the supplementation of cellulases with other enzymes of relevant activities and the inclusion of enzyme-activity-enhancing chemicals to ensure most of the saccharides in the biomass are converted to their reduced and fermentable forms are common practices. The supplementary enzymes and accessories play a complementary role in effective biomass bio-depolymerization (Gao et al., 2011). The role and benefits of some of the biological and chemical additives commonly reported in the literature are discussed as follows:

Biological Supplements

Hemicellulases

Hemicellulases are enzymes responsible for the breakdown of the hemicellulose sheath linking core cellulose and the outward lignin of the cell wall of plants. The hemicellulose substrate is the second most abundant plant polymer after cellulose (Peng & She, 2014; Rubin, 2008), and consist of easy-hydrolysable compounds including pentoses (e.g., arabinose and xylose), hexoses (e.g., mannose, galactose and glucose), and sugar acids (Hendriks & Zeeman, 2009; Imman, Arnthong, Burapatana, Laosiripojana, & Champreda, 2013). Generally, hemicellulases share common functionality with cellulases, in that they hydrolyze the β-1,4-glycosidic bonds within hemicellulose (Chang et al., 2011). Hemicellulases are also GHs and possess CMBs and other functional modules which support the functionality of the catalytic domains (CDs). Some CDs exhibit carbohydrate esterase (CE) functionality instead of the common GH-functionality (Shallom & Shoham, 2003). The GH-type catalytic domain hydrolyzes glycosidic linkages whereas CE-type hydrolyzes ferulic acid side groups or ester bonds of acetate (Shallom & Shoham, 2003). Xylanases, xylosidases, and arabinofuranosidases are the most common hemicellulases essential for biomass depolymerization (Ratanakhanokchai, Kyu, & Tanticharoen, 1999). However, mannanases, glucuronidases and esterases are also hemicellulases with distinct activities.

Cellulases and hemicellulases complementarily affect the degree of polymerization of the cellulosic substrate, resulting in a high level of sugar monomers (Pala, Mota, & Gama, 2007). Hemicellulase activity on its feedstock clears the way for cellulases to attack the core cellulose (Doi, 2008). According to Gao et al. (2011), more than 80% of the theoretical glucose yield is achievable using an optimized blend of cellulases and hemicellulases. Gao et al. (2014) compared the yield of reduced sugar (i.e., glucose and xylose) from corn stover (CS) pretreated by ammonium fibre expansion (AFEX), dilute acid (DA) and ionic liquid (IL) with and without hemicellulase supplementation. For IL-CS, they reported 88% glucose and 53% xylose yields in the presence of hemicellulases as against 82% glucose and 12% xylose yields in the absence of hemicellulases within 48 h. The hemicellulase-assisted hydrolysis of AFEX-CS resulted in 99% glucose and 55% xylose yields as against 84% glucose and 10% xylose yields for the hemicellulase-devoid system in 48 h. Lastly, the DA-CS gave close to 97% glucose and 68% xylose yields in the presence of the synergistic hemicellulase supplementation as compared to 88% glucose and 28% xylose yields for raw cellulase cocktail in 48 h. The hemicellulase helped in relieving bound cellulases from the substrate and that led to an improved recovery (Gao et al., 2014). Similar elucidations have been reported for steam-pretreated CS and hybrid poplar (Bura, Chandra, & Saddler, 2009) and barley straw (García-Aparicio et al., 2007). Notably, the glucose released in the presence of hemicellulases has a direct linear relationship with the concurrent release of xylose (Kumar & Wyman, 2009).

Laccases

Laccases (EC 1.10.3.2) are multicopper oxidases responsible for the one-electron oxidation of various feedstocks, including phenolic and non-phenolic subunits of lignin (Chandel, Gonçalves, Strap, & da Silva, 2015; Dwivedi, Singh, Pandey, & Kumar, 2011; Lahtinen et al., 2009). The active sites of laccases have four copper atoms viz. Type-1 (blue copper centre), Type-2 (normal copper) and Type-3 (coupled binuclear copper centres) located at three different centres (Dwivedi et al., 2011). The copper atoms oxidize cellulose substrates at C-1, C-4, and C-6 atoms positions (Segato et al., 2014). It is worth noting that the functionality of laccases is enforced by electron transfer and hydrogen atom mediators. Lignin peroxidase (EC 1.11.1.14) and manganese-dependent peroxidase (EC 1.11.1.13) have also been identified to oxidatively attack phenolic and non-phenolic aromatic lignin moieties (Manavalan, Manavalan, & Heese, 2015; Wan & Li, 2012).

The principal substrate of laccases is lignin, which is a biopolymer composed of mixed phenylpropanoid units (Meyer, Lupoi, & Smith, 2011). For that matter, the supplementation of canonical cellulases with laccases could take care of the lignin residues which remain after the pretreatment lignocellulose. This is necessary because residual lignin in pretreated biomass directly inhibits and impedes the movement of the cellulases along the cellulose chain (Berlin, 2013; Zhang & Lynd, 2004). According to Moilanen, Kellock, Galkin, & Viikari (2011), laccases are capable of liberating trapped cellulases from unproductive adsorption. Moreover, laccases could potentially address phenolic compound inhibition of cellulases during biomass hydrolysis. For instance, Hyeon et al. (2014) obtained a 2.6 fold increase in the reduced sugar yield upon the involvement of laccases in the saccharification of pretreated barley straw. Moilanen et al. (2011) also reported a 12% increase in hydrolysis yield from pretreated spruce, using laccases and commercial cellulases. Furtado, Ribeiro, Lourenzoni, and Ward (2013) and Ribeiro et al. (2011) have also demonstrated the synergy and associated catalytic performance improvement when laccases are fused to other enzymes for biomass hydrolysis.

Lytic Polysaccharide Mono-oxygenases (LPMOs)

Lytic polysaccharide mono-oxygenases (LPMOs) are a recent discovery in the lignocellulose depolymerization pathway. Intriguingly, their inception has been vital in the understanding of how saprophytes breakdown biomass for their energy demands. LPMOs were previously thought of as being endoglucanase due to their ability to exhibit weak endocellulase functionalities (Karkehabadi et al., 2008; Karlsson et al., 2001). However, modern structural and functional elucidations have necessitated their reclassification as auxiliary activity (AA) family enzymes.

Interestingly, the functional insights of LPMOs have challenged and reformed the classical concept of cellulose saccharification by canonical cellulases (Hemsworth, Davies, & Walton, 2013a). Therefore, some research has been geared at fully elucidating their functional distinctions and associated mechanisms to aid in their possible supplementation with cellulases.

Like laccases, LPMOs is also an oxidative enzyme. However, LPMS have a monomeric type II copper ions (Cu^{2+}) in the centre of the active sites for substrate interaction (Hemsworth et al., 2013b; Quinlan et al., 2011). The active site is positioned within an extended flat-face structure which is different from the common tunnel-shaped structures shielding the active sites of cellulases (Hemsworth et al., 2013a; Isaksen et al., 2014). The catalytic activity of LPMOs dwells on the binding of active atmospheric oxygen (O2) to the type II Cu^{2+} ion which then facilitates its interaction with C-1 and C-4 bonds along with the cellulose polymer (Hemsworth et al., 2013a; Walton & Davies, 2016). The presence of molecular oxygen, an external electron donor and possibly CBM is key for LPMO functionality. The external electron donor could be provided by residual lignin present within the cellulose matrix (Westereng et al., 2015).

LPMOs complement cellulases during the breakdown of cellulose by causing chain breaks (via oxidation reactions) in the cellulose matrix thereby improving the accessibility of cleavage sites to cellulases (Horn, Vaaje-Kolstad, Westereng, & Eijsink, 2012; Vaaje-Kolstad et al., 2010). In technical terms, the enzyme causes the abstraction of hydrogen atoms to aid in the cleavage of the bonds between the most accessible and most reactive C-H (i.e. C-1 and C-4) (Obeng et al., 2017). The strong synergism of LPMOs with other cellulases is believed to be due to their ability to attack highly crystalline and recalcitrant spots of cellulose where other enzymes cannot (Harris, Xu, Kreel, Kang, & Fukuyama, 2014). Jung, Song, Kim, and Bae (2015) reported an accelerated synergistic effect of 56 and 174% for the blend of LPMOs and cellulases on pretreated kenaf and oak, respectively. A similar observation (i.e. 60% more glucose) from LPMOs with Celluclast® on dry lignocellulosic biomass has been reported (Müller, Várnai, Johansen, Eijsink, & Horn, 2015).

Non-hydrolytic Accessory Proteins

Expansins and swollenins are the common non-hydrolytic proteins for lignocellulosic biomass deconstruction. They can loosen the cell wall of plants and alter the crystallinity of cellulosic material (Nakashima, Endo, Shibasaki-kitakawa, & Yonemoto, 2014). Expansins are plant proteins whereas swollenins are expansin-derivatives from fungi and bacteria. These proteins disrupt the hydrogen bonds within the cellulose structure to reduce the crystallinity thereby enhancing the cellulose accessibility for enzymatic attacks (Harris et al., 2014). Nakatani, Yamada, Ogino, and Kondo (2013)

reported a 2.9-fold increase in cellulase activity on phosphoric acid swollen cellulose (PASC) by co-displaying cellulase and expansin-like proteins on yeast cells. Also, Nakashima et al. (2014) reported a 35% increase in substrate digestibility by fusing endoglucanase with expansins.

Recently, there is also the practice of using non-enzymatic proteins such as bovine serum albumin (BSA), peptone, yeast extract, soybean protein and processing wastes from the meat, fish and milk industries as lignin blockers (Yang & Wyman, 2013). In simple terms, these proteinaceous materials help to either enhance cellulase adsorption or reduce unproductive adsorption of the cellulases onto lignin by interacting with (or "blocking") lignin. The blocking effect improves the accessibility of cellulases to cellulose to promote efficient cellulose depolymerization. Also, the inclusion of lignin blockers reduces the intensiveness of pre-treatment method (Wang, Kobayashi, & Mochidzuki, 2015), enzyme loading (Luo et al., 2019), and operation time (Brondi, Vasconcellos, Giordano, & Farinas, 2019); thus, improving the economics of biomass saccharification. For instance, Ko, Kim, Ximenes, and Ladisch (2015) supplemented cellulases with BSA for the hydrolysis of hydrothermally pre- treated hardwood. The work reported about a 72% increase in hydrolysis yield compared with 17% for saccharification without BSA. Similarly, Wang et al. (2015) tested the influence of BSA, peptone and yeast extract on the hydrolysis yield of pre-treated rice straw using different commercial enzymes. The work reported 14 – 20% increase in hydrolysis yield upon the inclusion of these blocking agents. Also, Luo et al. (2019) reported the supplementation of pre-treated lignocellulose with soybean protein reported an improved enzymatic conversion 40%, 30%, and 41% for eucalyptus, bamboo, and Masson pine, respectively. The inclusion of soybean protein reduced enzyme loading by 8 times. Similarly, Florencio, Badino, and Farinas (2019) obtained up to 86% improvement sugarcane bagasse hydrolysis. Seki et al. (2015) have also reported about 2.9-fold improvement in saccharification yield due to the effects of non-enzymatic proteins in cattle saliva on cellulose degradation.

Non-biological Additives

The common non-biological additives for enhancing cellulase performance include salts, surfactants and chelating agents. These chemicals provide cellulase activity improvement by either serving as metal cofactors, activators or stabilizers. The associated effects are enzyme, enzyme preparation and concentration dependent.

Salts and Chelating Agent

Many salts have been used in the literature to enhance the activity of cellulases. These salts are dominated by divalent cation-associated salts. However, the anion aspects of the salts have not shown their clear-cut functionality on cellulase enhancement to

date. KCl, $MnCl_2$, $CaCl_2$, $CuCl_2$, $MgCl_2$ and $ZnSO_4$ are some of the common salts being reported in the literature. Each of the metal cations has shown specific affinities for one cellulase compared to another, although the cations may have the same valency number. The discrepancies could have a link with the atomic radius of the cation and dimensions of the active site cavity; however, this is yet to be proved.

Table 1 Impacts of additives in cellulose hydrolysis

Enzyme(s)	Source	Additive	Relative performance	References
Endoglucanase	*Alicyclobacillus vulcanalis*	$CaCl_2$ (10 mM) $MgCl_2$ (10 mM) EDTA (2 mM) Tween 20 (0.1%) Triton X-100 (0.1%)	97% 86% 98% 124 % 124 %	Boyce and Walsh, 2015
Endoglucanase and xylanase fusion protein (Xyl10g GS Cel5B)	*Gloeophyllum trabeum*	$CoCl_2$ (1 mM) $CaCl_2$ (1 mM) $FeCl_3$ (1 mM) KCl (1 mM) LiCl (1 mM) EDTA (1 mM) NaCl (1 mM)	139% (115%) 101% (95%) 100% (115%) 100% (95%) 101% (95%) 91% (88%) 101% (97%)	Kim, Jung, Lee, Song, and Bae, 2015
Exoglucanase	*Rhizopus stolonifera*	$CaCl_2$ (1 mM) KOH (1 mM) $MgSO_4$ (1 mM) $ZnSO_4$ (1 mM) Fe_2Cl_3 (1 mM) NH_4Cl (1 mM) EDTA (1 mM) Tween 20 (1 mM) SDS (1 mM) Triton X 100 (1 mM)	160% 95% 126% 75% 100% 99% 130% 80% 70% 55%	Navya, Bhoite, and Murthy, 2012
β-glycosidase	*Alicyclobacillus acidocaldarius*	Mg^{2+} (5 mM) Mn^{2+} (5 mM) Ca^{2+} (5 mM) Zn^{2+} (5 mM) Co^{2+} (5 mM) Cu^{2+} (5 mM) Ni^{2+} (5 mM) Zn^{2+} (5 mM) Co^{2+} (5 mM) EDTA (5 mM)	Not significant Not significant Not significant 33% 96% Not significant Not significant Not significant Not significant Not significant	Di Lauro, Rossi, and Moracci, 2006

NB: The reported relative performances have been rounded to the nearest whole number. The figures in bracket refer to the concentration of xylose.

One key property of these salts is the ability to dissociate in solution to yield a dielectric strength capable of resisting pH fluctuation, thus preserving/improving the functionality and stability of the enzymes present (Suplatov, Panin, Kirilin, Shcherbakova, & Kudryavtsev, 2014). For instance, Ca^{2+} has been identified as having the ability to improve ligand binding and cellulase stability, and maintain the structural integrity of enzymes (Abou-hachem et al., 2002; Bolam et al., 2004; Jamal, Nurizzo, Boraston, & Davies, 2004). According to Warren and Cheatum (1966), the salts contribute to the enzyme enhancement by modifying the organized structure of the protein macromolecule.

On the other hand, chelating agents improve enzymatic activity by trapping and forming complexes with material (e.g., metal ions). This property may or may not be beneficial since some of these enzymes have inherent metal cations and other chelatable structures. The commonly used chelating agent is ethylenediamine tetraacetic acid (EDTA), and it is known for its metal ion scavenging abilities (Naika & Tiku, 2011). Fontes and Gilbert (2010) opined that EDTA hinders the interaction of dockerins with cohesins (both are facets of most enzyme structures) whereas Ca^{2+} proves essential for dockerin stability and function. Table 1 shows some of the reported impacts of these additives in cellulose hydrolysis.

Surfactants

Surfactants, for example, Tween, Triton and polyethene glycol, have been vital in cellulase enhancement procedures. Similar to lignin blockers, surfactants commonly function by improving the adsorption and desorption catalytic activity of enzymes in a way to improve enzyme mobility and prevent non-specific enzyme attachments (Helle, Duff, & Coopes, 1993; Tu, Zhang, Paice, Mcfarlane, & Saddler, 2009). The amphiphilic surface-active chemical potentially could alter the surface area and composition of cellulosic feedstock to improve the accessibility of cleavage point (Helle et al., 1993). It is worth noting that different surfactants also influence cellulase activity differently and this may be attributed to their polarity (i.e. ionic, non-ionic or zwitterionic), which by extension affects the binding modules of an enzyme. Polyethene glycol (PEG4000), for instance, has been reported to improve the activity of beta-glucosidases and endoglucanase by 20% and 60%, respectively (Rocha-Martín, Martinez-Bernal, Pérez-Cobas, Reyes-Sosa, & García, 2017). However, the associated cost concerns have to be addressed. Table 1 displays some of the reported benefits of common surfactants.

CONCLUSION

Several additives have a pronounced complementary effect on cellulase performance. The successful blend of cellulases, hemicellulases, lignases, accessory proteins and other additives in a way that will promote progressivity, synergism and non-competition are crucial for the cellulose-based industry. The challenge lies in proportionating these enzymes and supplementations in a manner that could function optimally to ensure the complete digestion of lignocellulosic biomass to simple sugars. However, with the current trend of cellulose hydrolysis research, the future of green products form lignocellulose still looks promising.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this work.

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