

# Enhanced enzymatic hydrolysis of cellulose from waste paper fibers by cationic polymers addition

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## ABSTRACT

Cationic polymers (cationic polyacrylamide (CPAM), polyethyleneimine (PEI) or cationic starch (CS)) were used to enhance the enzymatic hydrolysis of waste paper fibers (WPFs) at 15% (w/w) solids concentration. Results showed that 0.05 g/L PEI, CPAM and CS resulted in 72.5%, 65.9% and 59.7% conversion of WPFs, increased by 15.4%, 8.8% and 2.6%, respectively, compared with control (57.1%). PEI was shown to have a larger effect than CPAM and CS, and generate a total sugar concentration of 73.9 g/L. Improvement in hydrolysis with cationic polymer addition is attributed to increased cellulase adsorption on cellulose through electrostatic attraction, rather than enhancement of cellulase activity. A patching/ bridging mechanism of cationic polymer enhancement of cellulose adsorption in cellulose is hypothesized. PEI exhibited maximum cellulose binding for polymers examined and appears to promote binding through a patching mechanism. CPAM and CS adsorbed a relatively low cellulase through bridging mechanism. In addition, enzyme loading could be reduced by addition of cationic polymers to obtain the same glucose yield, especially when PEI was used.

## 1. Introduction

Production of bio-fuels (ethanol, butanol, etc.) from lignocellulosic materials is an important way to resolve the current energy and ecological crisis (Bharathiraja et al., 2017; Gao, Xiang, Chen, Yang, & Yang, 2015; Govumoni, Koti, Kothagouni, Venkateshwar, & Linga, 2013; Singh, Krishna, Kumar, & Bhaskar, 2016; Tan & Lee, 2015). Lignocellulose is composed mainly of cellulose, hemicelluloses and lignin. Enzymatic hydrolysis of cellulose and hemicelluloses into fermentable sugars is an important part of lignocellulose bioconversion into bio-fuels. Waste paper fibers (WPFs) are becoming a prospective raw material for producing fermentable sugars by enzymatic hydrolysis because they have high cellulose content, low cost and are readily available (Brummer et al., 2014; Dubey, Gupta, Garg, & Naithani, 2012; Subhedar, Babu, & Gogate, 2015). It has been regarded as beneficial from both a resource and an environmental perspective to produce sugars by enzymatic hydrolysis of their cellulose fractions (Adu, Jolly, & Thakur, 2018; Subhedar et al., 2015).

Studies of enzymatic hydrolysis of lignocellulose have mainly focused on operating at low substrate concentration ( $\leq 10\%$  (w/w)) (Sun, Sun, Cao, & Sun, 2016; Yang et al., 2015; Yu et al., 2015). However, the low sugar concentration after enzymatic hydrolysis is one of the bottlenecks for commercialization of biofuels due to the low ethanol

concentration in the fermentation broth, which increases the energy needed for biofuel recovery (Ramos et al., 2015; Zhao, Dong, Chen, & Liu, 2013). Many researchers have indicated that carrying out enzymatic hydrolysis at high solids loadings will be necessary to achieve high sugar concentrations (Mohamed, Ghazi, M Nejib, Mohamed, & Issam, 2016; Szabo & Csiszar, 2017). In theory, higher sugar concentration translates into higher ethanol concentration, and thus makes the bio-fuels production more economically feasible by reducing energy use associated with the distillation process (Kristensen, Felby, & Jørgensen, 2009). In addition, less process water produces less waste water (Hodge, Karim, Schell, & Mcmillan, 2008). Unfortunately, enzymatic hydrolysis at high solids loadings is facing technical problems. Lacking of free water is one of the major challenges during enzymatic hydrolysis. Water is important for the interaction between lignocellulose and enzymes since it is directly correlated with rheology, that is, to viscosity and shear rate during mixing (Hodge et al., 2008). Thus, lower water content can cause increased mass transfer limitations between reactants, products and enzymes with high solids concentration during enzymatic hydrolysis (Zhao et al., 2013).

Use of additives such as non-ionic surfactants (Li, Sun, Ge, & Zhang, 2016) and polymers (Wang, Liu, Yang, Chen, & Ni, 2015; Yang et al., 2015) can be an efficient way to improve enzymatic hydrolysis. Several additives have been screened for effectiveness in enhancing cellulose

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conversion, including cationic and nonionic polymers (Akimkulova, Zhou, Zhao, & Liu, 2016; Strobel, Pfeiffer, Blanch, & Clark, 2016). Non-ionic surfactants are a class of well-known additives for improving the enzymatic hydrolysis of lignocellulosic materials. Chen et al. reported that a nonionic surfactant of Tween-80 could enhance the enzymatic hydrolysis of fines from recycled paper mill waste rejects which had a high mineral content (Chen, Zhao, & Xia, 2008; Min, Bhayani, Jampana, & Ramarao, 2015). The proposed mechanism of action of Tween-80 in enhancing hydrolysis of paper waste is alleviation of the non-productive binding of enzyme with the higher affinity on minerals (Min et al., 2015). Nonionic additives could also improve the enzymatic hydrolysis of materials that have high lignin content through reduction of non-productive adsorption of cellulose onto lignin. Lou et al. reported that polyethylene glycol (AL-PEG1000) could enhance the enzymatic hydrolysis of a mixture of the cellulose (Whatman filter paper) and lignin with the ratio of 7:3 (Lou et al., 2013). Sipos et al. compared the enzymatic hydrolysis of different lignocelluloses by addition of nonionic additives, such as poly (ethylene glycol) (PEG) (Sipos et al., 2011). They found that the degree of enhancement differed among the substrates, being highest on steam pretreated spruce (33.6% of lignin content) with no effect for steam pretreated corn stover (20.3% lignin content). In addition, recent study showed that the nonionic surfactants did not improve the enzymatic hydrolysis efficiency at 0 and 100 rpm but could enhance the enzymatic hydrolysis significantly at high agitation rate (200 and 250 rpm) (Lou et al., 2018).

In this study, waste paper fibers had a relative low lignin content (18.8%), and the stirring rate was 100 rpm. At high solid concentration, it is difficult to improve the mass transfer by using high agitation rates. In addition, both of cellulase and cellulose have a negative charge that is expected to in repulsion and decreasing affinity between cellulose and cellulase enzymes (Wang et al., 2015). Therefore, cationic polymers have been recognized as a potential class of additives for improving the enzymatic hydrolysis of cellulose fibers. Lin et al. reported that the cationic surfactant cetyltrimethylammoniumbromide (C16TAB) increased the enzymatic hydrolysis of avicel linearly from 42.1 to 61.4% with increasing surfactant concentration (Lin et al., 2016). In the work of Guan et al., the digestibilities were significantly improved showing 20% increase for glucan, and 10% for xylan with addition of cationic polyacrylamide (CPAM) or poly-diallyl-dimethyl-ammonium chloride (PDADMAC) (Guan, Haynes, & Lee, 2014). Cationic polyelectrolytes such as cationic polyacrylamide (CPAM), polyethyleneimine (PEI), and cationic starch (CS) possess a positive charge and have been widely used in papermaking as effective retention aids based on their charge neutralization mechanism (Gaudreault, Cesare, Ven, & Weitz, 2015; Li et al., 2016). Taking this technical advantage, cationic polymers addition are expected to improve enzymatic hydrolysis of cellulose, especially for high solids concentrations.

In WPFs, the main component is cellulose, which is hydrophilic. During pulping process, the oxidation bleaching process was carried out, where the hydroxyl (–OH) and reducing end of cellulose and carbonyl of lignin will be oxidized to carboxyl (–COOH). Under the condition of enzymatic hydrolysis (pH4.8), the –COOH will ionize to –COO–, thereby resulting the negative charge of cellulose. The charge repulsion will affect the affinity between cellulose and cellulase. In view of the charge characteristics of cellulose fiber and cellulase, the addition of cationic polymers appears to neutralize the negative charge of cellulose, thereby increasing the cellulase binding capacity by electrostatic repulsion. In this study, we explored a way to best utilize WPFs as a bioconversion feedstock by adding cationic auxiliaries. Three types of cationic polymers (PEI, CPAM, and CS) were used during enzymatic hydrolysis at a high solids concentration of 15% (w/w). The effect of cationic polymer loading on enzymatic hydrolysis was investigated. The variations of cellulase adsorption capacity, zeta potential and atomic composition of WPFs and cellulase activity with and without cationic polymers were analyzed to reveal the mechanism responsible for the beneficial action of cationic polymers in enzymatic hydrolysis

applications.

## 2. Materials and methods

### 2.1. Materials and scanning electron microscopy (SEM) analysis

The waste paper fibers (WPFs) deinked with alkaline flotation was kindly provided by Huatai Group (Shandong, China). The fibers were washed to neutral pH with distilled water and then stored at 4 °C. The average contents of cellulose, hemicelluloses, lignin, and ash were 61.3%, 7.4%, 18.8% and 6.5%, respectively. WPFs were dried at 40 °C for 24 h prior to SEM analysis. Then they were coated with gold–palladium in a sputter coater (E-1010, HITACHI, Japan). The morphological structure was detected by scanning electron microscopy (SEM) (S-3400 N, HITACHI, Japan).

Cationic polyacrylamide (CPAM, molecular weight 1000 kDa), polyethyleneimine (PEI, molecular weight 5 kDa) and cationic starch (CS, molecular weight 100 kDa) were purchased from Sigma-Aldrich. CPAM was dissolved in deionized water at 2 g/L.

### 2.2. Enzymatic hydrolysis

The effect of cationic polymers on enzymatic hydrolysis of WPFs was investigated. The fibers were hydrolyzed by 18 FPU/g-cellulose for cellulase and 25 CBU/g-cellulose for  $\beta$ -glucosidase at 48 °C and pH 4.8 with a substrate consistency of 15% (w/w) in a 100-mL reactor made of polytetrafluoroethylene (PTFE), which was loaded into a large stainless steel tank after sealing. The system (PTFE reactor + stainless steel tank) was placed in an air oven equipped with a shaft that could be rotated at different speeds to ensure uniform mixing of the liquid and the substrate. The filter-paper activity of cellulase (Celluclast 1.5 L, Sigma) was 90 filter-paper units (FPU)/mL, and the cellobiase activity of Novozyme 188 (Sigma) was 175 cellobiase units (CBU)/mL. Before the hydrolysis experiment, the desired amounts of cationic polymers (0.05, 0.1, 0.2 and 0.3 g/L) were mixed well with cellulase for 30 min in a beaker. Then 10 g of WPFs was put into the PTFE reactor, followed by the cellulase-polymer mixture. After sealing, the PTFE reactor was rotated at 100 rpm. The effect of cellulase loadings on enzymatic hydrolysis of WPFs was also investigated under optimal cationic polymer dosages. The cellulase loadings were 9, 12, 15 and 18 FPU/g-cellulose. After hydrolysis, the slurry was mixed and left to equilibrate following addition of 20 ml water to dilute the glucose for 30 min and make the liquid easy to transfer.

The transferred liquids were withdrawn and centrifuged at 10,000 g for 5 min. Then the supernatants were filtered through 0.22- $\mu$ m filters and properly diluted for further sugar analysis. The filtrate was collected to detect glucose concentrations by HPLC (Waters 2695e, USA) using an Aminex HPX-87 P column (Bio-Rad, USA) at 85 °C and a refractive index detector at 35 °C. The glucose yield of the substrate was calculated from the glucose content as a percentage of the theoretical glucose available in the substrate. A cellulose:glucose ratio of 1:1.11 (g/g) was assumed.

### 2.3. Cellulase adsorption and surface analysis by X-ray photoelectron spectroscopy (XPS)

Adsorption of cellulases on WPFs was carried out in 100-mL conical flasks with different cationic polymer loadings. The substrate concentration was adjusted to 5% (w/w) by deionized water. The cellulase loading was 18 FPU/g-cellulose, which was similar to that used in enzymatic hydrolysis. The adsorption experiment occurred in a shaking incubator (150 rpm) at 4 °C for 2 h. At the end of cellulase adsorption, the liquid-solid mixture was centrifuged at 10,000 g for 10 min. The supernatants were filtered through 0.45- $\mu$ m filters for protein content analysis. Free cellulase was determined in accordance with the Coomassie Brilliant Blue (CBB) measurement. A control without any

enzyme was used for background correction. The difference between free and total protein was the bound cellulase.

Samples before and after cellulase adsorption with 0.2 g/L cationic polymers were washed 6 times with 50 mL of deionized water to avoid the influence of free components, and then freeze dried for XPS analysis. The X-ray photoelectron spectra analyses were carried out using a scanning XPS microprobe (Quantera SXM) equipped with a monochromatic Al-K alpha X-ray source (1486.60 eV, energy width 280 eV) operated at 23.7 W with a background pressure of  $5.9 \times 10^{-8}$  Torr.

#### 2.4. Cellulase activity analysis

Cationic polymers were added into the cellulase solution in the dosage of 0.2 g/L. Then the mixed solutions were incubated at 48 °C for 60 h. A total of 1 mL of the mixture was withdrawn periodically, and the enzyme activity with and without cationic polymers was assayed by the standard filter paper assay (Ghose, 2009). One unit of enzyme activity is defined as the amount of enzyme required to liberate 1  $\mu$ mol of reducing sugar per minute at pH 4.8 and 48 °C.

#### 2.5. Charge density analysis

A particle charge detector (Mutek PCD 03 titrator, Herrsching, Germany) was used to detect the charge density of the samples with a standard poly dimethyl diallyl ammonium chloride (PDADMAC) solution (1mN) or a poly(vinyl sulfate) potassium salt (PVSK) solution (1 mN), as described in reference (Wang et al., 2015). 0.5 g/L of CPAM and PEI solutions were prepared in 150-mL Erlenmeyer flasks. The cellulase and cellulase-polymer mixtures were diluted 1000 times in 250-mL Erlenmeyer flasks. The 0.5 g/L cellulase solution was neutralized by excess standard PDADMAC solution and shaken for 120 min to completely neutralize the surface negative charge of cellulose, after which the supernatant filtered from the fritted glass filter was titrated.

### 3. Results and discussion

#### 3.1. Composition and SEM analysis of waste paper fibers (WPFs)

The waste paper fibers (WPFs) were composed of 61.3% cellulose, 7.4% hemicellulose, 18.8% lignin and 6.5% ash. A mass balance lacking 100% is due to the presence of plastics, adhesives, and other organics in waste papers (Min & Ramarao, 2017). It is noticed that the carbohydrates (cellulose and hemicellulose) of WPFs account for 68.7%, indicating that WPFs are preferable candidates for bio-ethanol production. Min et al. reported that old corrugated containerboard (OCC) had lower lignin content (4%) and higher ash content (41%) compare with WPFs in the present study (Min et al., 2015). WPFs used in the present study were produced mainly from old newspapers. Generally, newspapers were produced using mechanical pulp as a material. During mechanical pulping process, most of lignin was retained in mechanical pulp. Therefore, the lignin content in WPFs was relatively high. In addition, the WPFs were taken from floatation cell, where most of ink particles were removed. Thereby, the ash content was relatively low.

The microstructures of WPFs were detected by SEM, as shown in Fig. 1. According to Fig. 1(a), the morphology of WPFs are different. Not only macrofibers but also fines appeared in raw materials. Some fibers were curved (Fig. 1(a)). Pores (Fig. 1(b)) and fragments (Fig. 1(d)) appeared on the surface of fibers. Moreover, broken fiber (Fig. 1(c)) was observed in WPFs. It has been suggested that disruption of the highly ordered and tightly packed regions of fiber structure facilitated the enzyme access to cellulose, which was expected to speed the hydrolytic attack of cellulases (Arantes & Saddler, 2011). Therefore, these WPF features are expected to be beneficial for enzymatic hydrolysis.

#### 3.2. Effects of cationic polymers on enzymatic hydrolysis at high solids concentration

The effect of CPAM, PEI or CS on enzymatic hydrolysis of WPFs at a high solids concentration of 15% (w/w) was investigated. Fig. 2 shows the glucose yield after 60 h hydrolysis with and without cationic polymers. The glucose yield first increased and then decreased with increasing CPAM, PEI and CS (Fig. 2). This result indicated that a moderate amount of CPAM, PEI and CS could substantially enhance enzymatic hydrolysis. The highest glucose yield of 72.5% was obtained with addition of 0.05 g/L PEI. The second-highest glucose yield of 65.9% was observed with 0.05 g/L CPAM. After this, as the dosages of CPAM and PEI were increased, glucose yields decreased. Compared to CPAM and PEI, CS did not noticeably improve the enzymatic hydrolysis. Obviously, the increment in glucose yield after adding PEI was better than adding CPAM or CS at the high solids concentration of enzymatic hydrolysis. Interestingly, when more than 0.2 g/L of CPAM was used, the glucose yield was even less than with no CPAM at all. However, all glucose yields after adding PEI were greater than that of the raw material. Guan et al. demonstrated that cationic polymers (CPAM and poly-diallyl-dimethyl-ammonium chloride (PDADMAC)) improved the enzymatic hydrolysis of pulp mill sludge at a solid concentration of 10% (w/w), and an increase of 20% in glucose yield was obtained after 96 h hydrolysis (Guan et al., 2014). An increase in glucose yield of approximately 15% observed in this study was lower than that found by Guan et al. The washed pulp mill sludge was composed of 70.6% cellulose, 16.1% hemicellulose, 7.3% lignin and 6.0% ash. By comparison, the lignin content in WPFs (18.8%) was higher than that in pulp mill sludge. In addition, the solid loading (15% (w/w)) was high and hydrolysis time (60 h) was low in this study. These differences likely caused the lower increase in glucose yields for WPFs compared to pulp mill sludge with cationic polymer addition. Overall, addition of cationic polymers provided net increase of the total sugar concentration to a level appropriate for subsequent fermentation. With addition of 0.05 g/L PEI, the sugar concentration increased from 57.3 g/L to 73.9 g/L. Energy requirement for distillation is significantly reduced if the ethanol concentration is above 4%. In order to meet this ethanol yield, glucose yield must be at least 8% (w/w) (assuming that an ethanol yield of 0.5 g/g-glucose). In this study, the highest glucose concentration of 7.39% was obtained, which was close to 8%.

#### 3.3. Effects of cationic polymers on cellulase adsorption

Mass transfer will be decreased when enzymatic hydrolysis is performed at high solids loadings (Kristensen et al., 2009), which could reduce the adsorption capacity of cellulase to cellulose. Therefore, increasing cellulase binding to cellulose is significant for enzymatic hydrolysis at high solids concentration. In this study, the cellulase binding capacity of WPFs was investigated before and after adding cationic polymers, as shown in Fig. 3. Cellulase binding was carried out at a solids concentration of 5% (w/w), which is different from that of enzymatic hydrolysis (15%, w/w) due to uniformity of adsorption and ease of sampling. Earlier studies showed that adsorption achieved equilibrium in 90–120 min (Yu, Tang, Xing, Zhu, & Jiang, 2013). In this study, cellulase binding was performed for 2 h to reach maximum adsorption. Fig. 3 shows that the cellulase binding trend was similar for the three cationic polymers. The cellulase binding capacity of WPFs was increased when a suitable dose of cationic polymers was used. This could, in part, explain the increase in glucose yield after adding cationic polymers (Fig. 2). The highest cellulase binding capacity was obtained by adding PEI; it increased from 30.2 mg/g-substrate (original WPFs) to 37.1 mg/g-substrate with an increase in the concentration of PEI from 0 to 0.2 g/L. The second-highest binding capacity (32.8 mg/g-substrate) was observed using 0.2 g/L CPAM. CS showed the lowest cellulase binding capacity (31.9 mg/g-substrate) with a loading of 0.2 g/L. Therefore, the greatest increase in enzymatic hydrolysis was obtained



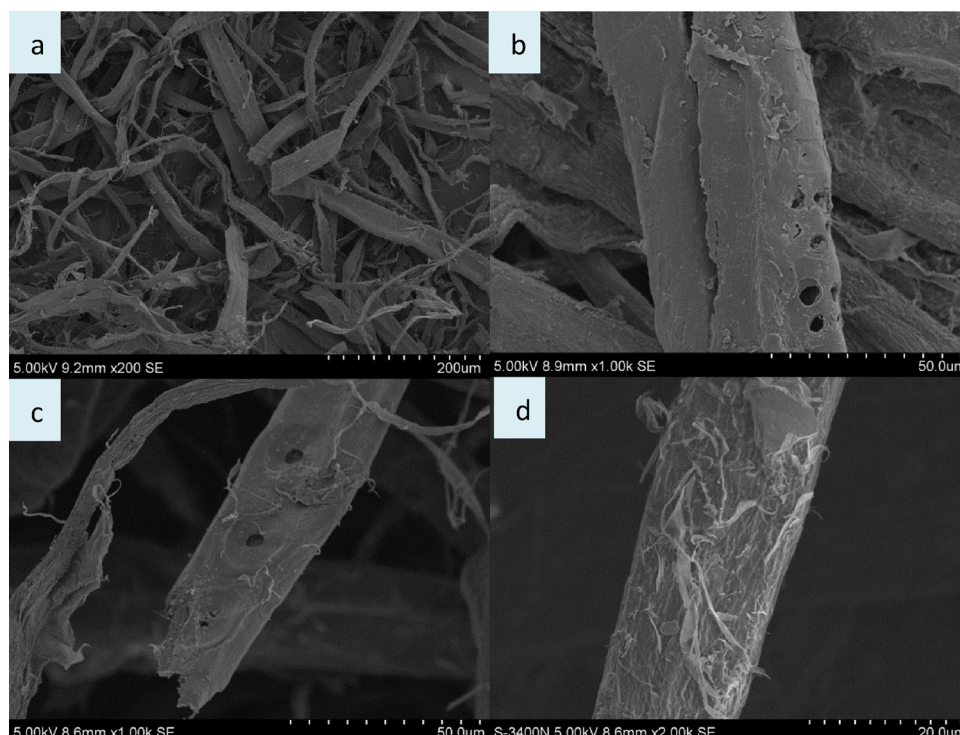


Fig. 1. Scanning electron microscopy (SEM) analysis of waste paper fibers.

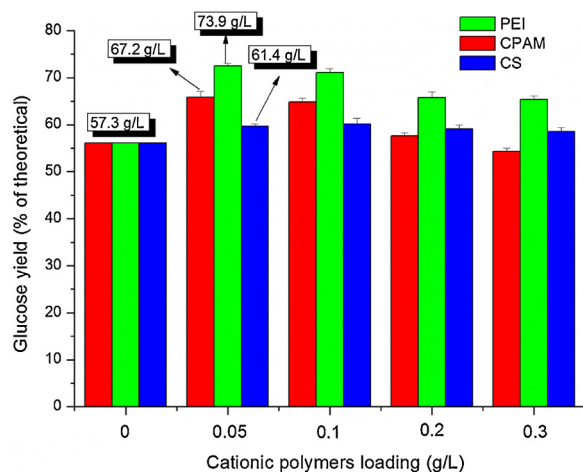


Fig. 2. Glucose yield after 60 h hydrolysis with different cationic polymers. CPAM is cationic polyacrylamide; PEI, polyethyleneimine; CS, cationic starch. The enzyme loading was 18 FPU/g-cellulose for cellulase and 25 CBU/g-cellulose for  $\beta$ -glucosidase.

by adding PEI during enzymatic hydrolysis, as illustrated in Fig. 2. After this point, continuing to increase the dose of cationic polymer (CPAM, PEI or CS) did not improve cellulase binding capacity. On the contrary, cellulase adsorption was decreased. This could explain the decrease in glucose yield during enzymatic hydrolysis at high solids concentration when more cationic polymer was used (Fig. 2).

XPS analysis revealed a change in elementary composition on the surface of original WPFs and WPFs with 0.2 g/L cationic polymers addition before and after cellulase adsorption, which is shown in Table 1. Carbon and oxygen are the main elements of the WPFs surface. The theoretical O/C values of cellulose and lignin are 0.83 and 0.33, respectively (Pang et al., 2012; Yu et al., 2013). According to Table 1, the O/C of original WPFs was 0.61, which falls between 0.83 and 0.33. It indicates that lignin and cellulose both exist on the surface of WPFs. In addition, nitrogen was not detected on the surface of WPFs. After PEI or

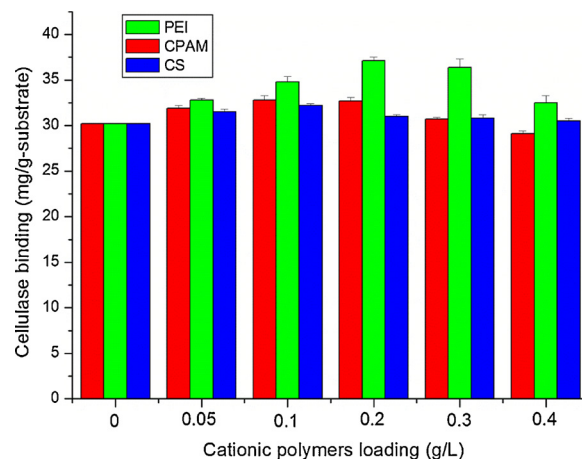


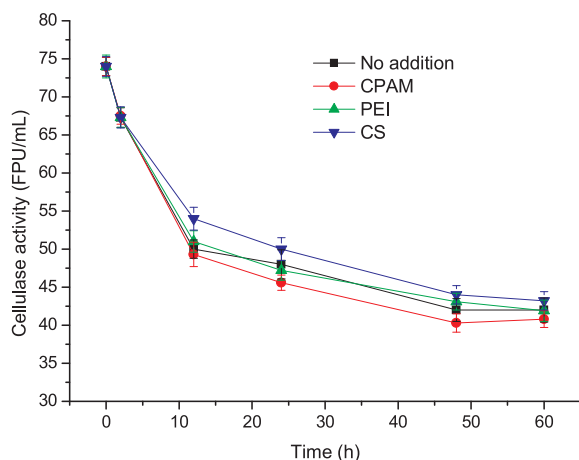
Fig. 3. Cellulase adsorption capacities of waste paper fibers with different cationic polymers. CPAM is cationic polyacrylamide; PEI, polyethyleneimine; CS, cationic starch.

Table 1

Atomic composition on waste paper fibers (WPFs) before and after cellulase adsorption.

	Before cellulase adsorption			After cellulase adsorption		
	C(%)	O (%)	N (%)	C (%)	O (%)	N (%)
Original WPFs	60.9	37.5	–	62.9	34.5	1.1
PEI + WPFs	64.2	30.9	3.4	67.6	23.4	8.3
CPAM + WPFs	64.8	32.1	2.1	67.5	27.5	4.9
CS + WPFs	62.2	36.5	–	63.1	33.8	1.9

CPAM addition, the nitrogen appeared on the surface of WPFs, indicating that PEI and CPAM were adsorbed onto WPFs. However, nitrogen was not detected with addition of CS. This is because CS does not have nitrogen. After cellulase adsorption, the nitrogen contents of all



**Fig. 4.** Time courses of cellulase activity incubated with cationic polymers in the hydrolyzed solution without the substrate. CPAM is cationic polyacrylamide; PEI, polyethyleneimine; CS, cationic starch. All cationic polymers were added at a concentration of 0.2 g/L.

samples increased, suggesting that cellulase was bound onto WPFs. When PEI was added, the increase in nitrogen content was 4.9% (increased from 3.4% to 8.3%), which was higher than adding CPAM (2.8%), CS (1.9%) and original WPFs (1.1%), respectively. This indicates that PEI could result in more cellulase adsorption onto WPFs, agreeing with the result showed in Fig. 3.

All cationic polymers were added at a concentration of 0.2 g/L. CPAM is cationic polyacrylamide; PEI, polyethyleneimine; CS, cationic starch.

### 3.4. Effects of cationic polymers on cellulase activity

Fig. 4 signifies the stability of the cellulase incubated with 0.2 g/L of CPAM, PEI and CS, respectively for 60 h. Without cationic polymer, the cellulase activity declined drastically at 48 °C and only 62.4% activity remained after 60 h incubation. With addition of PEI, a similar reduction in cellulase activity was observed. When CPAM was added, the cellulase activity was decreased compared to surfactant free solutions. The reduction was insignificant with reference to the experimental error. Interestingly, the cellulase activity was slightly increased with CS addition. It has been reported that some nonionic surfactants have the function of stabilizing enzymes, and may improve the enzymatic activity, stability and prevent deactivation of the cellulase during the hydrolysis process, thereby increasing the enzymatic hydrolysis. Jia et al. pointed out the cellulase activity increased by 27.5% with PEG4000 addition (Jia et al., 2010). Kaar and Holtzapple proposed that Tween-80 protected the enzymes from thermal deactivation during hydrolysis of pretreated corn stover (Kaar & Holtzapple, 2015). Cheng et al. reported that the addition of nonionic surfactant of Triton X-100 increased the cellulase activity significantly, which were 3.75-fold of the control at 96 h of enzymatic hydrolysis (Cheng, Yu, & Zhu, 2014). However, in our study, no obvious improvement in cellulase activity was observed with addition of cationic polymers. Fig. 2 showed the highest glucose yield was obtained as PEI was used, and the second highest glucose yield was acquired when CPAM was added. However, they did not improve the cellulase activity. Therefore, the improvement in enzymatic hydrolysis of WPFs with addition of CPAM or PEI appears to be due to their increase in cellulase adsorption onto cellulose.

### 3.5. Charge density of cellulase-polymer mixtures

Cellulase cocktail (Celluclast), used in this manuscript, contains various different cellulases (endoglucanase(EG), cellobiohydrolase (CBH) and  $\beta$ -glucosidase( $\beta$ G) and hemicellulases (xylosidase) (Stals,

**Table 2**

Charges of cellulase, cellulose, and cationic polymers.

Cellulase ( $\mu$ eq/mL)	Waste paper fibers ( $\mu$ eq/g)	CPAM ( $\mu$ eq/g)	PEI ( $\mu$ eq/g)	CS ( $\mu$ eq/g)
−191	−75.5	412.8	2965.6	175.7

Sandra, Devreese, Van Beeumen, & Claeysens, 2004). Therefore, the electronegativity of the individual component enzymes depends on the pH of system. The Celluclast cellulase preparation in this study is derived from *Trichoderma reesei*, where the dominant protein present in terms of mass is the cellobiohydrolase Cel7A. The pI of this enzyme ranges between 3.5 and 4.2 (Stals, Sandra, Devreese et al., 2004; Stals, Sandra, Geysens et al., 2004). Therefore, under the enzymatic hydrolysis condition of pH 4.8, Cel7A carries a negative charge. Usually, the WPFs will have negative charge due to the oxidation bleaching during pulping process. As shown in Table 2, the charge density of cellulase was −191  $\mu$ eq/mL. However, the pI of some of the other enzymes will be well above a pH of 4.8, such as the hemicellulases, and therefore will be positively charged. In this study, the WPFs were used as the cellulosic material, and WPFs has a charge density of −75.5  $\mu$ eq/mL (Table 2). Due to the fact that both cellulase and cellulosic fibers bear negative charges, there would be repulsion force between the two, hence reducing the cellulase adsorption. Reye et al. have postulated that cationic polyelectrolytes promote enzymatic hydrolysis by neutralizing the repulsive charge between cellulose surface and the enzyme (Reye, Maxwell, & Banerjee, 2011).

CPAM is cationic polyacrylamide; PEI, polyethyleneimine; CS, cationic starch.

Before enzymatic hydrolysis, the cellulase was mixed well with the cationic polymers for 30 min. Table 3 shows the charge density of the enzyme solution mixed with different cationic polymers. The charge density of cellulase increased with increasing CPAM, PEI and CS dosage (Table 3). Apparently, the overall negative charge of the enzyme solution was neutralized by the addition of the cationic polymers. After the isoelectric point of cellulase was reached, the cellulase-polymer system showed positive electric charge with increasing cationic polymer dosage. It is hypothesized that reducing the negative charge of cellulase will decrease the repulsive force between cellulase and WPF, thereby increasing the binding capacity. In addition, the low positive charge density of the cellulase-polymer system is beneficial for enzymatic hydrolysis because it can increase the binding of enzymes to WPF by electrostatic attraction. When a larger dose of cationic polymer was used, the cellulase surface will take on a positive charge, which decreased cellulase binding capacity by electrostatic repulsion. For this reason, larger doses of cationic polymers decreased glucose yield at high solids concentration (Fig. 2).

CPAM is cationic polyacrylamide; PEI, polyethyleneimine; CS, cationic starch.

Different adsorption modes occur when different cationic polymers are used; they involve distinct patching and bridging mechanisms as shown in Fig. 5. Our previous study showed that CPAM could improve the cellulase treatment efficiency of hardwood kraft-based dissolving pulp, which was mainly attributed to the patching/bridging mechanism (Wang et al., 2015). Shorter-chain cationic polymers are more suitable for patching, whereas longer-chain cationic polymers are more suitable

**Table 3**

Charges of cellulase-polymer mixtures with different cationic polymers.

Concentration (g/L)	CPAM ( $\mu$ eq/mL)	PEI ( $\mu$ eq/mL)	CS ( $\mu$ eq/mL)
0.05	−121.4	160.4	−171.4
0.1	−62.9	290.2	−90.5
0.2	225.2	360.1	192.8
0.3	338.9	570.8	278.6

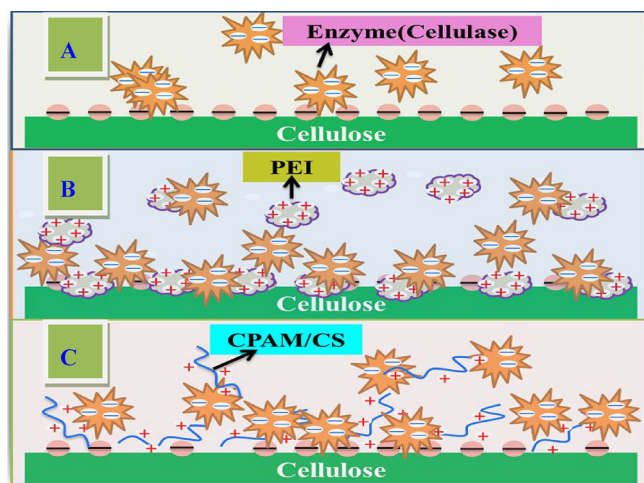


Fig. 5. Schematic of cationic polymers enhancing cellulase adsorption onto cellulose. A, interaction of cellulase and cellulose; B, PEI enhancing cellulase adsorption onto cellulose through a patching mechanism; C, CPAM enhancing cellulase adsorption onto cellulose through a bridging mechanism.

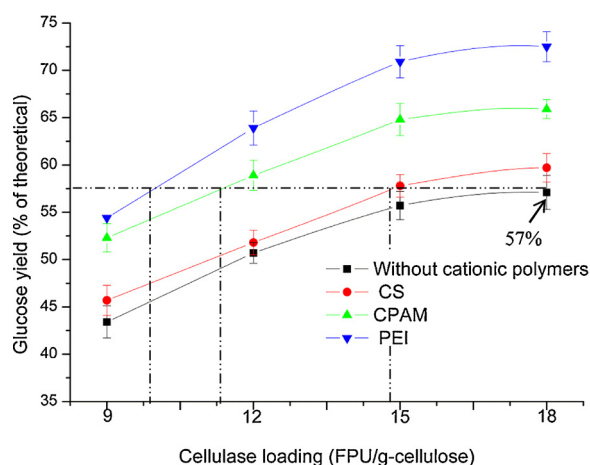


Fig. 6. Glucose yield after 60 h hydrolysis with different cellulase loadings. CPAM is cationic polyacrylamide; PEI, polyethyleneimine; CS, cationic starch.

for bridging. CPAM and CS have long molecular chains and therefore can bind cellulase through a bridging mechanism, as indicated in Fig. 5(C). However, short-chain PEI polymers are expected to bond cellulase through a patching mechanism, as shown in Fig. 5(B). Compared with the patching mechanism, the binding mechanism in bridging involves steric hindrance due to the characteristics of the long-chain structure. Therefore, it can be speculated that the increase in cellulase binding on cellulose observed with PEI is due to a patching mechanism and is greater than that with CPAM or CS, bonding through a patching mechanism. Thereby, the glucose yield after adding PEI will be higher than after adding CPAM or CS, as seen in Fig. 2. CPAM and CS are both long-chain polymers and they will have the same binding mechanism. The difference in their binding capacity may be due to their differences in charge density. The charge density of CS was 175.7  $\mu\text{eq/g}$ , which was lower than that of CPAM (412.8  $\mu\text{eq/g}$ ). Therefore, CS will bind less cellulase.

### 3.6. Enzymatic hydrolysis at different cellulase loadings

Under the optimal dosage of cationic polymers (0.05 g/L), four cellulase loadings were used to evaluate enzymatic hydrolysis at a high solids concentration of 15% (w/w). The cellulase loadings were 9, 12, 15 and 18 FPU/g-cellulose. Glucose yield increased as the cellulase

loading increased from 9 to 18 FPU/g-cellulose. Under all enzyme dosage points, enzymatic hydrolysis increased using cationic polymers. In agreement with previous research results, PEI showed the greatest improvement in enzymatic hydrolysis for all cellulase loadings. When cellulase loading was less than 15 FPU/g-cellulose, glucose yield increased rapidly with the use of cationic polymers. However, the increase in glucose yield slowed down when cellulase loading was greater than 15 FPU/g-cellulose. This indicated that the improvement of enzymatic hydrolysis with cationic polymers had reached a maximum. Therefore, cationic polymers can improve enzymatic hydrolysis markedly under low enzyme loadings. It is known that the price of enzymes is very high, which has hindered industrialized and large (Bharathiraja et al., 2017). From Fig. 6, a relatively low enzyme dosage can reach the same glucose concentration when cationic polymers are used. For example, if 58 g/L of glucose concentration is required, approximately 57% of glucose yield will be achieved when enzymatic hydrolysis is carried out at 15% (w/w) solids concentration. According to Fig. 6, in order to meet above glucose yield, the cellulase loadings are about 18 (without cationic polymers), 15 (with CS addition), 11 (with CPAM addition) and 9.5 (with PEI addition) FPU/g-cellulose, respectively.

## 4. Conclusions

With addition of PEI, CPAM or CS, the glucose hydrolysis yields at 15% (w/w) solids concentration was improved. After 60 h hydrolysis, the glucose yield increased from 57.1% to 72.5%, 65.9% and 59.7% with addition of 0.05 g/L PEI, CPAM and CS, respectively. It is suggested that the improvement in glucose yield caused by cationic polymers may be due to the increase in cellulase adsorption through electrostatic attraction. In addition, when the same glucose yield was obtained, cationic polymers addition could reduce the cellulase loading, especially when PEI was used. This may provide practical assistance for industrialized production of biofuels.

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## References

- Adu, C., Jolly, M., & Thakur, V. K. (2018). Exploring new horizons for paper recycling: A review of biomaterials and biorefinery feedstocks derived from wastepaper. *Current Opinion in Green and Sustainable Chemistry*, 13, 21–26.
- Akimkulova, A., Zhou, Y., Zhao, X., & Liu, D. (2016). Improving the enzymatic hydrolysis of dilute acid pretreated wheat straw by metal ion blocking of non-productive cellulase adsorption on lignin. *Bioresource Technology*, 208, 110–117.
- Arantes, V., & Saddler, J. N. (2011). Cellulose accessibility limits the effectiveness of minimum cellulase loading on the efficient hydrolysis of pretreated lignocellulosic substrates. *Biotechnology for Biofuels*, 4(1), 3–10–16.
- Bharathiraja, B., Jayamuthunagai, J., Sudharsana, T., Bhargavi, A., Praveenkumar, R., Chakravarthy, M., & Yuvaraj, D. (2017). Biobutanol – An impending biofuel for future: A review on upstream and downstream processing techniques. *Renewable and Sustainable Energy Reviews*, 68(1), 788–807.
- Brummer, V., Jurena, T., Hlavacek, V., Omelkova, J., Bebar, L., Gabriel, P., & Stehlik, P. (2014). Enzymatic hydrolysis of pretreated waste paper - source of raw material for production of liquid biofuels. *Bioresource Technology*, 152(1), 543–547.
- Chen, M., Zhao, J., & Xia, L. (2008). Enzymatic hydrolysis of maize straw polysaccharides for the production of reducing sugars. *Carbohydrate Polymers*, 71(3), 411–415.
- Cheng, J., Yu, Y., & Zhu, M. (2014). Enhanced biodegradation of sugarcane bagasse by *Clostridium thermocellum* with surfactant addition. *Green Chemistry*, 16(5), 2689–2695.
- Dubey, A. K., Gupta, P. K., Garg, N., & Naithani, S. (2012). Bioethanol production from waste paper acid pretreated hydrolyzate with xylose fermenting *Pichia stipitis*. *Carbohydrate Polymers*, 88(3), 825–829.



- Gao, W., Xiang, Z., Chen, K., Yang, R., & Yang, F. (2015). Effect of depth beating on the fiber properties and enzymatic saccharification efficiency of softwood kraft pulp. *Carbohydrate Polymers*, 127, 400–406.
- Gaudreault, R., Cesare, N. D., Ven, T. G. M. V. D., & Weitz, D. A. (2015). Structure and strength of flocs of precipitated calcium carbonate induced by various polymers used in papermaking. *Industrial & Engineering Chemistry Research*, 54(24), 6234–6246.
- Ghose, T. K. (2009). Measurement of cellulase activities. *Pure and Applied Chemistry*, 59(2), 257–268.
- Govumoni, S. P., Koti, S., Kothagouni, S. Y., Venkateshwar, S., & Linga, V. R. (2013). Evaluation of pretreatment methods for enzymatic saccharification of wheat straw for bioethanol production. *Carbohydrate Polymers*, 91(2), 646–671.
- Guan, W., Haynes, R. D., & Lee, Y. Y. (2014). Effects of cationic polyelectrolytes on enzymatic hydrolysis of pulp mill sludge under high solid loading. *J-FOR-Journal of Science & Technology for Forest Products and Processes*, 4, 36–43.
- Hodge, D. B., Karim, M. N., Schell, D. J., & Mcmillan, J. D. (2008). Soluble and insoluble solids contributions to high-solids enzymatic hydrolysis of lignocellulose. *Bioresource Technology*, 99(18), 8940–8948.
- Jia, O., Dong, Z. W., Song, X. Y., Xin, L., Mu, C., & Qiang, Y. (2010). Improved enzymatic hydrolysis of microcrystalline cellulose (Avicel PH101) by polyethylene glycol addition. *Bioresource Technology*, 101(17), 6685–6691.
- Kaar, W. E., & Holtzapfel, M. T. (2015). Benefits from tween during enzymic hydrolysis of corn stover. *Biotechnology and Bioengineering*, 59(4), 419–427.
- Kristensen, J. B., Felby, C., & Jørgensen, H. (2009). Yield-determining factors in high-solids enzymatic hydrolysis of lignocellulose. *Biotechnology for Biofuels*, 2(1), 11–18.
- Li, Y., Sun, Z., Ge, X., & Zhang, J. (2016). Effects of lignin and surfactant on adsorption and hydrolysis of cellulases on cellulose. *Biotechnology for Biofuels*, 9(1), 1–9.
- Lin, X., Cai, C., Lou, H., Qiu, X., Pang, Y., & Yang, D. (2016). Effect of cationic surfactant cetyltrimethylammonium bromide on the enzymatic hydrolysis of cellulose. *Cellulose*, 24(1), 1–8.
- Lou, H., Wang, M., Lai, H., Lin, X., Zhou, M., Yang, D., & Qiu, X. (2013). Reducing non-productive adsorption of cellulase and enhancing enzymatic hydrolysis of lignocelluloses by noncovalent modification of lignin with lignosulfonate. *Bioresource Technology*, 146, 478–484.
- Lou, H., Zeng, M., Hu, Q., Cai, C., Lin, X., Qiu, X., ... Pang, Y. (2018). Nonionic surfactants enhanced enzymatic hydrolysis of cellulose by reducing cellulase deactivation caused by shear force and air-liquid interface. *Bioresource Technology*, 249, 1–8.
- Min, B. C., & Ramarao, B. V. (2017). Mechanisms of the inhibition of enzymatic hydrolysis of waste pulp fibers by calcium carbonate and the influence of nonionic surfactant for mitigation. *Bioprocess and Biosystems Engineering*, 40(6), 1–8.
- Min, B. C., Bhayani, B. V., Jampana, V. S., & Ramarao, B. V. (2015). Enhancement of the enzymatic hydrolysis of fines from recycled paper mill waste rejects. *Bioresources and Bioprocessing*, 2(1), 40.
- Mohamed, A. J., Ghazi, B. M., M Nejjib, M., Mohamed, M., & Issam, S. (2016). Physico-chemical characterization and enzymatic functionalization of *Enteromorpha* sp. cellulose. *Carbohydrate Polymers*, 135, 274–279.
- Pang, C. S., Xie, T. J., Lin, L., Zhuang, J. P., Liu, Y., Shi, J. B., et al. (2012). Changes of the surface structure of corn stalk in the cooking process with active oxygen and MgO-based solid alkali as a pretreatment of its biomass conversion. *Bioresource Technology*, 103(1), 432–439.
- Ramos, L. P., Silva, L. D., Ballem, A. C., Pitarello, A. P., Chiarello, L. M., & Silveira, M. H. L. (2015). Enzymatic hydrolysis of steam-exploded sugarcane bagasse using high total solids and low enzyme loadings. *Bioresource Technology*, 175, 195–202.
- Reye, J. T., Maxwell, K. E., & Banerjee, S. (2011). Cationic polyacrylamides promote binding of cellulase and amylase. *Journal of Biotechnology*, 154(4), 269.
- Singh, R., Krishna, B. B., Kumar, J., & Bhaskar, T. (2016). Opportunities for utilization of non-conventional energy sources for biomass pretreatment. *Bioresource Technology*, 199, 398.
- Sipos, B., Szilágyi, M., Sebestyén, Z., Perazzini, R., Dienes, D., Jakab, E., ... Réczey, K. (2011). Mechanism of the positive effect of poly (ethylene glycol) addition in enzymatic hydrolysis of steam pretreated lignocelluloses. *Comptes Rendus Biologies*, 334(11), 812–823.
- Stals, I., Sandra, K., Devreese, B., Van Beeumen, J., & Claeysens, M. (2004). Factors influencing glycosylation of *Trichoderma reesei* cellulases. II: N-glycosylation of Cel7A core protein isolated from different strains. *Glycobiology*, 14(8), 725–737.
- Stals, I., Sandra, K., Geysens, S., Contreras, R., Van Beeumen, J., & Claeysens, M. (2004). Factors influencing glycosylation of *Trichoderma reesei* cellulases. I: Postsecretorial changes of the O- and N-glycosylation pattern of Cel7A. *Glycobiology*, 14(8), 713–724.
- Strobel, K. L., Pfeiffer, K. A., Blanch, H. W., & Clark, D. S. (2016). Engineering Cel7A carbohydrate binding module and Linker for reduced lignin inhibition. *Biotechnology and Bioengineering*, 113(6), 1369–1374.
- Subbhadra, P. B., Babu, N. R., & Gogate, P. R. (2015). Intensification of enzymatic hydrolysis of waste newspaper using ultrasound for fermentable sugar production. *Ultrasonics Sonochemistry*, 22, 326.
- Sun, S., Sun, S., Cao, X., & Sun, R. (2016). The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials. *Bioresource Technology*, 199, 49.
- Szabo, O. E., & Csizsar, E. (2017). Some factors affecting efficiency of the ultrasound-aided enzymatic hydrolysis of cotton cellulose. *Carbohydrate Polymers*, 156, 357–363.
- Tan, I. S., & Lee, K. T. (2015). Solid acid catalysts pretreatment and enzymatic hydrolysis of macroalgae cellulosic residue for the production of bioethanol. *Carbohydrate Polymers*, 124, 311.
- Wang, Q., Liu, S., Yang, G., Chen, J., & Ni, Y. (2015). Cationic polyacrylamide enhancing cellulase treatment efficiency of hardwood kraft-based dissolving pulp. *Bioresource Technology*, 183, 42–46.
- Yang, X., Bu, L., Sun, D., Liu, Z., Liu, S., & Jiang, J. (2015). High glucose recovery from direct enzymatic hydrolysis of bisulfite-pretreatment on non-detoxified furfural residues. *Bioresource Technology*, 193, 401.
- Yu, H., You, Y., Lei, F., Liu, Z., Zhang, W., & Jiang, J. (2015). Comparative study of alkaline hydrogen peroxide and organosolv pretreatments of sugarcane bagasse to improve the overall sugar yield. *Bioresource Technology*, 187, 161.
- Yu, H. L., Tang, Y., Xing, Y., Zhu, L. W., & Jiang, J. X. (2013). Improvement of the enzymatic hydrolysis of furfural residues by pretreatment with combined green liquor and hydrogen peroxide. *Bioresource Technology*, 147(11), 29–36.
- Zhao, X. B., Dong, L., Chen, L. A., & Liu, D. H. (2013). Batch and multi-step fed-batch enzymatic saccharification of Formiline-pretreated sugarcane bagasse at high solid loadings for high sugar and ethanol titers. *Bioresource Technology*, 135(2), 350.