

# Comparison of the Interaction Modes Between Cellulase and Bovine Serum Albumin /Lignosulfonate /Polyethylene Glycol in Promoting Lignocellulose Saccharification

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## Research

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# Abstract

**Background:** Bovine serum albumin (BSA), polyethylene glycol (PEG) and lignosulfonate (LS) have been extensively employed as synergistic agents in lignocellulose saccharification, albeit it has not been fully understood how they interact with enzymes from the perspectives of molecular interactions. Herein, we attempted to unveil the promotion mechanisms of BSA, PEG and LS for lignocellulose saccharification from the perspective of their respective interaction with cellulase using Quartz Crystal Microbalance with Dissipation monitoring (QCM-D), Surface Plasmon Resonance (SPR), and Small Angle X-ray Scattering (SAXS) to investigate their respective interaction and the complex formation. In the meanwhile, we compared the effects of adding these additives into the enzymatic hydrolysis of pure cellulose (Avicel) and green liquor-pretreated lignocellulose (GL).

**Results:** The results showed that BSA and LS could bind to cellulase to form complexes, whereas PEG did not. However, PEG had a high affinity to lignin or lignin derivatives. In term for Avicel and GL substrates, the results showed that BSA and PEG promoted the enzymatic hydrolysis of both substrates, while LS had a promoting effect for GL only and inhibited some extent for Avicel.

**Conclusions:** This study showed that synergistic agents of LS, BSA, and PEG have different interaction modes with cellulase. BSA and LS form complexes with cellulase and the formed complexes prevent from nonproductive binding by residue lignin; whereas PEG prevents from nonproductive binding by forming a thin layer on residue lignin which actually serve as steric hindrance. This investigation will help us to understand the sophisticated interactions among the components in the complicated enzymatic system, especially the interactions between enzymes and synergistic agents. It will be helpful in the design and utilization of synergistic additives in the lignocellulose biorefinery process as well.

## 1. Background

Lignocellulosic biomass resources are abundant and environmentally friendly on earth. The liquid fuel produced by lignocellulose especially ethanol is an alternative energy with great development potential [1]. In recent years, lignocellulose has become a hot spot of modern biomass research. However, it is well known that enzymatic hydrolysis of lignocellulose is easily restricted due to the complex structure of lignocellulose. It is currently believed that the main reason affecting the hydrolysis of lignocellulose is the non-productive adsorption of the substrate lignin and cellulase, thereby reducing the efficiency of cellulase [2, 3].

Many studies have found that adding some additives (or synergic agents) into the lignocellulose enzymatic hydrolysis system can enhance the enzymatic hydrolysis effect by reducing the non-productive adsorption of lignin and cellulase. Lignosulfonate (LS), polyethylene glycol (PEG) and Bovine Serum Albumin (BSA) are three synergic agents under extensive studies for enzymatic hydrolysis of lignocellulose [4–8]. The chemical structures of three synergists are quite different; therefore, the promoting modes of three synergists should be different. However, it has not been fully understood how

they promote the enzymatic hydrolysis efficiency. In literature, there are some controversies in the explanation of their promoting effects.

LS is a by-product of sulfite pulping and also a by-product of SBOL pretreatment[9]. Zhou et al. [10] suggested that LS can act as a surfactant to prevent the non-productive binding of substrate lignin to cellulase, thereby promoting saccharification. Wang et al. [9] proposed that LS can bind to cellulase and the formed complex increases the amount of negative charges carried by cellulase, thereby increasing the electrostatic repulsion between cellulase and residual lignin in substrate, as a consequence reducing cellulase non-productive adsorption of substrate lignin. By adding LS to the enzymatic hydrolysate of different pretreatment substrates, Wang et al. [11] found that the pretreatment method will affect the effect of LS. However, these studies were carried out in a complicated hydrolysis system, where both residual lignin and soluble lignin coexisted. As such, the action mechanism between cellulase and lignin cannot be clearly identified which part of lignin involved. In order to understand the action mode of LS, researchers studied the mechanism of LS in the enzymatic hydrolysis of lignocellulose from the perspective of substrate/substrate lignin- enzyme adsorption [12, 13]. However, at present, when studying the mechanism of LS in lignocellulose hydrolysis system, the influence of substrate lignin cannot be ruled out.

Adding PEG to the lignocellulose hydrolysate can reduce the amount of enzymes used and increases the conversion rate of cellulose [6, 8, 14]. It is currently believed that PEG as a nonionic surfactant can promote enzymatic hydrolysis of cellulose. The main role of surfactants in substrate containing lignin is that lignin with adsorbed surfactants can inhibit the interaction between enzymes and lignin, which makes the efficiency of lignin-binding enzymes low [15]. However, it was found in the research that the efficiency of PEG is not always positively correlated with the lignin content[16]. Therefore, some researchers believe that the reason why PEG can enhance the efficiency of enzymatic hydrolysis is due to that the addition of PEG can increase the activity of cellulase [17, 18].

BSA is widely used in biochemical studies, and plays an active role in the enzymatic hydrolysis of cellulose [19, 20]. BSA can be used as a synergist for lignocellulose hydrolysis by reducing the non-specific adsorption of lignin to cellulase [5, 19]. Some researchers believe that in addition to preventing the non-productive adsorption of cellulase by lignin, BSA can also reduce the inactivation of exoglycanase, thereby improving the hydrolysis of microcrystalline cellulose [21]. Jia et al. [22] compared the effects of adding BSA on the lignocellulose conversion rate of acid-pretreated poplar wood, ethanol-washing acid-pretreated poplar wood, and delignified acid-pretreated poplar wood and they found that BSA acts as a synergistic agent by coating the substrate lignin, thereby blocking the binding site of the substrate lignin.

From the literature study, it can be found that whether to form a complex between synergistic agent and cellulase is very critical in the discussion how they promote the enzymatic hydrolysis. Consequently, how synergistic agent interact with cellulase is very important to understand their role-played in enzymatic hydrolysis. Whereas in the literature, the system was too complicated, e.g., in the LS promotion cases,

both residual and soluble lignin were coexisted. Therefore, it is difficult to distinguish the exact roles residual and soluble lignin played. In order to make better use of synergistic agents, it is necessary to study the mechanism of synergistic agents in the hydrolysis process. As for our best understanding, no relevant studies have investigated interactions between cellulase and synergistic agents alone. This may be attributed to both cellulase and synergistic agents are soluble in the enzymatic hydrolysis conditions.

QCM-D and SPR are two commonly used noninvasive techniques to study interface effects in recent years [23–27]. QCM is made according to the principle of piezoelectric effect of quartz crystal. When the quality on the electrode chip changes, the electrical signal  $\Delta f$  output by the quartz crystal system will change accordingly. The quality change of the chip can be calculated based on the data of  $\Delta f$  obtained. The accuracy can reach nanogram level [28]. Sauerbrey equation [29] shows that the frequency of crystal oscillation is inversely proportional to the mass of sensor coupling. However, this formula only applies to thin, uniform, rigid films, otherwise we think the quantity of chips will be underestimated [30, 31]. Recently, some literatures have reported that QCM-D can be used to monitor the formation of thin films in real time and explore the properties of each film layer-by-layer adsorption of substances on the chip [32–34]. In this case, the viscoelastic Voigt model can be established to estimate the adsorption capacity of each layer more accurately [35]. The viscous modulus, elastic modulus, and thickness of each film can be obtained by this model. SPR is a method to study intermolecular interactions by using optical properties. We usually study the information change of analyte by the Angle change of SPR obtained. Small Angle X-ray scattering (SAXS) can be used to analyze the size and shape of molecules and obtain the aggregation information of proteins in solution [36].

In this investigation, we attempted to simply the system to study the interaction between the synergistic agents and cellulase alone, ignoring the influence of morphology and chemistry (e.g., residual lignin content, chemical difference, and distribution), using QCM-D and SPR techniques to monitor the interaction between cellulase that was immobilized onto the sensors of QCM-D and SPR and synergistic agents. In our previous work, we probed the cellulase biosensor formation in situ and in real time on gold chips [31]. Based on the cellulase biosensor we monitored the interactions between cellulase and BSA/LS/PEG in situ and in real time. In addition, SAXS was used to study whether cellulase and additives form a complex and to evaluate the size of the complex. At the same time, the effect of adding these additives to the enzymatic hydrolysis system of pure cellulose (Avicel) and lignocellulose (green liquor-pretreated lignocellulose (GL)) on the glucose conversion rate was investigated thoroughly.

## **2. Results And Discussion**

### **2.1 Interaction study between cellulase and enzymatic synergistic agents**

#### **2.1.1 Interaction between BSA and cellulase**

The Interactions study between cellulase (Cell) and BSA was conducted on a QCM-D E4 and a MP-SPR. The monitored curves are shown in Fig. 1. In Fig. 1a, the formation of self-assembled monolayer of MUA/MPA with carboxylic groups and the activation were carried out in the first 90 min. Once stable curves of frequency and dissipation value were obtained, the enzyme solution was injected into the system (point 1). Cellulase was covalently bond to the chip, evidencing by sharply decreased frequency ( $\Delta f$ ).  $\Delta f$  was decreased by about 20 Hz. Then, the buffer solution was pumped in (point 2) for rinsing, and the frequency increased slightly. This showed that the enzyme was firmly attached to the gold chips. After the BSA solution was injected (point 3), the  $\Delta f$  dropped dramatically down by 20 Hz. The buffer was then pumped in to rinse the unbound and loosely bound BSA. This legend system applies to Figs. 2 ~ 5. The frequency of the chips went up slightly. Regarding to the thickness of adlayer on QCM sensors, both fitted thickness values derived from Sauerbrey equation (open symbols) and viscoelastic modeling (solid symbols) were plotted together in Fig. 1b for easy comparison. The immobilized cellulase thickness was calculated to be 8.9 and 8.2 nm, respectively, for before and after rinsing. The thickness of BSA was 5.2 and 4.8 nm, respectively, for before and after rinsing. Based on Sauerbrey estimation, all according values were less than half of the values based on viscoelastic modeling, indicating the layer formed was very soft and therefore the Sauerbrey estimation was substantially underestimated the layer thickness of cellulase and/or BSA [37].

The same procedure was conducted on MP-SPR and the SPR angle changed with time is reported in Fig. 1c. The thickness of each layer based on MP-SPR was calculated and reported in Table 1. The adsorption thickness of BSA on cellulase was 2.0 nm after rinsing. The SPR data also showed that the thickness of cellulase layer was about 6.9 nm after rinsing. Since the molecular weight of cellulase is about 58 kDa, while that of BSA was 66.4 kDa. Thus, one BSA molecule can bind to four cellulase molecules to form a complex (BSA-Cell) in the planar adsorption as conducted in our system. One thing must be noted that the adsorption of BSA on immobilized cellulase is different than that in practice since there is only one interface is available for BSA adsorption while there is multiple direction for BSA binding in solution.

Table 1  
The calculated adsorption thickness of cellulase and BSA which acquired by SPR/QCM

Layer	Thickness (nm)		Coupled water (%)
	SPR	QCM	
Cellulase: before rinsing	7.1	8.9	20.2
after rinsing	6.9	8.2	15.9
BSA: before rinsing	2.6	5.2	50.0
after rinsing	2.0	4.8	58.3

Since QCM-D detects the wet-mass of the adlayer on the surface, while SPR probes the dry-mass, the coupled water of cellulase layer and BSA layer could be derived based on the different thickness examined by QCM-D and SPR techniques and the results are reported in the last column of Table 1. It can be noted that the cellulase layer was much more compact while BSA much softer, since the former contained less than half of the coupled water as the latter.

## 2.1.2 Interactions between Lignosulfonate and cellulase

Interactions study between cellulase (Cell) and lignosulfonate (LS) are present in Fig. 2. As stated previously, through the change of QCM frequency and SPR angle, it can be seen that LS can be adsorbed strongly and irreversibly on the cellulase film. The QCM frequency decreased by 15Hz after LS solution was loaded. It can be seen from Fig. 2b that the thickness of the LS film adsorbed on the cellulase is about 3.0 nm, and the height of the stably adsorbed LS film after buffer cleaning is about 1.7 nm. According to SPR data (Table 2), the adsorption thickness of LS on cellulase was 0.73 nm after washing. In summary, cellulase and LS can form complexes (LS-Cell). This was consistent with the conclusions made by Wang et al [9]. Through our adsorption experiments, it can be estimated that the formed complex (LS-Cell) composed of four LS and one cellulase molecules in the planar adsorption situation as conducted in our system.

Table 2  
The calculated adsorption thickness of cellulase and lignosulfonate (LS) which acquired by SPR/QCM

Layer	Thickness (nm)		Coupled water (%)
	SPR	QCM	
Cellulase: before rinsing	10.3	12.0	14.2
after rinsing	10.3	12.0	14.2
LS: before rinsing	1.29	8.3	84.5
after rinsing	0.73	3.2	77.2

The coupled water of cellulase layer and LS layer derived was reported in the last column of Table 2. The coupled water content in cellulase adlayer was about 14.2%, which agreed very well our results in the formation of BSA-Cell complex. However, the coupled water content of LS layer was about 80%, indicating LS was much extended in solution and the LS adlayer was very soft.

Figure 2d showed that the experimental scattering intensity of the mixed solution of cellulase and LS was not the same as their theoretical scattering intensity without any interaction, and the experimental intensity was always greater than that of the theoretical one, indicating some complexes of LS-Cell formed. This is consistent with the QCM-D and SPR measurements addressed previously.

The pH of the buffer will affect the adsorption of LS on the cellulase film [31]. Our previous study [31] has shown that LS adsorbed more cellulase under high pH condition. Some researchers also found that with

the increase of pH, the enzymatic hydrolysis efficiency of cellulose would increase [9]. According to the theory of electrostatic adsorption, it carries some negative charge but the intensity is not very strong, since the isoelectric point of cellulase is about 3.6 at pH 4.8 [9]. Therefore, it can form complex with LS likely at pH 4.8. Whereas at pH 7.5, cellulase carries a number of negative charges and its zeta potential can reach down to -30 mV [9] and therefore it is unlikely to form complex of LS-Cell in this situation. But in our previous research [31], the opposite result was observed. This indicates that electrostatic interaction is not the only factor that predominates the formation of complexes between cellulase and LS.

The above results showed that LS and cellulase could form complexes in different solutions, but with different structures, and the formation of this complex is not only related to electrostatic adsorption. This opinion is different from previous reports in the literature [9].

### **2.1.3 Interaction between PEG and cellulase**

The interaction between PEG on cellulase film monitored by QCM-D is shown in Fig. 3a. After the PEG solution was injected, the frequency ( $\Delta f$ ) basically had no fluctuation. There was no frequency change either after the buffer rinse. Accordingly, there was no fluctuation in dissipation values after PEG loading and rinsing. This suggested that PEG did not adsorb on cellulase film.

In order to verify the results of QCM, we used SPR to study the interaction between cellulase and PEG. It can be seen from Fig. 3b. Similarly, after PEG solution was injected, SPR angle remained unchanged.

At last, the small-angle X-ray scattering signal of the mixture of cellulase and PEG in different proportion (10: 1, 1: 1 and 1: 10) are plotted in Fig. 3c, along with the simulated signals with the volume ratio with cellulase and PEG. It can be found that the experimental scattering intensity of the mixed solution of PEG and enzyme was almost overlapped with the simulated scattering intensity assuming no interaction between PEG and cellulase.

Figure 3d showed that pH did not affect the interaction between PEG and cellulase films. Although it is a fact that PEG can improve the enzymatic hydrolysis ability of lignocellulose [9, 38]. However, the above results all proved that PEG and cellulase did not combine to form complex. If look at the last phase of Fig. 2b, where PEG was loaded after LS forming. PEG actually had an adsorption thickness of about 0.2 nm on LS film (point 4). Even after rinsing, there was still about 0.1 nm PEG remained on LS film, indicating that PEG has a relative strong affinity to lignin or lignin-derived materials. This provides some evidences that PEG can be adsorbed on the surface of residual lignin in substrate, albeit this adsorption is not strong. The authors in other studies have similar findings [39].

## **2.2 Enzymatic efficiency assessment of enzymatic synergistic agents for Avicel and green liquor treated substrate**

In this study, we aimed to elucidate the effect of addition on enzymatic hydrolysis reactions of pure microcrystalline cellulose, i.e., Avicel, and poplar pulp pretreated with green liquid containing lignin, to explore the mechanism of these addition in the process of cellulase hydrolysis.

Figure 4 showed that by adding BSA the enzymatic hydrolysis efficiency of Avicel was increased from 46–69% while the efficiency of GL was increased from 69–83%. The addition of PEG increased the enzymatic hydrolysis efficiency of microcrystalline cellulose to 80% and the enzymatic hydrolysis efficiency of GL to 87%. Unlike them, the addition of LS inhibited the enzymatic hydrolysis of microcrystalline cellulose (35%), but it enhanced the enzymatic hydrolysis efficiency of GL (75%). In conclusion, BSA and PEG can be effective to increase the enzymatic hydrolysis efficiency for both pure cellulose and lignocellulose sample with residual lignin; while LS can also enhance the enzymatic hydrolysis efficiency of lignocellulose with residual lignin, but inhibits the enzymatic hydrolysis of pure cellulose. Similar results have been found in other studies [11, 22].

## 2.3 Proposed interaction mechanisms

Based on our interaction study between cellulase and BSA/PEG/LS, and the saccharification promotion assessment for Avicel and green liquor treated substrate, the proposed interaction mechanisms of synergists of BSA/PEG/LS are presented in Fig. 5.

Without synergist, it has been widely recognized that during the enzymatic hydrolysis of cellulose, the substrate lignin (i.e., residual lignin) adsorbs cellulase unproductively, thus reducing the enzymatic hydrolysis efficiency of lignocellulose as illustrated in Fig. 5a [2, 3].

As illustrated in Fig. 5b, LS particles can bind with cellulase to form complexes through electrostatic interaction forces and other forces that not fully recognized and understood. Actually, the formed complex of LS-Cell has a lower enzymatic activity than their native one. It inhibits the enzymatic hydrolysis of cellulose in the absence of substrate lignin. Nevertheless, in the situation where residual lignin presents in the substrate, the LS-Cell complex formed in the solution can eventually reduce the nonproductive adsorption of cellulase on residual lignin in substrate. Therefore, the lignocellulose saccharification promotion effect of LS for the enzymatic hydrolysis is very complicated. It should be balanced the benefits from the reduced non-productive binding to residual lignin and the costs of reduced enzymatic activity of LS-Cell complex. When there was non- or less residual lignin presence in the matrix of lignocellulose, the reduced enzymatic activity of LS-Cell complex cannot be overcome by the reduced non-productive binding, the total effect should be negative. While when the residual lignin in the lignocellulose matrix is substantial, the non-productive binding is dominant in the enzymatic hydrolysis. In this situation, the promotion effect of LS should be obvious since the gain in the nonproductive binding reduction can beat the loss in enzymatic activity. In practice, the pretreatment removes part of lignin from substrate, substantial residual lignin remains in the substrate. Therefore, LS can still be considered as a good synergistic agent in lignocellulose enzymatic hydrolysis.

As illustrated in Fig. 5c, Unlike LS particles, PEG does not form complex with cellulase. It can enhance the activity of cellulase on one hand. On the other hand, PEG can adsorb on substrate lignin, preventing cellulase from adsorbing on substrate lignin nonproductively. This hypothesis was brought up by Lei et al [39]. that PEG forms a physical barrier to reduce the adsorption of cellulase onto substrate lignin. It was confirmed by our interaction study that PEG does have a high affinity to lignin and it does form a physical barrier to prevent nonproductive binding of cellulase.

As illustrated in Fig. 5d, like LS, BSA does form BSA-Cell complex, whereas the BSA-Cell complex plays an active role in the enzymatic hydrolysis for microcrystalline cellulose. This can be attributed to that the BSA-Cell stabilizes cellulase activity [14]. In sum, BSA can increase the sugar conversion rate of lignocellulosic biomass in two aspects, one is increased enzymatic activity induced by enzyme stabilization; the other is exhibited in nonspecific adsorption on residual lignin to occupy the binding sites for cellulase. It was found that with the increase of enzyme load, the improvement of enzymatic hydrolysis effect by adding BSA gradually decreased [22]. It supports the action mechanism brought up by us.

### 3. Conclusions

Although all three representatives of enzymatic hydrolysis synergistic agents can promote the enzymatic hydrolysis of lignocellulose, we found that LS, BSA and PEG have totally different action modes. LS and BSA can form complexes with cellulase, while the complexes formed with the former reduce the cellulase activity, as comparison, the latter enhances the cellulase activity. The complex of LS-Cell promotes the enzymatic hydrolysis of lignocellulose only in the presence of residual lignin of substrate due the electrostatic repulsion between LS-Cell complex and residual lignin. Unlike LS, BSA complex works both from its enhanced cellulase activity and reducing the nonspecific adsorption of cellulase and substrate lignin. Although PEG does not bind to cellulase, it can also enhance cellulase activity for pure cellulose due to its surfactant properties, while for lignocellulose containing residual lignin due to the reduced nonproductive adsorption of cellulase since it prefers to bind to residual lignin to form a steric physical barrier. This investigation shed some lights on the molecular mechanisms of how different enzymatic hydrolysis synergistic agents work from the perspective of their interactions with enzymes. It will be helpful in the practical enzymatic hydrolysis process and in designing of enzyme engineering.

## 4. Experimental

### 4.1 Materials

Chemicals of 11-mercaptoundecanoic acid (MUA, 98%), 3-mercaptopropionic acid (MPA, 98%), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 98%), N-hydroxysulfosuccinimide sodium salt (NHS, 98%) and bovine serum albumin (BSA, 96%) were purchased from Aladdin Corp. (Shanghai, China). Commercial soluble liginosulfonate (Reax 85A, with an averaged MW of 10,000) was supplied by

Mead Westvaco (Atlanta, GA). Polyethylene glycol (PEG, with an averaged MW of 4000) was procured from Shenggong Bioengineering Corp. (Shanghai, China).

Lignocellulosic biomass material was a poplar pulp, which was pretreated with green liquor as described elsewhere [40]. Poplar chips were collected from the north of Jiangsu Province, China. The cellulase (Cellic®CTec2) for cellulose hydrolysis was provided by Novozymes Corp. (Franklinton, NC) and the activity determined by Whatman No. 1 filter paper was 180 FPU/mL through the standard method recommended by IUPAC Commission Biotechnology. All other reagents were purchased from Nanjing Reagent Co., LTD (Nanjing, China).

QCM-D sensors with gold surface (Product number QSX 301) were supplied by Biolin Scientific Co. Ltd (Gothenburg, Sweden), while SPR sensors by BioNavis Co. Ltd. (Tampere, Finland).

## **4.2 Real time interaction probing by QCM-D and SPR**

The cleaning procedures of gold QCM-D and SPR chips and the in situ immobilization of cellulase on gold chips can refer to our previous work [31]. Briefly, the chips were modified using a mixture of thiol-containing organic molecules (MUA and MPA), which resulted to form a mixed self-assembled monolayer with carboxylic groups as the exposed tail group. The cellulase then covalently bonds to the chips through a peptide bond.

Once a uniform cellulase layer was immobilized onto the gold chips of QCM or SPR sensors, an acetate buffer solution (pH = 4.8, 0.05M) was flushed to the system and a stable baseline was obtained. Then the respective synergistic agent solution was introduced into the chambers of QCM-D or SPR and interacted with the immobilized cellulase until equilibrium. LS solution (Reax 85A) with a concentration of 4.5 mg/mL was pumped into the reaction tank at a rate of 0.100 mL/min. After adsorption equilibrium, the buffer solution was injected for rinsing. Similarly, the effects of BSA/PEG and cellulase were studied in the same way. PEG solution with a concentration of 4.5 mg/mL, whereas BSA had a concentration of 0.05 mg/mL (50 ppm). All synergistic agents were dissolved in the same buffer that was administered when the baseline was obtained.

## **4.3 SAXS measurements**

X-ray small Angle scattering was used to study whether the complexes of cellulase and LS/PEG formed and what are the sizes of the complexes. This part of experiment was performed in Shanghai Light source center BL19U2 biological small Angle station. The sample moves at a speed of 10  $\mu$ L/s in the capillary. The main technical indicators were beam intensity of 240 mA, energy of 12 keV, detector for Pilatus 1 M, sample to probe distance of 2.6 m, and the wavelength of 1.03 Å.

## **4.4 Enzymatic hydrolysis experiments**

Enzymatic hydrolysis was performed at 2% (w/v) in 50 mL of pH = 4.8 and 0.05M acetic acid - sodium acetate buffer in a corked conical flask at 50 °C and 180 rpm for 72 h. The substrate, buffer, synergic agent, and cellulase were added in turn. The cellulase loading was 20 FPU/g glucan. The addition

amount of LS, PEG and BSA was 0.2 g/g, 1 g/L and 5 g/L, respectively, based on the optimal dosage in literature [11, 22, 41]. After enzymatic hydrolysis, the hydrolysate was boiled for 10 min to inactivate cellulase. After that, all the mixtures were filtered through a 0.22  $\mu\text{m}$  aqueous phase membrane. The glucose content in hydrolysate was measured by liquid chromatography (Waters ACQUITY Arc, UAS). The mobile phase was 5 mM  $\text{H}_2\text{SO}_4$ , the flow rate was 0.6 mL/min, and the column temperature was 55  $^\circ\text{C}$ . All the data were averaged from the two groups and their errors were calculated.

## Abbreviations

BSA: Bovine serum albumin; PEG: polyethylene glycol; LS: liginosulfonate; GL: green liquor-pretreated lignocellulose; MUA: Chemicals of 11-mercaptoundecanoic acid; MPA :3-mercaptopropionic acid; EDC: N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride; NHS: N-hydroxysulfosuccinimide sodium salt; QCM-D: Quartz Crystal Microbalance with Dissipation monitoring; SPR: Surface Plasmon Resonance; SAXS: Small Angle X-ray Scattering.

## Declarations

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### Authors' contributions

Peipei Wang: Investigation, Data curation, Methodology, Writing-original draft. Tian Liu: Validation. JiaqiGuo: Writing-review & editing. Yongcan Jin: Funding acquisition. Huining Xiao: Supervision. Jinyou Lin: Methodology. Junlong Song: Conceptualization, Data curation, Methodology, Funding acquisition, Writing-review & editing.

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### Availability of data and materials

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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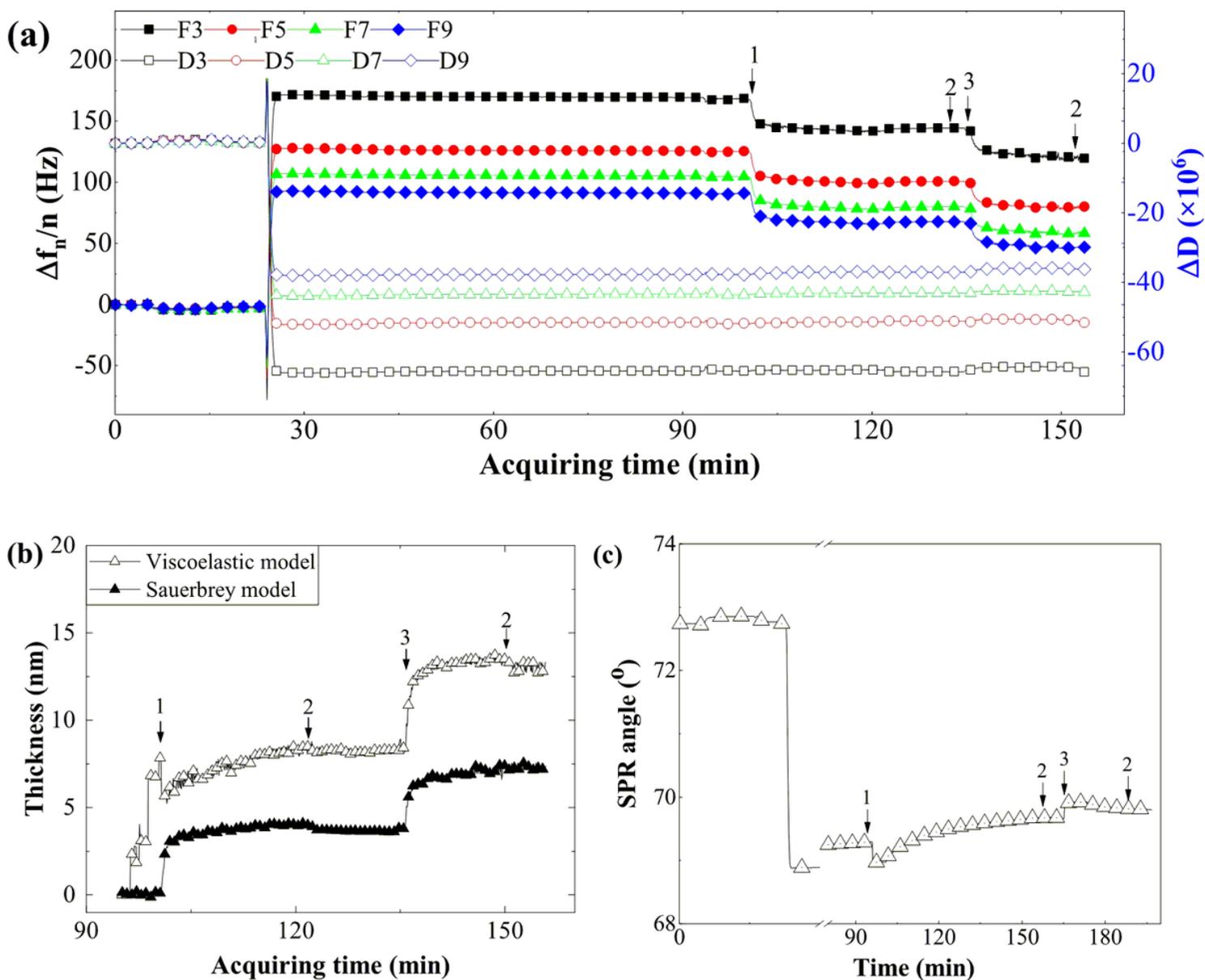
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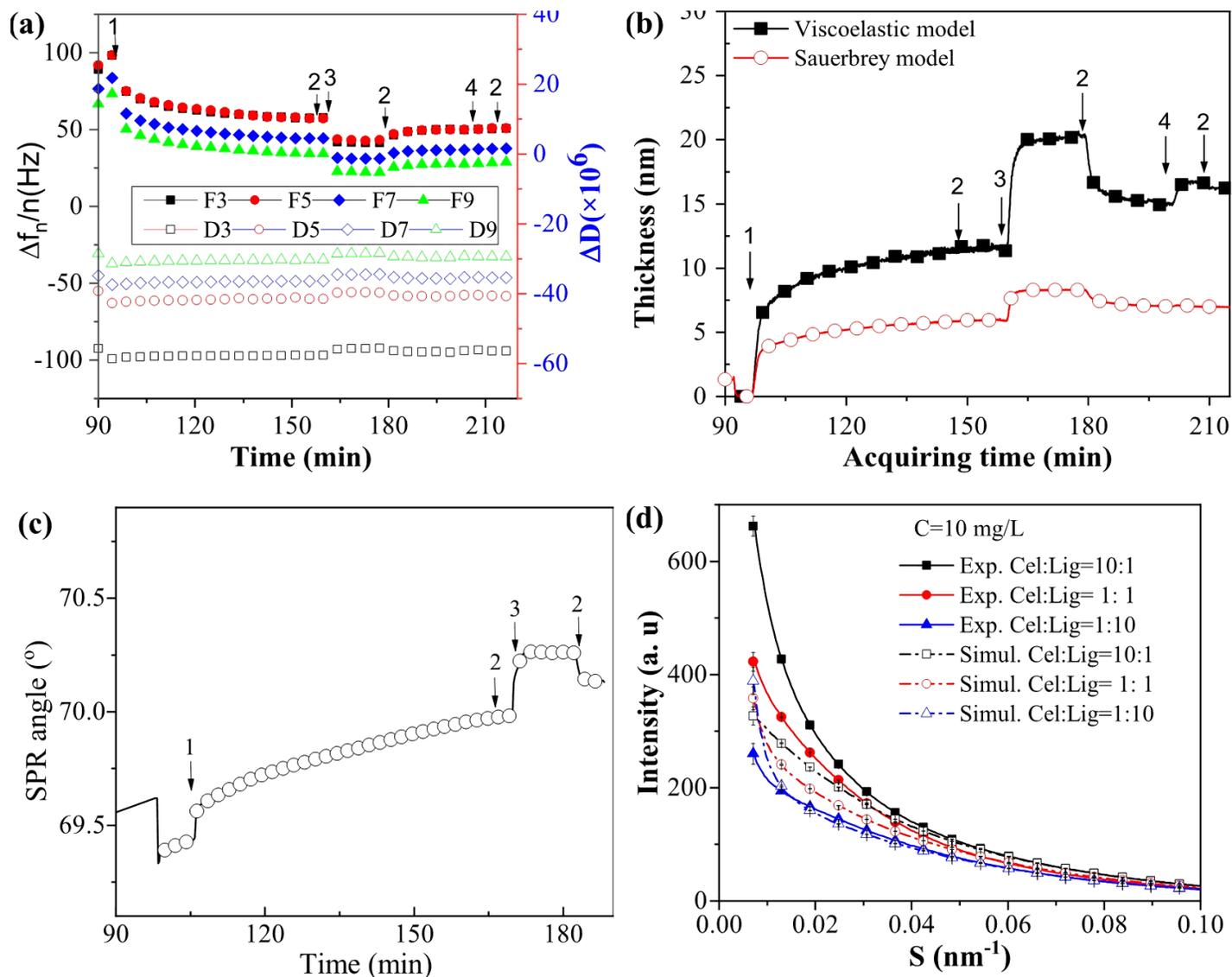
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## Figures



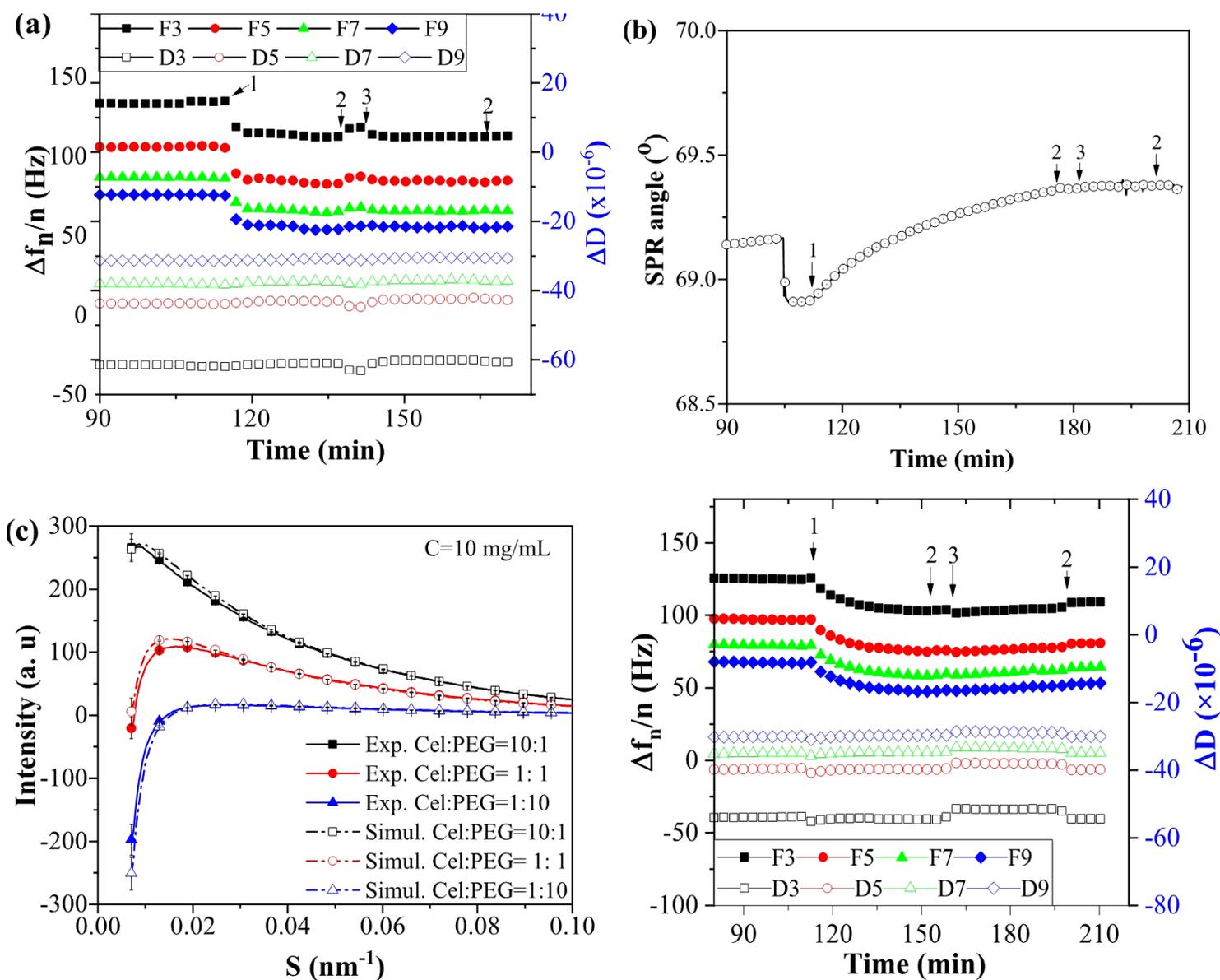
**Figure 1**

Interaction study between cellulase (Cell) and BSA. (a) Frequency change and energy dissipation for the interaction between cellulase and BSA was obtained on a QCM-D E4 equipment in-situ and in real time. (b) Fitted thickness of the layer on cellulase film with the addition of BSA. Open symbols represent the data extracted from viscoelastic modeling while solid symbols derived from Sauerbrey equation directly. (c) SPR signal change for the interaction between BSA and cellulase was obtained on a MP-SPR.



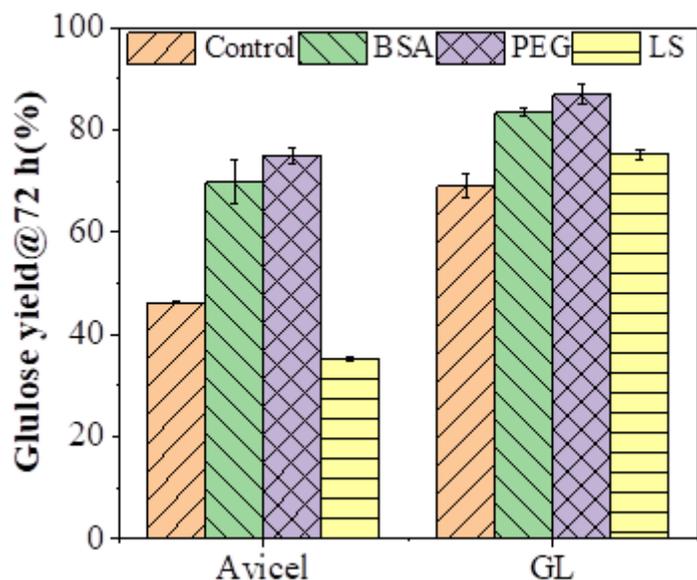
**Figure 2**

Interactions study between cellulase (Cell) and lignosulfonate (LS). (a) Frequency change and energy dissipation for the interaction between cellulase and LS was obtained on a QCM-D E4 equipment in-situ and in real time. (b) Fitted thickness of the layer on cellulase film with the addition of LS. Solid symbols and stars represent the data extracted from viscoelastic modeling while open symbols derived from Sauerbrey equation directly (c) SPR signal change for the interaction between LS and cellulase was obtained on a MP-SPR. (d) The small-angle X-ray scattering signal of the mixture of cellulase and LS in different proportion. (Cellulase and LS concentrations were both 10 mg/mL and dissolved in 0.05M, pH 4.8 acetate buffer solution).



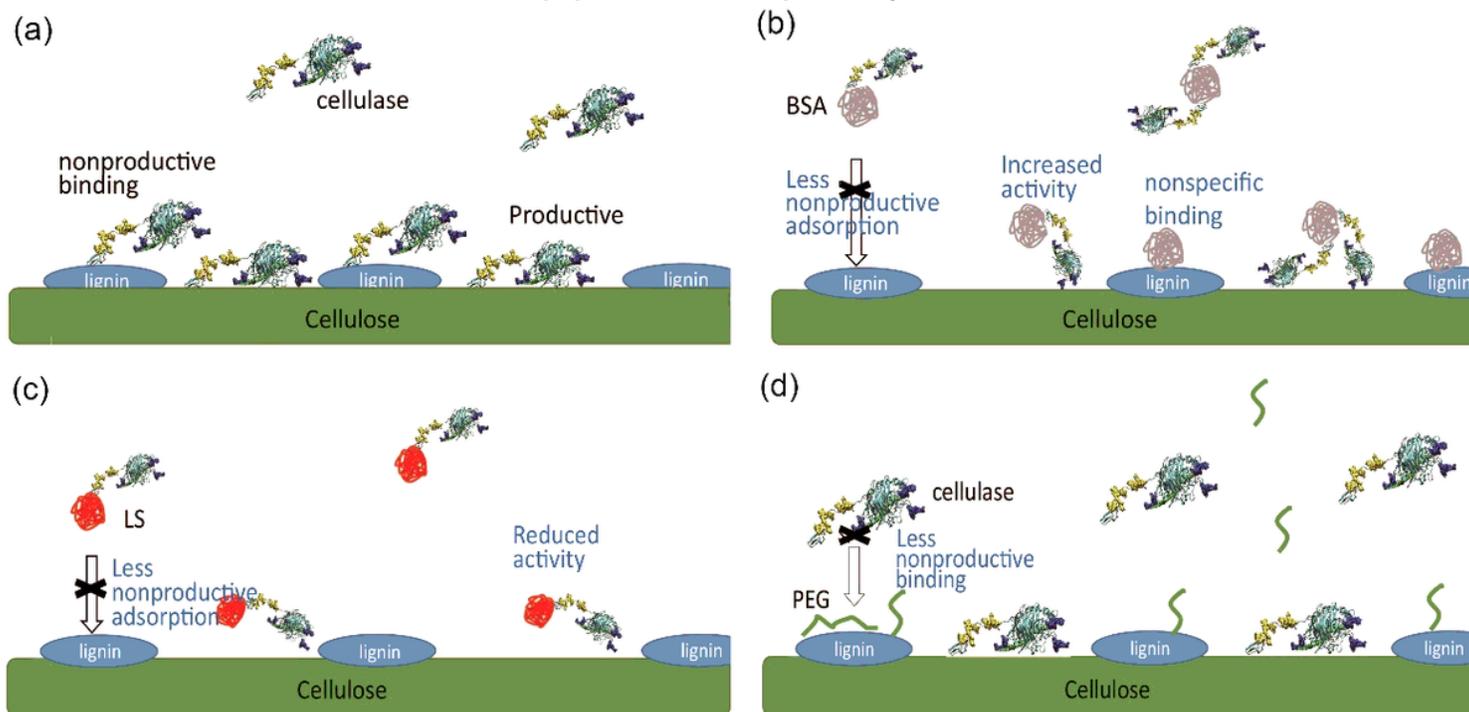
**Figure 3**

Interactions study between cellulase (Cell) and PEG. (a, d) Frequency change and energy dissipation for the interaction between cellulase and PEG was obtained on a QCM-D E4, and (b) SPR signal change for the interaction between PEG obtained on a MP-SPR. (c) The small-angle X-ray scattering signal of the mixture of cellulase and PEG in different proportion. Acetate buffer (0.05M, pH 4.8) was used in a, b and c, while phosphate buffer (0.05M, pH 7.5) was used in d.



**Figure 4**

Activity of the mixture of cellulase and enzymatic synergistic agents. The glucose conversion rates of Avicel and GL at 72h were measured with or without the addition of synergistic agents, in which dosages of LS, PEG, and BSA were 0.2, 1, and 5g/g-substrate, respectively.



**Figure 5**

Proposed saccharification promotion mechanisms of (a) without synergetic agent addition, and with synergetic agent addition of (b) BSA, (c) LS and (d) PEG.