Protective Effects of Five Surfactants on Cellulase in the Saccharification of Corn Stover Based on the Impeded Michaelis-Menten Model

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Protective effects of five surfactants were investigated relative to the saccharification of lignocellulose using the impeded Michaelis-Menten model (IMM). The yield of total reducing sugar (Y_{trs}) and cellulase activity were indexed as the effect of surfactant. The IMM was used to fit the correlation between Y_{trs} and reaction time to obtain the index ($K_{obs,0}$) reflecting the accessibility between cellulose and lignocellulose and the comprehensive index (K_i) reflecting cellulase inactivation and non-specific site adsorption. Results showed that the strongest protective effect was found from polyoxyethylene (80) sorbitan monooleate, followed by rhamnolipid. The surfactants protected cellulase from inactivation and nonspecific site adsorption of lignocellulose in the saccharification, leading to enhanced cellulase activity, especially with respect to carboxymethyl cellulase (CMCase) and filter paper enzyme (FPase) activities. The maximum Y_{trs} was obtained when the CMCase activity was 136.2 U/mL, while the FPase and β-glucosidase activities should be as high and low as possible, respectively, under the optimized condition. These findings lay the foundation for improving the saccharification efficiency of cellulase and reducing the cost of saccharification of biomass cellulose.

Keywords: Surfactant; Impeded Michaelis-Menten model; Nonspecific site adsorption; Enzymatic activity; Saccharification

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INTRODUCTION

Low saccharification efficiency is the bottleneck of bioconversion of lignocellulose into ethanol, butyl alcohol, or sugar. Many reports have shown that surfactants can enhance the saccharification efficiency to produce more reducing sugar, leading to an increased conversion rate of ethanol (Castanon and Wilke 1981; Ooshima *et al*. 1986; Park *et al*. 1992; Kristensen *et al*. 2007). However, little data is available about the mechanism underlying the enhancing effects of surfactants on saccharification efficiency of lignocellulose, and different conclusions have been achieved so far, as follows: (1) surfactants change the substrate structure, making cellulase more accessible to cellulose (Helle *et al*. 1993); (2) surfactants effectively decrease the thermal denaturation of cellulase and increase the stability of these enzymes (Reese 1980; Kaar and Holtzapple 1998); and

(3) surfactants protect the adsorbed enzyme against denaturation and enhance the interaction between cellulase and cellulose (Kurakake *et al*. 1994; Eriksson *et al*. 2002).

Surfactants can be divided into synthetic surfactants and biosurfactants. Polyoxyethylene (80) sorbitan monooleate (Tween 80), polyoxyethylene (20) sorbitan monolaurate (Tween 20), octylphenol Ethoxylate with 10 moles of ethylene oxide (OPE-10), polyethylene glycol, and sodium dodecyl sulfate (SDS) are commonly used synthetic surfactants. Biosurfactants are the metabolic products derived by bacteria, yeasts, and fungi. Compared with the synthetic surfactants, biosurfactants have special advantages, such as better biodegradability, low toxicity, low solubility, and insensitivity to extreme temperature and pH. Betaine, rhamnolipid, and glycine are commonly used biosurfactants (Cooper 1986; Mulligan 2005). Polyoxyethylene (80) sorbitan monooleate and rhamnolipid are glycolipid surfactants, SDS is organic salt surfactant, betaine is alkaloid surfactant, and glycine was amino acid surfactant. Betaine is a cation surfactant, whereas SDS is an anionic surfactant. Polyoxyethylene (80) sorbitan monooleate and rhamnolipid are nonionic surfactants. These five surfactants possess different sources, structure, and mechanisms and properties. Thus they were selected to study their protective effect on cellulase. Different surfactants have various protective effects on different enzymes.

A kinetic model plays an essential role in describing the reaction process to analyze the effect of a surfactant on saccharification. For enzyme kinetics, the classical Michaelis-Menten model is practically suitable in a homogeneous system. In fact, the lignocellulose hydrolysis is conducted in a heterogeneous system. In heterogeneous systems, cellulose is hydrolyzed into cellobiose by endo-β-1,4-glucanase and exo-β-1,4-glucanase, and cellobiose is hydrolyzed into glucose by β-glucosidase. Various alternative models have been proposed to simulate the enzymatic reactions in a heterogeneous system, such as Michaelis-Menten-based models, empirical models, Langmuir adsorption isotherm-based models, the jammed Michaelis kinetics model, the fractal Michaelis kinetics model, and the kinetic model based on shrinking-particle theory and the Langmuir isotherm concept (Movagharnejad and Sohrabi 2003; Xu and Ding 2007; Bansal *et al*. 2009). However, these models contain several complicated equations that should be solved and many parameters that cannot be uniquely determined, and even some parameters are arbitrarily chosen rather than from a fitting process based on experiments (Ye and Berson 2011).

In lignocellulose saccharification, many factors affect the reaction process, including mass-transfer resistance, interactions of enzyme and lignocellulosic biomass, and enzyme inhibition (Gan *et al*. 2003). Based on these factors, Yang and Fang have constructed the impeded Michaelis-Menten model (IMM) (Yang and Fang 2015a,b) with advantages as follows: (1) the parameters in the model can be accurately determined; and (2) the model can provide some information for practical applications, such as system design and optimization. In the authors' previous study, the IMM was used to assess the kinetics of cellulase saccharification of corn stover (CS) after pretreatment with lignin peroxidase and H_2O_2 (Zhang *et al*. 2016), and the effect of pretreatment condition on the oxidative degradation of lignin has been analyzed (Liu *et al*. 2019a). In the present study, the IMM is used to analyze the protective effects of five surfactants on cellulase in the saccharification of CS pretreated by a combination of steam-explosion and NaOH (SE-NaOH) treatments.

EXPERIMENTAL

Materials

The CS was obtained from a local farm and crushed into a fine powder of less than 0.25 mm. The main components of CS powder included 26.5% hemicellulose, 34.1% cellulose, and 15.5% lignin. *Trichoderma reesei* cellulase was obtained from Guangzhou Global Green Group Tech. Ltd. (Guangzhou, China). The activities of carboxymethyl-cellulase (CMCase), filter paper enzyme (FPase), β-glucosidase, and xylase were 5.97 × 10^4 U/mL, 1.71 × 10^4 FPU/mL, 1.68 × 10^4 U/mL, and 2.84 × 10^4 U/mL, respectively. All chemicals were of analytic grade and purchased from East China Chemical and Glass-Instruments Co., Ltd. (Zhenjiang, China).

SE-NaOH Pretreatment

The dried CS pretreated by SE at 1.5 MPa for 400 s was washed and dried at 80 °C, followed by treatment with 2% NaOH for 1 h. The pretreated substrate was washed with distilled water to neutral pH, dried at 80 °C to constant weight, and preserved at room temperature.

Enzymatic Saccharification

Several surfactants, including glycine, betaine, SDS, polyoxyethylene (80) sorbitan monooleate (POESM), and rhamnolipid, were compared in the current experiment. All saccharification experiments of the pretreated CS were conducted in a 100-mL Erlenmeyer flask. Briefly, 1 g CS pretreated by SE-NaOH was added to 20 mL 0.1 mmol/L acetate buffer containing 1% cellulase (pH = 4.4) and various surfactants at different concentrations (Table 1). After the mixture was fully blended, the initial concentration of the reducing sugar was determined and denoted as C_0. The saccharification reaction was maintained at 47 °C in a shaking water bath (160 rpm) for 32 h. Subsequently, samples were withdrawn at 1, 2, 4, 8, 16, and 32 h. Each sample was immediately cooled in an ice bath to room temperature to terminate the reaction and then centrifuged at 4,000 rpm for 15 min. The supernatant was used for determination of the yield of total reducing sugar (Y_{trs}) and activities of CMCase, FPase, and β-glucosidase.

Table 1. Effects of Different Concentrations of Surfactants on the Saccharification Reaction

Surfactants	Concentration (%)				
Glycine	0.02	0.05	0.10	0.20	0.50
Betaine	0.02	0.05	0.10	0.20	0.50
SDS	0.02	0.05	0.10	0.20	0.50
POESM	0.025	0.05	0.10	0.25	0.50
Rhamnolipid	0.03	0.06	0.12	0.24	0.48

Analysis

The concentration of reducing sugar was determined according to the 3,5-dinitrosalicylic acid method (Miller 1959) and denoted as C_1. The yield of total reducing sugar (Y_{trs}) was calculated according to Eq. 1,

$$Y_{trs}\ (\%) = (C_1 - C_0) \times V/G \times 100 \quad (1)$$

where V is the volume of the reaction solution (mL), G is the weight of total dry substrate (g), and C_0 and C_1 are the reducing sugar concentrations (g/mL) at 0 h and t h of reaction, respectively.

FPase and CMCase activities were determined by the methods described by Ghose (1987) and Eveleigh *et al.* (2009), respectively, and the β-glucosidase activity was determined by the method described by Ghose (1987). One unit of enzyme activity was defined as the amount of enzyme required to release 1 μmol of reducing sugar (expressed as glucose) per min from the original substrate under the experimental conditions. The lignin contents in CS were determined respectively by two-step acid hydrolysis according to the NREL LAP method described by Sluiter *et al.* (2008). The lignin content was defined as the sum of acid-soluble and -insoluble portions; the latter was measured by gravimetric analysis, and the former by UV-Vis spectroscopy. Cellulose and hemicellulose content was measured according to the methods described by Mussatto and Roberto (2006).

IMM

The saccharification of CS is carried out in a heterogeneous system. Many factors impact the saccharification reaction kinetics of CS. These factors include mass-transfer resistance, interactions of enzyme and lignocellulosic biomass, and enzyme inhibition. According to the Michaelis–Menten model with a modification, the "impeded" Michaelis model applied in this study describes the saccharification in a heterogeneous system. Several assumptions in IMM are made: (1) the adsorption of enzymes on the solid substrate is very much faster than the enzymatic reactions, (2) the saccharification actions of cellulase on the inert and non-reactive materials, the product inhibition, and the mass-transfer resistance for cellulases are combined as the impeded reaction of enzymes with a time-dependent decay coefficient, (3) the saccharification is considered as the combined effect of the cellulase system on the substrate, and (4) the effect of the pretreatment for changing the structure of substrate is reflected in the corresponding reaction coefficients.

According to IMM, the Y_{trs} reflecting saccharification efficiency depended on the accessibility of cellulase to the cellulose in CS and residual cellulase activity in reaction solution, and their correlation could be described as follows,

$$\frac{dY_{trs}}{d_t} = K_{obs,0} \times \frac{1}{1+\alpha t}(1-Y_{trs}) \quad (2)$$

where $K_{obs'0}$ reflects the accessibility of cellulase to cellulose, and a is a constant. The coefficient of the time-dependent inactive enzyme (K_i) can be calculated according to the constant a using the following equation:

$$K_i = \frac{2\alpha}{1+\alpha t} \quad (3)$$

Equation 2 can be solved and then rearranged as,

$$\frac{t}{-\ln(1-Y_{trs})} = \frac{1}{K_{obs,0}} + \frac{\alpha}{K_{obs,0}} t \quad (4)$$

where Eq. 3 was applied to fit the experimental data by plotting $t/(-\ln(1 - Y_{trs}))$ *versus* t. The $K_{obs,0}$ is the reciprocal of the constant, and a is the coefficient of t divided by the constant.

All data were expressed as means ± standard errors. All the regression equations were conducted using SPSS 17.0 software and the analysis of variance (ANOVA) was performed by Dunnett's test, where $p<0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Effects of Glycine on Saccharification

Figure 1 shows the effects of glycine concentration and reaction time on the changing trends of Y_{trs}. The authors found that with the extension of reaction time, Y_{trs} was rapidly increased in the initial 4 h and it slowly increased in the later 28 h, whereas Y_{trs} gradually decreased with the increase of glycine concentration. Therefore, it was deduced that it was impossible to increase saccharification efficiency using glycine. Glycine is an amino acid-based surfactant. Holmberg believes that although amino acid-based surfactants are environmentally friendly, they cannot increase the cellulase activity during saccharification (Holmberg 2018). Holmberg's idea supported the authors' result.

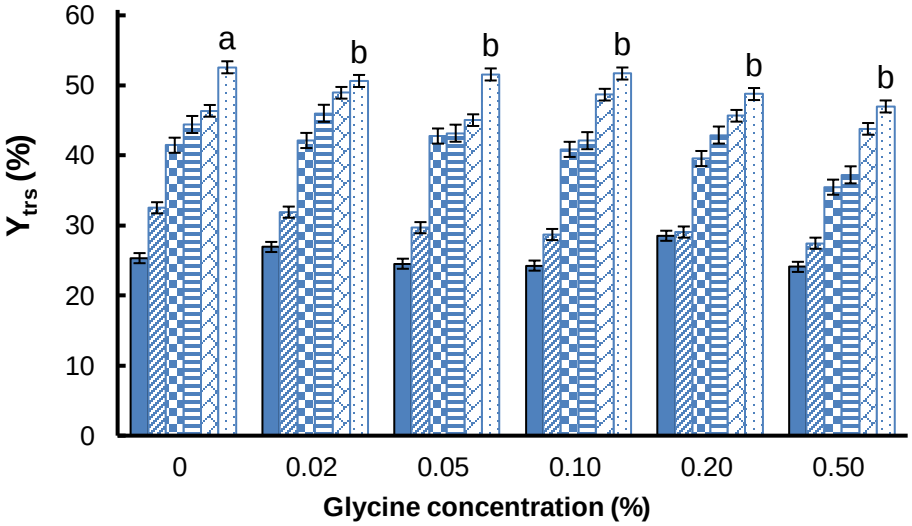

Fig. 1. Effect of glycine concentration on Y_{trs} at different reaction time points: ■: 1 h; ▨: 2 h; ⊠: 4 h; ▤: 8 h; ▨: 16 h; and ⊡: 32 h. a: the control; b: significantly less than the control ($p < 0.05$)

To analyze the effect of glycine in the saccharification process, the data of Fig. 1 were fit by IMM (Table 2). All R^2 higher than 0.9 and near adj-R^2 indicated that IMM achieved an excellent fit the data in Fig. 1. Table 2 reveals that $K_{obs,0}$ and K_i concurrently increased or decreased with the increase of glycine concentration compared with those in the absence of glycine. The increase of $K_{obs,0}$ indicated the enhanced accessibility between cellulase and substrate, and the increase of K_i exhibited enzyme deactivation. The factors causing enzyme deactivation include lignin adsorption, nonspecific site adsorption of lignocellulose (Lou *et al*. 2013), and feedback inhibition of saccharification end-production. Thus, it is speculated that the nonspecific site adsorption of lignocellulose might be the key factor among these factors.

Table 2. Effect of Glycine on Saccharification Kinetic Model of Pretreated CS

Concentration (%)	$t/-\ln(1 - Y_{trs})$	R^2	Adj-R^2	$K_{obs,0}$ (h^{-1})	K_i (h^{-1})
0	1.2786x + 2.8255	0.994	0.992	0.3539	0.0585
0.02	1.3438x + 2.0846	1.000	1.000	0.4797	0.0596
0.05	1.3170x + 2.9606	0.992	0.990	0.3378	0.0584
0.10	1.2816x + 3.0502	0.998	0.997	0.3278	0.0582
0.20	1.4238x + 2.4468	0.998	0.998	0.4087	0.0593
0.50	1.4790x + 3.3930	0.996	0.995	0.2947	0.0583

Table 3. Effect of Glycine on Cellulase Activity (U/mL)

Concen-tration (%)	Enzyme*	1 h	2 h	4 h	8 h	16 h	32 h
0	CMC	98.9 ± 3.1	66.2 ± 3.2	128.4 ± 9.0	100.7 ± 11.2	88.2 ± 11.0	81.5 ± 10.5
	FP	0.77 ± 0.05	0.78 ± 0.07	0.41 ± 0.08	0.55 ± 0.05	0.35 ± 0.05	0.29 ± 0.03
	β-G	0.93 ± 0.10	0.99 ± 0.10	0.80 ± 0.10	1.05 ± 0.10	1.24 ± 0.13	0.84 ± 0.11
0.02	CMC	99.5 ± 3.8	67.0 ± 6.9	128.6 ± 8.6	94.5 ± 8.1	100.2 ± 5.6	82.8 ± 4.6
	FP	0.67 ± 0.02	0.69 ± 0.03	0.43 ± 0.06	0.51 ± 0.17	0.44 ± 0.08	0.24 ± 0.06
	β-G	1.09 ± 0.15	1.08 ± 0.15	0.90 ± 0.09	0.95 ± 0.08	1.04 ± 0.06	1.00 ± 0.19
0.05	CMC	100.9 ± 5.8	67.2 ± 8.5	130.7 ± 11.7	87.9 ± 13.1	95.6 ± 9.6	81.0 ± 12.8
	FP	0.77 ± 0.02	0.62 ± 0.10	0.50 ± 0.07	0.51 ± 0.05	0.40 ± 0.10	0.23 ± 0.07
	β-G	0.91 ± 0.02	1.04 ± 0.06	0.80 ± 0.08	1.06 ± 0.17	0.90 ± 0.06	0.68 ± 0.15
0.10	CMC	100.5 ± 11.1	62.5 ± 4.8	128.4 ± 13.3	98.6 ± 7.6	85.4 ± 6.2	82.0 ± 13.2
	FP	0.91 ± 0.05	0.64 ± 0.02	0.57 ± 0.07	0.50 ± 0.03	0.31 ± 0.05	0.28 ± 0.01
	β-G	0.89 ± 0.10	0.98 ± 0.07	0.82 ± 0.27	0.94 ± 0.12	0.66 ± 0.11	0.72 ± 0.12
0.20	CMC	102.3 ± 3.1	62.5 ± 3.5	126.9 ± 11.3	101.9 ± 7.7	82.7 ± 10.0	80.1 ± 8.8
	FP	0.63 ± 0.07	0.66 ± 0.04	0.56 ± 0.05	0.43 ± 0.17	0.45 ± 0.03	0.19 ± 0.07
	β-G	0.93 ± 0.12	1.12 ± 0.15	0.88 ± 0.09	0.89 ± 0.12	0.75 ± 0.10	0.94 ± 0.09
0.50	CMC	99.6 ± 3.7	62.2 ± 3.2	127.9 ± 10.8	98.2 ± 10.2	87.5 ± 7.6	76.6 ± 6.9
	FP	0.69 ± 0.07	0.70 ± 0.04	0.51 ± 0.12	0.49 ± 0.04	0.35 ± 0.05	0.21 ± 0.02
	β-G	1.06 ± 0.09	0.89 ± 0.09	0.77 ± 0.08	0.86 ± 0.05	0.87 ± 0.08	0.89 ± 0.11
*CMC, FP, and β-G were expressed as CMCase, FPase, and β-glucosidase, respectively							

The activities of CMCase, FPase, and β-glucosidase were analyzed to classify the deactivation effect of glycine on cellulase. Table 3 shows that the CMCase activity rapidly decreased in the initial 2 h, increased between 2 h and 4 h, and then slowly decreased after 4 h. Such fluctuating activity was explained as follows. In the initial 2 h, cellulase rapidly adsorbed on the surface of lignocellulose, which led to the decreased CMCase activity, and then hydrolysis of lignocellulose resulted in cellulase release, resulting in increased CMCase activity again. After 4 h, slow deactivation of enzyme caused the decrease of enzyme activity. The CMCase activity was not significantly changed in the presence of glycine at various concentrations compared with that in the absence of glycine ($p < 0.05$).

Table 3 shows that when the glycine concentration was less than 0.01%, the FPase activity in the presence of glycine was not obviously changed compared with that in the absence of glycine ($p > 0.05$). However, when the glycine concentration was higher than 0.01%, the FPase activity in the absence of glycine was significantly decreased. Table 3 exhibits that the presence of 0.05 to 0.1% glycine could reduce the β-glucosidase activity. This finding could explain why the effect of glycine at a low concentration (< 0.05%) on β-glucosidase was not significant. In contrast, glycine at a high concentration could protect β-glucosidase activity.

Effect of Betaine on the Saccharification

Under the same conditions, the Y_{trs} of lignocellulose saccharification significantly increased when the betaine concentration increased from 0 to 0.02% ($p < 0.05$), while Y_{trs} maintained stable when the betaine concentration increased from 0.02% to 0.05% ($p > 0.05$) (Fig. 2). However, when the betaine concentration was further increased, the Y_{trs} gradually decreased. The largest Y_{trs} (68.74%) was obtained in the presence of 0.02% betaine at 32 h, which increased 30.19% compared to in the absence of betaine. Therefore, the optimum betaine concentration was 0.02%.

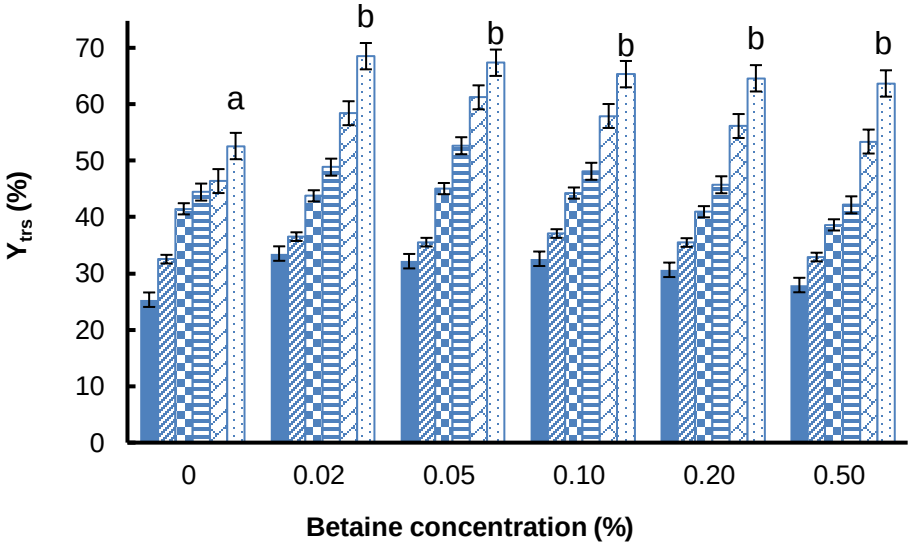

Fig. 2. Effect of betaine concentration on Y_{trs} at different reaction time points: ■: 1 h; ▨: 2 h; ⊠: 4 h; ▤: 8 h; ▨: 16 h; and ⊡: 32 h. a: the control; b: significantly higher than the control ($p < 0.05$)

Table 4. Effect of Betaine on Saccharification Kinetic Model of Pretreated CS

Concentration (%)	$t/-\ln(1 - Y_{trs})$	R^2	Adj-R^2	$K_{obs,0}$ (h^{-1})	K_i (h^{-1})
0	1.2786x + 2.8255	0.994	0.992	0.3539	0.0585
0.02	0.7913x + 3.5601	0.970	0.962	0.2809	0.0548
0.05	0.8114x + 3.0723	0.992	0.989	0.3255	0.0559
0.10	0.8697x + 3.2214	0.983	0.979	0.3104	0.0560
0.20	0.8799x + 3.7152	0.976	0.970	0.2692	0.0552
0.50	0.8952x + 4.4159	0.962	0.952	0.2265	0.0542

Table 5. Effect of Betaine on Cellulase Activity (U/mL)

Concen-tration (%)	Enzyme*	1 h	2 h	4 h	8 h	16 h	32 h
0	CMC	98.9 ± 3.1	66.2 ± 3.2	128.4 ± 9.0	100.7 ± 11.2	88.2 ± 11.0	81.5 ± 10.5
	FP	0.77 ± 0.05	0.78 ± 0.07	0.41 ± 0.08	0.55 ± 0.05	0.35 ± 0.05	0.29 ± 0.03
	β-G	0.93 ± 0.10	0.99 ± 0.10	0.80 ± 0.10	1.05 ± 0.10	1.24 ± 0.13	0.84 ± 0.11
0.02	CMC	136.2 ± 11.7	139.7 ± 8.7	136.6 ± 12.4	134.4 ± 8.0	135.8 ± 6.8	141.4 ± 10.6
	FP	0.97 ± 0.09	0.93 ± 0.11	0.64 ± 0.11	0.68 ± 0.02	0.45 ± 0.06	0.21 ± 0.03
	β-G	1.06 ± 0.07	0.82 ± 0.08	1.43 ± 0.10	1.05 ± 0.05	1.05 ± 0.17	0.53 ± 0.06
0.05	CMC	136.4 ± 5.7	140.8 ± 9.3	131.7 ± 11.9	137.3 ± 8.9	141.0 ± 4.0	139.5 ± 5.1
	FP	1.02 ± 0.07	0.92 ± 0.10	0.80 ± 0.07	0.47 ± 0.05	0.25 ± 0.10	0.25 ± 0.05
	β-G	0.87 ± 0.15	0.78 ± 0.08	0.92 ± 0.14	0.85 ± 0.05	0.85 ± 0.06	0.62 ± 0.01
0.10	CMC	136.0 ± 4.7	143.1 ± 3.6	136.9 ± 8.4	137.1 ± 1.1	136.2 ± 10.5	129.8 ± 4.7
	FP	1.02 ± 0.10	0.71 ± 0.08	0.69 ± 0.07	0.49 ± 0.02	0.31 ± 0.09	0.34 ± 0.05
	β-G	0.83 ± 0.09	0.77 ± 0.03	0.90 ± 0.10	0.88 ± 0.04	0.86 ± 0.06	0.45 ± 0.07
0.20	CMC	137.5 ± 7.1	137.3 ± 6.3	131.0 ± 7.9	134.6 ± 5.0	131.1 ± 7.9	143.1 ± 13.8
	FP	0.96 ± 0.09	0.79 ± 0.00	0.59 ± 0.09	0.54 ± 0.05	0.51 ± 0.07	0.28 ± 0.03
	β-G	0.97 ± 0.05	1.15 ± 0.14	0.85 ± 0.09	0.74 ± 0.09	0.89 ± 0.07	0.46 ± 0.05
0.50	CMC	136.4 ± 12.5	135.0 ± 7.9	134.6 ± 9.8	136.8 ± 13.0	135.6 ± 10.4	145.9 ± 5.1
	FP	1.01 ± 0.07	0.81 ± 0.10	0.61 ± 0.01	0.59 ± 0.10	0.50 ± 0.06	0.20 ± 0.04
	β-G	0.88 ± 0.17	0.72 ± 0.08	0.67 ± 0.09	0.90 ± 0.13	1.06 ± 0.09	0.51 ± 0.08

*CMC, FP, and β-G were expressed as CMCase, FPase, and β-glucosidase, respectively

After the data of Fig. 2 were fit by IMM, all R^2 higher than 0.9 and near to adj-R^2 (Table 4) indicated that the fitting result was reliable. All $K_{obs,0}$ and K_i in the presence of betaine were less than those in the absence of betaine (Table 4). These results demonstrated that betaine decreased the accessibility of cellulase to lignocellulose, leading to the deactivation of enzyme in the process of saccharification. Obviously, a betaine-induced increase of Y_{trs} depended on the ability of betaine to protect cellulase from inactivation and nonspecific site adsorption. To verify the above-mentioned findings, the CMCase, FPase, and β-glucosidase activities were analyzed. Table 5 shows that the CMCase activity reached the highest in the presence of 0.02% betamine. Although the FPase activity gradually decreased in the process of saccharification, the FPase activity in the presence of betaine at corresponding time points was higher compared with that in the absence of betaine. The results exhibited that betaine decreased the adsorption of substrate to enzyme and reduced the inactivation in the CMCase and FPase assays. Taherzadeh-Ghahfarokhi *et al.* (2019) used rice straw as substrate of *Trichoderma reesei* solid-state fermentation to study the effects of surfactants on cellulase activity. They found that betaine can increase CMCase and FPase activities. Their findings are consistent with the authors' results. However, in the presence of betaine, inactivation of β-glucosidase became significant in the later stages of saccharification compared with that in the absence of betaine. Pollard and WynJones found that betaine is not toxic to bacterial β-glucosidase (Pollard and WynJones 1979). Their findings are inconsistent with the authors' results, which could have been attributed to the source of β-glucosidase.

Effect of SDS on the Saccharification

Figure 3 shows that when the SDS concentration was less than 0.02%, Y_{trs} rapidly increased with the increase of SDS concentration, while when the SDS concentration was higher than 0.02%, Y_{trs} was negatively correlated with the SDS concentration. The highest Y_{trs} (71.61%) was obtained in the presence of 0.02% SDS at 32 h, which was increased 35.63% compared with that in the absence of SDS.

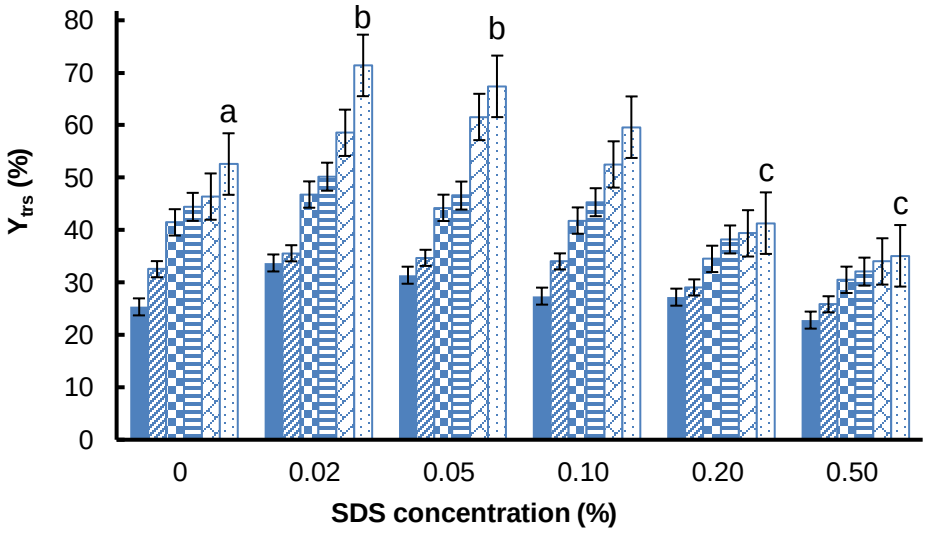

Fig. 3. Effect of SDS concentration on Y_{trs} at different reaction time points: ■: 1 h; ▨: 2 h; ▩: 4 h; ▤: 8 h; ▧: 16 h; and ▦: 32 h. a: the control; b: significantly higher than the control ($p < 0.05$), c: significantly less than the control ($p < 0.05$)

After the data of Fig. 3 were fit by IMM, all R^2 were higher than 0.9 and near to adj-R^2 (Table 6). The fitting result was very reliable. Table 6 shows that when the SDS concentration increased from 0 to 0.02%, $K_{obs,0}$ and K_i decreased from 0.3539 h^{-1} to 0.2726 h^{-1}, and from 0.0585 h^{-1} to 0.0540 h^{-1}, respectively. When the SDS concentration increased from 0.02% to 0.50%, $K_{obs,0}$ and K_i increased from 0.273 h^{-1} to 0.481 h^{-1}, and from 0.0540 h^{-1} to 0.0607 h^{-1}, respectively. The results suggested that low concentration SDS could protect CMCase and FPase from nonspecific site adsorption of lignocelluloses. The SDS interacts with most proteins to form a complex of SDS-protein at a concentration well below its critical micelle concentration. Because SDS is an anionic surfactant, the complex of SDS-protein contains a great deal of negative charge. These complexes with negative charge cannot be combined with the nonspecific site adsorption (Zhou *et al*. 2015a). However, when the SDS concentration is higher than its critical micelle concentration, SDS can cause enzyme deactivation and decrease the Y_{trs} of saccharification (Xiang *et al*. 2006; Holmberg 2018).

Table 7 shows that CMCase and FPase exhibited higher activities in the presence of 0.02% SDS, while the β-glucosidase activity was significantly decreased in the process of saccharification compared with that in the absence of SDS. The CMCase and FPase activities in the presence of 0.02% SDS at 32 h were 96.1 ± 7.4 U/mL and 0.46 ± 0.04 U/mL, respectively. When the SDS concentration was higher than 0.02%, these three enzyme activities all significantly decreased with the increase of SDS concentration. The changing trends of CMCase and FPase activities with SDS concentration were consistent with those of Y_{trs} and K_i during saccharification.

Table 6. Effect of SDS on Saccharification Kinetic Model of Pretreated CS

Concentration (%)	$t/-\ln(1 - Y_{trs})$	R^2	Adj-R^2	$K_{obs,0}$ (h^{-1})	K_i (h^{-1})
0	1.2786x + 2.8255	0.994	0.992	0.3539	0.0585
0.02	0.7319x + 3.6679	0.954	0.943	0.2726	0.0540
0.05	0.7976x + 3.5903	0.975	0.969	0.2785	0.0548
0.10	1.0251x + 3.3731	0.989	0.986	0.2966	0.0567
0.20	1.8133x + 1.9873	0.999	0.999	0.5032	0.0604
0.50	2.2394x + 2.0777	1.000	1.000	0.4813	0.0607

Effect of Polyoxyethylene (80) Sorbitan Monooleate (POESM) on Saccharification

POESM is a non-ionic surfactant. A non-ionic surfactant can stop nonspecific site adsorption on the lignocellulose of cellulase and increase the Y_{trs}. The authors studied the effect of POESM on Y_{trs}. The Y_{trs} gradually increased when the POESM concentration increased from 0 to 0.05% ($p < 0.05$), while it did not increase when the POESM concentration increased from 0.05% to 0.5%. The highest Y_{trs} (68.3%) was obtained in the presence of 0.05% POESM at 32 h. The smallest change in Y_{trs} with POESM concentration increase was found when POESM exceeded 0.05%. This result was consistent with some

reports on the increasing hydrolysis rate of pure-cellulosic materials in the presence of surfactant (Helle *et al*. 1993; Yang *et al*. 2011; Okino *et al*. 2013; Liu *et al*. 2019b).

Table 7. Effect of SDS on Cellulase Activity (U/mL)

Concen -tration (%)	Enzyme*	1 h	2 h	4 h	8 h	16 h	32 h
0	CMC	98.9 ± 3.1	96.2 ± 3.2	98.4 ± 9.0	90.7 ± 11.2	88.2 ± 11.0	81.5 ± 10.5
	FP	0.77 ± 0.05	0.78 ± 0.07	0.41 ± 0.08	0.55 ± 0.05	0.35 ± 0.05	0.29 ± 0.03
	β-G	0.93 ± 0.10	0.99 ± 0.10	0.80 ± 0.10	1.05 ± 0.10	1.24 ± 0.13	0.84 ± 0.11
0.02	CMC	99.5 ± 16.9	99.5 ± 11.7	98.6 ± 5.2	97.1 ± 2.1	95.4 ± 5.1	96.1 ± 7.4
	FP	0.72 ± 0.05	0.73 ± 0.06	0.55 ± 0.07	0.51 ± 0.05	0.45 ± 0.02	0.46 ± 0.04
	β-G	0.72 ± 0.05	0.73 ± 0.06	0.55 ± 0.07	0.51 ± 0.05	0.45 ± 0.02	0.46 ± 0.04
0.05	CMC	95.3 ± 4.4	89.4 ± 11.8	85.8 ± 3.3	85.4 ± 7.7	88.9 ± 6.3	63.5 ± 3.9
	FP	0.73 ± 0.09	0.70 ± 0.03	0.53 ± 0.03	0.47 ± 0.08	0.33 ± 0.02	0.26 ± 0.02
	β-G	0.73 ± 0.09	0.70 ± 0.03	0.53 ± 0.03	0.47 ± 0.08	0.33 ± 0.02	0.26 ± 0.02
0.10	CMC	89.8 ± 2.0	92.2 ± 2.9	86.7 ± 7.3	83.8 ± 4.5	84.5 ± 2.7	62.6 ± 14.7
	FP	0.68 ± 0.02	0.65 ± 0.03	0.51 ± 0.04	0.44 ± 0.05	0.40 ± 0.02	0.26 ± 0.04
	β-G	0.68 ± 0.02	0.65 ± 0.03	0.51 ± 0.04	0.44 ± 0.05	0.40 ± 0.02	0.26 ± 0.04
0.20	CMC	84.9 ± 8.2	81.6 ± 6.3	40.4 ± 7.5	43.0 ± 3.9	40.2 ± 8.0	35.1 ± 3.3
	FP	0.69 ± 0.04	0.63 ± 0.03	0.37 ± 0.06	0.46 ± 0.05	0.36 ± 0.02	0.36 ± 0.05
	β-G	0.69 ± 0.04	0.63 ± 0.03	0.37 ± 0.06	0.46 ± 0.05	0.36 ± 0.02	0.36 ± 0.05
0.50	CMC	27.8 ± 5.9	27.6 ± 7.3	26.9 ± 2.5	25.2 ± 4.7	24.7 ± 6.1	21.4 ± 3.2
	FP	0.58 ± 0.05	0.44 ± 0.07	0.23 ± 0.04	0.33 ± 0.05	0.31 ± 0.01	0.20 ± 0.03
	β-G	0.58 ± 0.05	0.44 ± 0.07	0.23 ± 0.04	0.33 ± 0.05	0.31 ± 0.01	0.20 ± 0.03
*CMC, FP, and β-G were expressed as CMCase, FPase, and β-glucosidase, respectively							

Table 8. Effect of POESM on Saccharification Kinetic Model of Pretreated CS

Concentration (%)	$t/-\ln(1 - Y_{trs})$	R^2	Adj-R^2	$K_{obs,0}$ (h^{-1})	K_i (h^{-1})
0	1.2786x + 2.8255	0.994	0.992	0.3539	0.0585
0.025	0.8832x + 3.6841	0.978	0.972	0.2714	0.0553
0.05	0.7945x + 3.7691	0.971	0.964	0.2653	0.0544
0.10	0.8200x + 3.4853	0.979	0.974	0.2869	0.0552
0.25	0.9032x + 3.1441	0.987	0.984	0.3181	0.0564
0.50	0.8398x + 3.5345	0.977	0.971	0.2829	0.0552

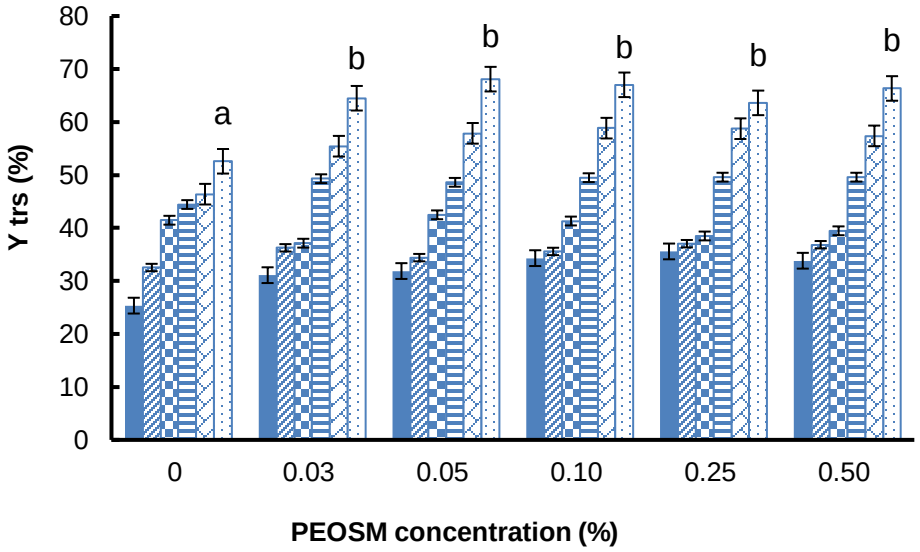

Fig. 4. Effect of POESM concentration on Y_{trs} at different reaction time points: ■: 1 h; ▨: 2 h; ▩: 4 h; ▤: 8 h; ▨: 16 h; and ▨: 32 h, a: the control; b: significantly higher than the control ($p < 0.05$)

After data of Fig. 4 were fit by IMM, all R^2 were higher than 0.9 and near to adj-R^2 (Table 8). The fitting result was highly credible. Table 8 shows that when the POESM concentration increased from 0 to 0.05%, $K_{obs,0}$ and K_i decreased from 0.3539 h^{-1} to 0.2653 h^{-1}, and from 0.0585 h^{-1} to 0.0544 h^{-1}, respectively. When the POESM concentration increased from 0.05% to 0.50%, $K_{obs,0}$ and K_i were not increased. The mechanism underlying the POESM decreased K_i, namely decreased nonspecific site adsorption, could be because POESM formed the anti-micelle to wrap CMCase and FPase, and protect CMCase and FPase from damage caused by shear and heat (Eckard *et al.* 2014).

Table 9 shows that CMCase maintained higher activity (approximately 205 U/mL) during saccharification with extension of reaction time, and when the POESM concentration increased from 0.03% to 0.5%, its activity was much higher compared with that in the absence of POESM. However, the activities of FPase and β-glucosidase gradually decreased with extension of reaction time. Activity of FPase in the presence of POESM at various concentrations was almost same and higher than that in the absence of POESM. In the presence of POESM, the β-glucosidase activity was higher than that in the presence of other surfactants. Such higher CMCase, FPase, and β-glucosidase activities implied that POESM possessed a better power to protect enzymes from shear and heat damage and from nonspecific site adsorption of lignocellulose. Similar results have been reported in other studies (Castanon and Wilke 1981; Yang *et al*. 2011).

Effect of Rhamnolipid on the Saccharification

Figure 5 shows that when the rhamnolipid concentration increased from 0 to 0.06%, the Y_{trs} rapidly increased, when the rhamnolipid concentration increased from 0.06% to 0.12%, the Y_{trs} almost kept the constant, and when the rhamnolipid concentration was further increased from 0.12% to 0.48%, the Y_{trs} gradually decreased. All Y_{trs} in the presence of rhamnolipid was significantly higher than that in the absence of rhamnolipid ($p < 0.05$). The highest Y_{trs} (75.60%) was obtained at 32 h in the presence of 0.12% rhamnolipid.

Table 9. Effect of Tween 80 on Cellulase Activity (U/mL)

Concen-tration (%)	Enzyme*	1 h	2 h	4 h	8 h	16 h	32 h
0	CMC	98.9 ± 3.1	66.2 ± 3.2	128.4 ± 9.0	100.7 ± 11.2	88.2 ± 11.0	81.5 ± 10.5
	FP	0.77 ± 0.05	0.78 ± 0.07	0.41 ± 0.08	0.55 ± 0.05	0.35 ± 0.05	0.29 ± 0.03
	β-G	0.93 ± 0.10	0.99 ± 0.10	0.80 ± 0.10	1.05 ± 0.10	1.24 ± 0.13	0.84 ± 0.11
0.03	CMC	198.6 ± 7.0	205.4 ± 7.1	204.5 ± 12.0	208.5 ± 13.6	207.0 ± 10.9	209.8 ± 15.1
	FP	1.03 ± 0.08	0.83 ± 0.04	0.77 ± 0.05	0.57 ± 0.04	0.47 ± 0.04	0.35 ± 0.03
	β-G	1.00 ± 0.13	1.04 ± 0.08	1.15 ± 0.07	0.89 ± 0.09	0.90 ± 0.11	0.67 ± 0.07
0.05	CMC	205.0 ± 8.1	204.7 ± 9.4	206.3 ± 6.4	208.7 ± 7.2	211.1 ± 15.0	203.6 ± 7.0
	FP	0.71 ± 0.08	0.75 ± 0.06	0.58 ± 0.03	0.54 ± 0.05	0.38 ± 0.04	0.31 ± 0.04
	β-G	0.90 ± 0.11	0.97 ± 0.07	1.04 ± 0.10	1.09 ± 0.17	0.78 ± 0.10	0.76 ± 0.04
0.10	CMC	201.7 ± 7.3	207.8 ± 5.6	207.0 ± 19.9	206.3 ± 12.8	211.6 ± 3.7	203.4 ± 3.1
	FP	0.81 ± 0.12	0.87 ± 0.04	0.68 ± 0.08	0.50 ± 0.05	0.42 ± 0.05	0.33 ± 0.06
	β-G	0.95 ± 0.14	0.99 ± 0.09	1.05 ± 0.07	0.90 ± 0.05	0.83 ± 0.08	0.52 ± 0.13
0.25	CMC	207.4 ± 11.9	208.3 ± 2.2	206.0 ± 10.8	210.7 ± 15.0	203.9 ± 3.1	207.6 ± 8.9
	FP	0.96 ± 0.07	0.98 ± 0.07	0.50 ± 0.04	0.55 ± 0.04	0.42 ± 0.06	0.27 ± 0.07
	β-G	0.95 ± 0.13	1.00 ± 0.11	1.15 ± 0.14	1.01 ± 0.09	0.71 ± 0.12	0.82 ± 0.09
0.50	CMC	208.1 ± 9.9	203.8 ± 6.7	200.3 ± 6.2	198.8 ± 7.6	205.4 ± 10.5	207.4 ± 16.7
	FP	0.81 ± 0.08	0.82 ± 0.04	0.59 ± 0.04	0.61 ± 0.07	0.39 ± 0.06	0.31 ± 0.04
	β-G	0.95 ± 0.09	0.98 ± 0.12	1.06 ± 0.17	0.96 ± 0.09	0.83 ± 0.06	0.81 ± 0.05
*CMC, FP, and β-G were expressed as CMCase, FPase, and β-glucosidase, respectively							

Wang *et al.* found that rhamnolipid can increase the Y_{trs} to a different extent in the hydrolysis process of wheat straw pretreated by various methods (Wang *et al*. 2011). Their findings supported the authors' conclusion. Among the tested five surfactants, the Y_{trs} in the presence of rhamnolipid was the highest. The result exhibited that the protective effect of rhamnolipid on Y_{trs} was strong in the process of saccharification.

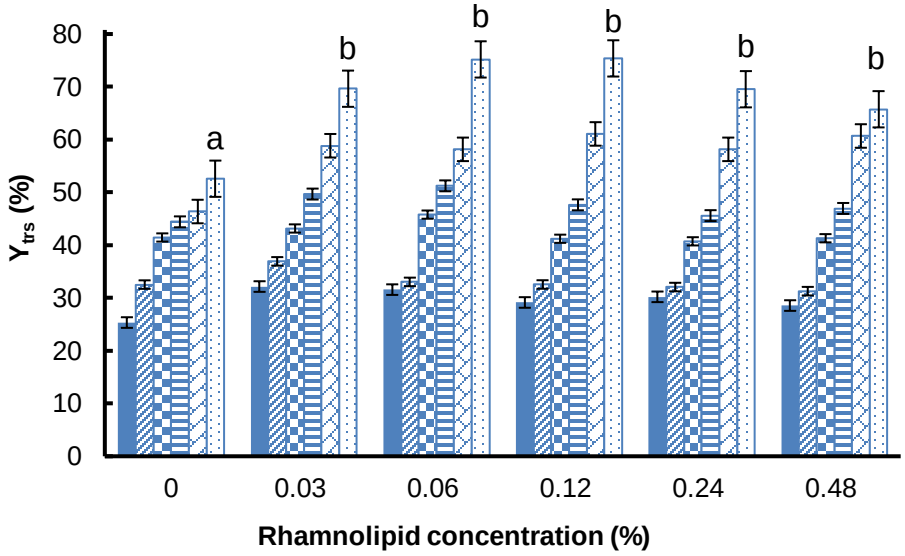

Fig. 5. Effect of rhamnolipid concentration on Y_{trs} at different reaction time points: ■: 1 h; ▨: 2 h; ⊠: 4 h; ⊟: 8 h; ▧: 16 h; and □: 32 h. a: the control; b: significantly higher than the control ($p < 0.05$).

Table 10 presents the data in Fig. 5 fit by IMM. $K_{obs,0}$ and K_i decreased from 0.3539 h^{-1} to 0.2096 h^{-1}, and from 0.0585 h^{-1} to 0.0503 h^{-1}, respectively, when the rhamnolipid concentration increased from 0 to 0.012%. Moreover, when the rhamnolipid concentration was further increased, $K_{obs,0}$ and K_i also increased. The changing trends of $K_{obs,0}$ and $K_i$were opposite to Y_{trs}. Rhamnolipid is lipophilic and can be adsorbed on the surface of lignocellulose through hydrophobic interactions (Kaar and Holtzapple 2015). Rhamnolipid adsorption on the surface of lignocellulose reduces the nonspecific site adsorption, $K_{obs,0}$ and K_i, which further reduces Y_{trs} (Zhou *et al*. 2015b).

Table 10. Effect of Rhamnolipid on Saccharification Kinetic Model of Pretreated CS

Concentration (%)	$t/-\ln(1 - Y_{trs})$	R^2	Adj-R^2	$K_{obs,0}$ (h^{-1})	K_i (h^{-1})
0	1.2786x + 2.8255	0.994	0.992	0.3539	0.0585
0.03	0.7629x + 3.6680	0.967	0.959	0.2726	0.0543
0.06	0.6455x + 4.2530	0.918	0.897	0.2351	0.0518
0.12	0.6126x + 4.7707	0.923	0.903	0.2096	0.0503
0.24	0.7446x + 4.4395	0.952	0.940	0.2253	0.0527
0.48	0.8235x + 3.8445	0.984	0.980	0.2601	0.0545

Table 11 indicates that the CMCase and β-glucosidase activities in the presence of rhamnolipid at various concentrations were higher in the saccharification than those in the absence of rhamnolipid. The FPase activity in the presence of 0.03%, 0.06%, and 0.12%

rhamnolipid was higher in the initial stage of saccharification, while it was rapidly deactivated after 2 h of saccharification. Similar results have been reported in previous studies (Wang *et al*. 2011; Zhang *et al*. 2009).

Table 11. Effect of Rhamnolipid on Cellulase Activity (U/mL)

Concen-tration (%)	Enzyme*	1 h	2 h	4 h	8 h	16 h	32 h
0	CMC	98.9 ± 3.1	66.2 ± 3.2	128.4 ± 9.0	100.7 ± 11.2	88.2 ± 11.0	81.5 ± 10.5
	FP	0.77 ± 0.05	0.78 ± 0.07	0.41 ± 0.08	0.55 ± 0.05	0.35 ± 0.05	0.29 ± 0.03
	β-G	0.93 ± 0.10	0.99 ± 0.10	0.80 ± 0.10	1.05 ± 0.10	1.24 ± 0.13	0.84 ± 0.11
0.03	CMC	149.1 ± 6.2	147.4 ± 6.3	152.7 ± 11.3	150.7 ± 13.1	149.4 ± 9.2	158.2 ± 11.2
	FP	0.98 ± 0.08	0.73 ± 0.04	0.64 ± 0.04	0.43 ± 0.05	0.45 ± 0.06	0.30 ± 0.10
	β-G	0.82 ± 0.04	0.56 ± 0.05	0.68 ± 0.07	0.90 ± 0.04	0.82 ± 0.10	0.79 ± 0.06
0.06	CMC	147.8 ± 9.4	145.0 ± 4.8	148.7 ± 12.2	150.5 ± 10.1	155.8 ± 8.6	148.3 ± 10.2
	FP	1.03 ± 0.10	0.74 ± 0.04	0.67 ± 0.06	0.50 ± 0.05	0.48 ± 0.04	0.25 ± 0.05
	β-G	0.87 ± 0.16	0.74 ± 0.05	0.94 ± 0.05	1.02 ± 0.09	0.90 ± 0.05	0.70 ± 0.07
0.12	CMC	150.5 ± 15.8	147.2 ± 10.8	146.0 ± 12.2	153.6 ± 8.9	157.3 ± 18.8	156.0 ± 5.6
	FP	0.94 ± 0.02	0.69 ± 0.09	0.76 ± 0.06	0.48 ± 0.05	0.43 ± 0.04	0.25 ± 0.08
	β-G	0.79 ± 0.10	0.56 ± 0.05	1.16 ± 0.12	0.98 ± 0.12	0.91 ± 0.05	0.61 ± 0.10
0.24	CMC	151.6 ± 4.7	138.5 ± 5.6	151.6 ± 7.4	151.4 ± 6.1	148.0 ± 14.3	152.9 ± 8.8
	FP	0.85 ± 0.05	0.79 ± 0.03	0.67 ± 0.07	0.52 ± 0.06	0.52 ± 0.04	0.41 ± 0.03
	β-G	0.86 ± 0.11	0.74 ± 0.06	0.72 ± 0.08	0.82 ± 0.06	0.85 ± 0.09	0.68 ± 0.10
0.48	CMC	150.0 ± 7.3	149.2 ± 1.6	152.4 ± 9.0	148.3 ± 1.4	149.1 ± 14.7	147.4 ± 5.9
	FP	1.01 ± 0.07	0.78 ± 0.06	0.52 ± 0.03	0.45 ± 0.06	0.48 ± 0.06	0.44 ± 0.02
	β-G	0.79 ± 0.07	0.81 ± 0.06	0.67 ± 0.07	0.81 ± 0.09	0.83 ± 0.05	0.74 ± 0.14
*CMC, FP, and β-G were expressed as CMCase, FPase, and β-glucosidase, respectively							

Analysis of Correlation between Y_{trs} and $K_{obs,0}$ or K_i

There were various potential mechanisms underlying the surfactant-increased Y_{trs}. To exactly disclose the mechanism, the Y_{trs} in Figs. 1 through 5 was used as a dependent variable, $K_{obs,0}$ and K_i in Tables 2, 4, 6, 8, and 10 were used as independent variables, and the correlation between Y_{trs} and $K_{obs,0}$ or K_i was analyzed by the following Eq. 5,

$$Y_{trs} = \alpha \times K_{obs,0} + \beta \times K_i + \gamma \quad (5)$$

where a and β are the coefficients of $K_{obs,0}$ and K_i, respectively, and γ is a constant.

Table 12 shows the relationship between Y_{trs} and $K_{obs,0}$ or K_i. The F-value of Fisher's test (113.589) and p-value of the t-test P (0.000) indicated that the correlation between Y_{trs} and $K_{obs,0}$ or K_i was extremely significant, and the analysis was very reliable. After the coefficient of each item in Table 12 was introduced into Eq.5, the following equation was generated:

$$Y_{trs} = 264.019 + 6.520K_{obs,0} - 3581.948K_i \quad (6)$$

Table 12. Coefficient of Each Item and ANOVA Results of Linear Regression Analysis Between the Y_{trs} and $K_{obs,0}$ or K_i

Item	Coefficient	T-value	p-value [a]
Constant	264.019	10.259	0.000
$K_{obs,0}$	6.520	-0.341	0.736
K_i	-3581.948	-6.512	0.000
[a] *F*-value = 113.598; Significance *p* = 0.000			

The t-value (6.512) and p (0.000) of K_i as well as the t-value (0.341) and p-value (0.736) of $K_{obs,0}$ indicated that the effect of K_i on Y_{trs} was extremely significant, while the effect of $K_{obs,0}$ on Y_{trs} was not significant. Therefore, the effects of surfactants on Y_{trs} mainly depended on nonspecific site adsorption and enzymatic activity. Significant increase of CMCase and FPase activities in Table 3, Table 5, Table 7, Table 9 and Table 11 d after adding surfactants was precisely because reduction of nonspecific site adsorption and deactivation of enzymes caused by surfactants. The negative coefficients of K_i indicated that the lower the enzyme deactivation or the less the nonspecific site adsorption of enzyme, the higher the Y_{trs}. Based on these results, the authors believed that surfactants increased Y_{trs} and improved the saccharification efficiency mainly *via* enhancing enzyme stability and activity and decreasing nonspecific site adsorption and deactivation of enzymes. Liu *et al.* studied the interactive relationship between surfactants (rhamnolipid and Tween 80) and enzymes (cellulase and xylanase) by fluorescence spectroscopy using pyrene as probe (Liu *et al*. 2011). Their results are consistent with the authors'. The effect of accessibility between enzyme and substrate on saccharification efficiency was negligible compared with that of surfactants. After comparing all K_i values in Tables 2, 4, 6, and 8, the authors found that rhamnolipid had the lowest K_i. Therefore, the authors deduced that rhamnolipid had a stronger power to maintain cellulase activity and protect cellulase from nonspecific site adsorption of lignocellulose.

Analysis of Correlation Between Y_{trs} and Activities of CMCase, FPase, and β-glucosidase

Cellulase is a complex enzyme, mainly including endo-β-glucanase, exo-β-glucanase, and β-glucosidase. Endo-β-glucanase and exo-β-glucanase are usually expressed as CMCase and FPase, respectively. To disclose effects of CMCase, FPase, and β-glucosidase on the saccharification, the correlation between Y_{trs} at 32 h in Figs. 1, 2,3, 4, and 5 as well as the CMCase, FPase, and β-glucosidase activities at 32 h in Tables 3, 5, 7, 9, and 11 were fit by the following equation,

$$Y_{trs} = \alpha + \sum_{i=1}^{3}(\beta_i \times F_i^2) + \sum_{i=1}^{3}(\gamma_i \times F_i) \quad _3 + \delta \quad (7)$$

where a is a constant, and F_i denotes the activity of CMCase, FPase, or β-glucosidase. F_i^2 is the interaction of the same enzyme, β_i and γ_i are the coefficients of F_i^2 and F_i, respectively, and δ is a standardized residual.

Table 13 reveals the relationship between Y_{trs} and various enzymes. The F-value of Fisher's test (13.237) and p-value of the t-test (0.000) indicated that the correlation between Y_{trs} and the activity of CMCase, FPase, or β-glucosidase was significant, and the analysis was very reliable. The t-value (4.642) and p (0.000) of F_1 (CMCase) exhibited that the effect of CMCase on Y_{trs} was significant. The t-value (1.277, 0.990) of F_2^2 and F_2 (FPase) was higher than t-value (0.177, 0.578) of F_3 (β-glucosidase), and the p-value (0.214, 0.332) of F_2^2 and F_2 was lower than p-value (0.861, 0.569) of F_3^2 and F_3, showing that the effect of FPase was more significant compared with β-glucosidase. After the coefficient of each item in Table 13 was introduced into Eq. 7, the following equation was generated:

$$Y_{trs} = -0.002F_1^2 + 0.545F_1 + 224.445F_2^2 - 111.160F_2 + 5.864F_3^2 - 23.881F_3 + 47.722 \quad (8)$$

The negative coefficients of F_1^2 (CMCase) (Table 13) exhibited that the curves of Y_{trs} and CMCase activity presented an inverted U-shaped relationship with a peak value of Y_{trs}. The positive coefficients of F_2^2 and F_3^2 (Table 13) exhibited that the curves of Y_{trs} and FPase or β-glucosidase activity presented a U-shaped relationship with a minimal value of Y_{trs}. To further analyze the correlation between Y_{trs} and three components of cellulase, the following equation was obtained by taking the derivation of Y_{trs} to F1, F2, and F3, respectively,

$$Y'_{trs} = -0.004F1 + 0.545 \quad (9)$$

$$Y'_{trs} = 448.89F2 - 111.16 \quad (10)$$

$$Y'_{trs} = 11.728F3 - 23.864 \quad (11)$$

where Y'_{trs} is the derivative of Y_{trs}. If $Y'_{trs} = 0$, then $F_1 = 136.25$ U/mL, $F_2 = 0.2476$ U/mL and $F_3 = 2.036$ U/mL.

When CMCase activity was 136.2 U/mL, the maximum Y_{trs} was obtained. After comparison of CMCase in Tables 3, 5, 7, 9, and 11, it clearly showed that the CMCase activity in the presence of betaine (Table 5) and rhamnolipid (Table 11) was close to 136.2 U/mL. Therefore, the maximum Y_{trs} was obtained in the presence of betaine or rhamnolipid. Furthermore, after comparison of FPase and β-glucosidase activities in Tables 3, 5, 7, 9, and 11, it was found that most FPase activities were higher than 0.248, and all β-glucosidase activities were less than 2.04. When $F_2 > 0.2476$ U/mL, $Y'_{trs} > 0$, namely, the Y_{trs} was positively correlated with FPase activity. When $F3 < 2.036$ U/mL, $Y'_{trs} < 0$, namely, the Y_{trs} was negatively correlated with β-glucosidase activity. This finding suggested that β-glucosidase was enough to meet the test requirements, and excessive β-glucosidase activity produced too much glucose that in turn inhibited CMCase and FPase activities by combining with the specific site of CMCase and FPase. After comparison of FPase and β-glucosidase activities in Tables 3, 5, 7, 9, and 11, it was observed that the FPase activity in the presence of betaine (Table 5) was less than that in the presence of rhamnolipid (Table 11), and the β-glucosidase activity in the presence of betaine was close to that in the presence of rhamnolipid. Therefore, the Y_{trs} in the presence of betaine was less than that in the presence of rhamnolipid.

Table 13. Coefficient of Each Item and ANOVA Results of Linear Regression Analysis Between Y_{trs} and CMCase, FPase, or β-glucosidase

Item	Coefficient	T-value	p-value [a]
Constant	47.722	2.673	0.014
F_1	0.545	4.642	0.000
F_1^2	-0.002	3.724	0.001
F_2	-111.160	0.990	0.332
F_2^2	224.445	1.277	0.214
F_3	-23.881	0.578	0.569
F_3^2	5.864	0.177	0.861
[a] F-value = 13.237; Significance p = 0.000; F_1, F_2, and F_3 were expressed as CMCase, FPase, and β-glucosidase, respectively			

Tables 9 and 11 show that the CMCase and FPase activities in the presence of 0.03% POESM at 32 h were 209.8 U/mL and 0.35 U/mL, respectively. The CMCase activity was much higher than 136.25 U/mL. Therefore, the authors designed the test again. In such test, the concentrations of cellulase and POESM were 0.6% and 0.03%, respectively, and other conditions remained the same as those described in the 'Methods' section. The CMCase, FPase, and β-glucosidase activities at 32 h were 148.4 U/mL, 0.27 U/mL, and 0.54 U/mL, respectively, and Y_{trs} was 80.3%. Therefore, it was deduced that the best surfactant for CS saccharification was POESM, followed by rhamnolipid, while glycine was not suitable for protecting cellulase in the saccharification of lignocellulose.

CONCLUSIONS

1. In the present study, several surfactants, including betaine, sodium dodecylsulfate (SDS), polyoxyethylene (80) sorbitan monooleate (POESM), and rhamnolipid, were used to improve the efficiency of lignocellulose saccharification. No significant protective effect of glycine was found. Among these surfactants, the protective effect of POESM was the best, followed by rhamnolipid.
2. After all data were fit by the impeded Michaelis-Menten model (IMM). The R^2 and Adj-R^2 values showed that IMM could fit all the tested data.
3. After addition of the surfactants, the $K_{obs,0}$ and K_i values were significantly decreased. The reduced $K_{obs,0}$ indicated that surfactants could not increase the accessibility of cellulase enzymes into the lignocellulose. Decreased K_i suggested that these surfactants could protect cellulase from inactivation and nonspecific site adsorption of lignocellulose in the saccharification, leading to enhanced cellulase activity, especially CMCase and FPase activities.
4. The CMCase activity played a crucial role in increasing the efficiency of lignocellulose saccharification in cellulase. The maximum yield of total reducing sugars Y_{trs} could be obtained when the CMCase activity was 136.25 U/mL, while FPase and β-glucosidase activities should remain as high and low as possible, respectively, under the optimized condition. The results helps to improve the saccharification efficiency of cellulase and reduce the cost of saccharification of biomass cellulose.

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