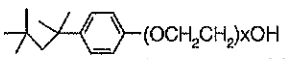
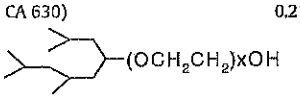
Table 1
Some basic parameters of polymers/nonionic surfactants.

Polymers or nonionic surfactants	CMC[b] (mM)	HLB[c]	Cloud point (°C)	Fractal kinetic parameters		Relative activity (%)[a]
				k	h	
Control (without lignin)				0.082	0. 7	
Control (with lignin)				0.077	0.87	
Polyethylene glycerol (PEG) or Polypropylene glycerol (PPG)						
PEG 4000	No micelle		>100	0.08	0.68	–
PEG 10000	No micelle		>100	0.082	0.67	–
PPG 2000	No micelle		Insoluble	0.077	0.79	–
Polyoxyethylene-polyoxypropylene copolymers (PEO-PPO-PEO)						
Pluronic L 61		3	15–19	0.08	0.74	100
Pluronic L 62		7	Double cloud points	0.081	0.72	100
Pluronic L 64		7–14	>60	0.083	0.69	100
Pluronic F 68		>24	>100	0.083	0.69	100
Sorbitan fatty acid esters						
Span 20 (Sorbitan monododecanoate)		8.6	Insoluble	0.078	0.85	100
Tween 80 (Polyoxyethylene sorbitan monooleate)	0.01–0.02	15		0.082	0.71	100
Polyoxyethylene glycol monoethers, $CH_3(CH_2)_{i-1}O(CH_2CH_2O)_x$ H						
Brij 30 (tetraethylene glycol dodecyl ether)			Dispersible	0.079	0.73	8
Brij 92v (diethylene glycol oleyl ether)			Insoluble	0.103	0.78	100
t-Octylphenoxy polyoxethylene ethers, 						
Triton X-45	0.103	9.8	Dispersible	0.08	0.71	18
Triton X-114	0.2	12.3	25	0.081	0.69	53
Triton X-100 (Igepal CA 630)	0.21	13.4	66	0.081	0.69	68
Tergitol TMN series, 						
Tergitol TMN-3 (TMN-3)		8.1	Insoluble	0.079	0.76	42

[a] relative biomass activity is determined at 0.2 g/100 ml nonionic surfactant/polymer in aqueous solution.
[b] CMC is the critical micelle concentration of a surfactant in an aqueous solution.
[c] HLB is the hydrophile-lipophile balance number of a surfactant.

changed with the different nonionic surfactant/polymer compositions in the aqueous solution. A relative biomass activity in a given polymer/nonionic surfactant aqueous solution was defined as the ratio of the glucose degradation rate in the polymer/nonionic surfactant solution to that of aqueous solution. The toxicity of the polymer/nonionic surfactant was indexed by the relative activity.

2.4. Glucose concentration analysis

Reducing sugar concentration was represented with glucose equivalent (Mizutani et al., 2002). The glucose concentration in a sample of an enzymatic saccharification of cellulose was determined by a standard 3,5-dinitrosalicylic acid method with a spectrophotometer (Miller, 1959). The basic analysis procedure was as follows: First, an analysis reagent was prepared: 6.3 g of 3,5-dinitrosalicylic acid was dissolved in 262 ml of 2 M NaOH solution. Potassium sodium tartrate tetrahydrate (185 g) was dissolved in 500 ml of hot water (about 45 °C). Both of them were combined and 5 g of anhydrous sodium sulfite and 5 g of phenol were added consecutively. Then the solution was diluted with distilled water to 1000 ml. Second, 2 ml of the diluted sample solution was mixed with 1.5 ml of the analysis reagent, which was reacted in a boiling water bath for 5 min. The reaction solution was cooled to room temperature and diluted to 25 ml with distilled water. Finally, the diluted solution was detected at a wavelength 540 nm with a spectrophotometer and the same aqueous solution without glucose followed the same procedure was used as a control.

2.5. Data analysis

A time course of enzymatic saccharification of cellulose is described by a fractal kinetic model (Wang and Feng, 2010).

$$X = \frac{C_0 - C}{C_0} = 1 - EXP(-k(1 + \frac{t^{1-h} - 1}{1-h})) \quad (1)$$

where X is the hydrolysis efficiency; C is the residual cellulose concentration and C_0 is the initial cellulose concentration; t is the time; k and h are two parameters of the fractal kinetic model, which are called as rate constant and fractal exponent, respectively. The hydrolysis efficiency increases with the increase of rate constant while decreases with the increase of fractal exponent. When $h = 1$, Eq. (1) is changed to

$$X = 1 - EXP(-k(1 + \ln t)) \quad (1a)$$

The hydrolysis cellulose concentration was converted into the data of reducing sugar concentration.

$$C_p = 1.1(C_0 - C) = 1.1C_0X \quad (2)$$

where C_p is the reducing sugar concentration (g/L).

A time course of enzymatic saccharification of cellulose was fitted with the fractal kinetic model. The model parameters were estimated by minimizing the residual sum of square errors (RSSE) between the simulated values and the experimental data with the same procedure as our previous work (Wang and Feng, 2010).

$$f = \sum_{i=1}^{n} \left(C_{pi}^s - C_{pi}^e\right)^2 \quad (3)$$

where C_{pi}^s was the simulated value of glucose concentration; C_{pi}^e was the experimental data of glucose concentration; n was the number of simulated or experimental value.

3. Results and discussion

3.1. Enzymatic saccharification of Avicel in aqueous solution

An enzymatic saccharification of Avicel in a buffer solution was determined as shown in Fig. 1. The rate of glucose production was retarded with the increase of hydrolysis time (Fig. 1A), which had a similar trend to the literature's reports (Bommarius et al., 2008;

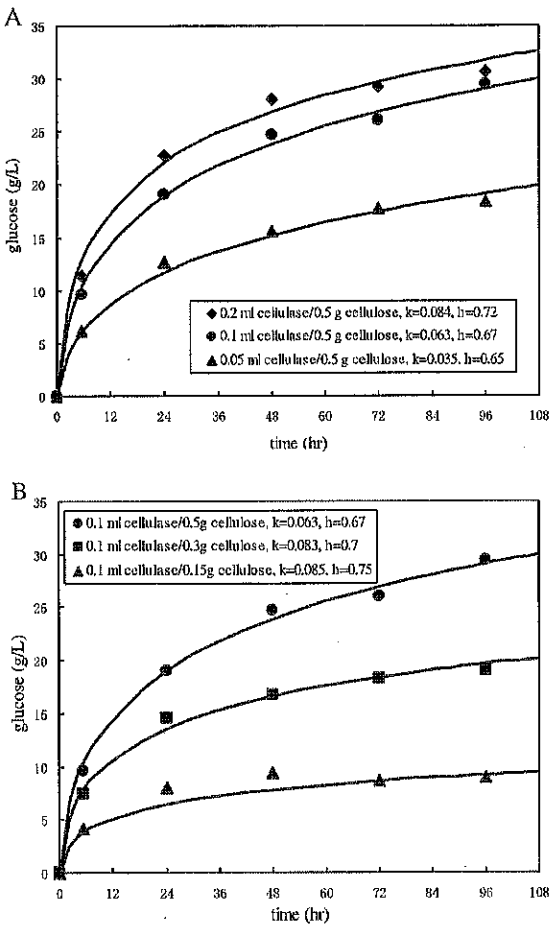


Fig. 1. Enzymatic saccharification of cellulose in an aqueous solution A: Effect of cellulase loading with Avicel loading 0.5 g in 10 ml reaction volume; B: Effect of cellulose with Accellerase 1500 loading 0.1 ml in 10 ml reaction volume.

Wu and Ju, 1998). The experimental data was fitted with the fractal kinetic model as detailed in the section of data analysis, in which both the rate constant and fractal exponent were increased with the increase of cellulase loading. The glucose concentration was also a function of cellulose loading under a certain cellulase loading condition (Fig. 1B). It was consisted with the nonlinear dependence of glucose yield on the cellulose loading (Wald et al., 1984). Both the rate constant and the fractal exponent were decreased with the increase of cellulose loading.

The data of fractal kinetic parameters in Fig. 1 were represented as a function of the ratio of cellulase to cellulose loading, i.e., the cellulase loading in per gram of cellulose (Fig. 2). Different from conventional reaction kinetic in an aqueous solution, the fractal kinetic parameters are changed with the experimental conditions, such as cellulase/cellulose loading, which is the intrinsic character of enzymatic saccharification of cellulose due to its fractal. The phenomenon is similar to cellulase activity analysis (FPU), where the cellulase solution must be diluted to release exactly 2 mg of glucose under the corresponding experimental condition, i.e., a certain cellulase and cellulose loading.

As shown in Fig. 2, with increasing ratio of cellulase to cellulose loading, the rate constant increased and then reached to a platform value. It may be related to the rate of enzymatic saccharification of Avicel is strongly depended on the cellulase adsorption capacity of cellulose and the adsorption of cellulase to cellulose is fitted with Langmuir isothermal model (Yang et al., 2006; Kumar and Wyman, 2009). An increasing ratio of cellulase to cellulose loading leads to

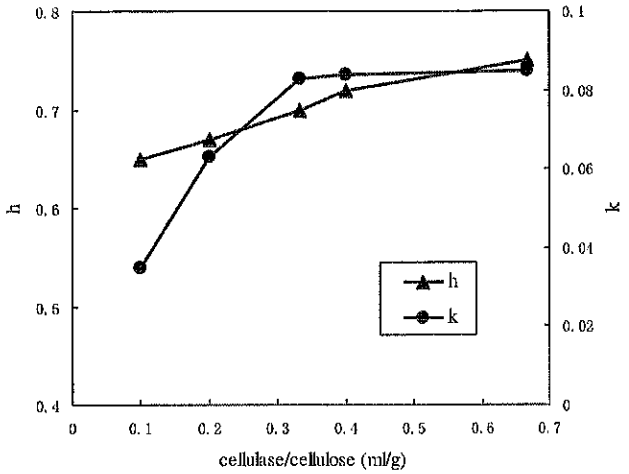


Fig. 2. Relationship between fractal kinetic parameters and ratio of cellulase to cellulose.

high rate constant, which can be ascribed to high adsorption of cellulase to cellulose. A further increasing ratio of cellulase to cellulose loading leads to no increase of rate constant, which may be related to the saturated adsorption of cellulase to cellulose, i.e., the excess of cellulase are presented in the aqueous solution and does not take part in the enzymatic saccharification of cellulose.

Although the fractal exponent in the fractal kinetic model can not exclude the effect of cellulase inactivity and product inhibition etc., the fractal exponent as shown in Fig. 2 gives a picture of the retarded rate of enzymatic saccharification of cellulose. On the one hand, the high ratio of cellulase to cellulose loading leads to crowding cellulase on cellulose surfaces, which limits the diffusion of cellulase on the cellulose surfaces (Xu and Ding, 2007). On the other hands, the two-domain structure of cellulase, one is hydrophilic and the other is hydrophobic (Palonen et al., 1999), may form aggregations in the aqueous solution at a high cellulase concentration, which also obstacles the diffusion of cellulase on the cellulose surfaces. All of those cause the high fractal exponent at high cellulase loading.

3.2. Inhibitive effect of lignin and counterbalance with high cellulase loading

Enzymatic saccharification of Avicel containing different ratio of lignin to cellulose was determined and analyzed with the fractal kinetic model (Fig. 3A). Despite of the differences between Avicel and lignin mixture and lignocellulose (pretreated biomass) (such as lignin is at isolated state in the Avicel and lignin mixture while interaction with cellulose in lignocellulose etc.), the fractal kinetic analysis gives an actual picture of lignin inhibition. The highest hydrolysis efficiency with fractal kinetic parameters $k = 0.082$ and $h = 0.7$ was obtained under the absence of lignin condition. The lignin inhibition on the enzymatic saccharification of Avicel was indexed by the increased fractal exponent while nearly no change of rate constant. An addition of lignin to cellulose (filter paper) reduces hydrolysis efficiency (Sewalt et al., 1997) while an elimination of lignin in lignocellulose improves hydrolysis efficiency (Eriksson et al., 2002; Börjesson et al., 2007; Qing et al., 2010) have also reported. It may be ascribed to the non-productive adsorption of cellulase onto lignin by the phenolic group content in lignin (Chandra et al., 2007). The high fractal exponent indexes the strongly retarded hydrolysis rate, which hints the presence of lignin mainly affects the enzymatic saccharification of cellulose at a prolonged hydrolysis time: It is consisted with that cellulase