Kinetic Modeling of Enzymatic Hydrolysis of Cellulosic Material

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Research Article

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Kinetic modeling of enzymatic hydrolysis of cellulosic material

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Abstract

Development of a kinetic model for analysis of processes for fermentable sugars production is a significant challenging issue due to the complexity of the enzymatic hydrolysis of cellulose. This paper presents a useful mathematical model for simulation and evaluation of enzymatic hydrolysis of cellulosic material. The simulation results were compared with a set of experimental results reported in the literature, in order to validate the proposed model. A comparison was also made between the proposed model and another model previously described in the literature Numerical results indicate that the proposed model gives a more accurate prediction of time course production of glucose, cellobiose, and cellulose during enzymatic hydrolysis cellulosic materials.

Keywords: Cellulosic material; Cellobiose, Enzymatic hydrolysis; Glucose; Kinetic model

1. Introduction

Cellulose as a major constituent of all plant materials is a linear biopolymer of glucose molecules, $(C_6H_{10}O_5)_n$ (Girometta, Zeffiro et al. 2017; Tian, Lu et al. 2017). Enzymatic hydrolysis of cellulose is performed by cellulases enzymes including Endo-Glucanase, Exo-Glucanase (cellobiohydrolases) and β-Glucosidase (cellobiase) under mild conditions (pH 4.5-5.0 and temperature 40–50°C)(Singhania, Saini et al. 2015; Niu, Shah et al. 2016). Enzymatic hydrolysis has various advantages compared to acid hydrolysis, including low utility consumption, less corrosion problems, higher glucose yield without sugar-degradation and producing inhibitory products (Tian, Lu et al. 2017). However, Enzymatic hydrolysis of cellulose is still considered as the main bottleneck of the biological production of ethanol due to the low hydrolysis rates and the high cost of enzymes (Szijártó, Siika-aho et al. 2008; Soudham, Alriksson et al. 2011; Zheng, Zhang et al. 2013). Many parameters including cellulose structure (accessible area, degree of polymerization, crystallinity) and cellulase system (enzyme activities, adsorption, synergism, and inhibition) play an important role in the enzymatic hydrolysis of cellulose(Hu, Zhang et al. 2018). However, in view of the difficulty in analysis and measurement of parameters such as crystallinity and degree of polymerization of cellulose, a model based on observable properties would be useful in the analysis of processes for fermentable sugars or bioethanol production.

Several authors investigated the kinetics of enzymatic hydrolysis of cellulosic material applying Michaelis–Menten's (M-M) equation due to its simplicity (Movagharnejad and Sohrabi 2003; Imai, Ikari et al. 2004; Peri, Karra et al. 2007; Zheng, Pan et al. 2009; Harun and Danquah 2011; Sakimoto, Kanna et al. 2017). Also, Ye and Berson (Ye and Berson 2011) proposed a mathematical model describing the kinetics of glucose formation from cellulose with first order inactivation of adsorbed cellulase. In this model, substrate reactivity (transformation in the degree of polymerization, crystal structure, substrate availability, etc), cellulose to cellobiose and cellobiose to glucose conversion have not been included. Some authors believed that the substrate reactivity (SR) is one of the important parameter affecting the enzymatic hydrolysis of cellulosic material (Klyosov 1990; Weimer, French et al. 1991; Gama, Teixeira et al. 1994; Desai and Converse 1997; Lynd, Weimer et al. 2002; Kadam, Rydholm et al. 2004).They detected cellulose hydrolysis rate increased 3–30 times in amorphous cellulose compared to crystalline cellulose. However, it is difficult to evaluate substrate structural shapes and specify the different enzymes adsorbed onto crystalline or amorphous cellulose regions. Therefore, the variation of SR may be a more feasible way to model the biphasic demeanor compare to an assumed dichotomy between crystalline and amorphous regions.

The objective of this work was to modify the model proposed by Ye and Berson (Ye and Berson 2011) to incorporate substrate reactivity, enzyme inactivation and the kinetic of cellobiose and glucose formation from cellulose. Afterward, the proposed model and another model from the literature were compared together and with the experimental data reported in the literatures.

2. Model description

In this work, the proposed model by Zheng, et al (Zheng, Pan et al. 2009) is evaluated by the experimental data reported in four literatures. Thereafter, the modified kinetic model was used proposed to predict concentrations of cellulose, cellobiose, and glucose during enzymatic hydrolysis. Reaction scheme for the modeling of the cellulose hydrolysis is depicted in Fig. 1.

Fig. 1

The computer program, AQUASIM, is used as a tool to simulate enzymatic hydrolysis of cellulose (Reichert 2014).

2.1. Kinetic model from the literature

The model proposed in the literature by Zheng et al (Zheng, Pan et al. 2009) assumes that hydrolysis of cellulose occurs in three steps ". The cellulose is hydrolyzed to soluble cellobiose by the synergistic action of endo-β-1,4-glucanase (EG) and exo-β-1,4-cellobiohydrolase (CBH), the cellulose is hydrolyzed to glucose by the synergistic action of CBH and exo-β-1,4-glucanase, and cellobiose is hydrolyzed to glucose by the action of β-glucosidase. Reaction scheme (Fig. 1(a)) involves hydrolysis reactions of r_1, r_2, and r_3. The reaction rates in the model are given by the following equations:

$$r_1 = \frac{k_{1r}.E.SR.C}{1 + \frac{G_2}{k_{1IG2}} + \frac{G}{k_{1IG}}} \quad (1)$$

$$r_2 = \frac{k_{2r}.E.SR.C}{1 + \frac{G_2}{k_{2IG2}} + \frac{G}{k_{2IG}}} \quad (2)$$

$$r_3 = \frac{k_{3r}.E.G_2}{k_{3M}.(1 + \frac{G}{k_{3IG}}) + G_2} \quad (3)$$

SR is substrate reactivity and expressed according to the following equation.

$$SR = \frac{C}{C_0} \quad (4)$$

This model was developed based on end-product inhibition. In these rate equations, k_{ir} (1, 2) are the reaction rate constants (ml/(mg h)); k_{3r} is the reaction rate constant (h^{-1}); $k_{1IG,}$ k_{1IG2} (1, 2) are inhibition constants (mg/ml) of glucose and cellobiose on enzymes, respectively; k_{3M} is cellobiose saturation constants (mg/ml); and C, $G_{2,}$ G are concentrations of cellulose, cellobiose, and glucose (mg/ml), respectively. C_0, and E are initial substrate concentration (g/l) and initial enzyme concentration (mg/ml), respectively. The mass balance equations of cellulose, cellobiose, and glucose can be written as:

$$\frac{dC}{dt} = -r_1 - r_2 \quad (5)$$

$$\frac{dG_2}{dt} = 1.056 r_1 - r_3 \quad (6)$$

$$\frac{dG}{dt} = 1.116 r_2 + 1.053 r_3 \quad (7)$$

2.2. Proposed kinetic model

The proposed model by Ye and Berson (Ye and Berson 2011) simulates straight conversion of cellulose to glucose. Moreover, this model did not consider the substrate reactivity. Thus, the modification of this model was conducted in an attempt to predict kinetic of cellobiose and glucose formation from cellulose and to consider substrate reactivity and enzyme inactivation.

Inactivation of cellulases is described in the model proposed in this work with the use of an exponential decay term. In the proposed model, it is assumed that hydrolysis of cellulose occurs in two steps: the cellulose is hydrolyzed first to soluble cellobiose by the synergistic action of β-1,4-glucan cellobiohydrolase and endo-β-1,4-glucanase, and the cellobiose is hydrolyzed to glucose by the action of β-glucosidase (Fig. 1(b)). To simplify the model r_2 in the previous model is ignored. The reaction rates and mass balance equations in the new model are demonstrated by the following equations:

$$K_c = \frac{k_r}{k_r + k_f} + \frac{k_f}{k_r + k_f}.\exp(-(k_r + k_f).t) \quad (8)$$

$$r_1 = \frac{k_{1r}.E.SR.K_c.C}{C + K_m} \quad (9)$$

$$r_2 = \frac{k_{2r}.E.K_c.G_2}{G_2 + K_m} \quad (10)$$

$$\frac{dC}{dt} = -r_1 \quad (12)$$

$$\frac{dG_2}{dt} = 1.056 r_1 - r_2 \quad (13)$$

$$\frac{dG}{dt} = 1.053 r_2 \quad (14)$$

In these rate equations, k_{1r} is the reaction rate constant (ml/ (mg h)); and k_{2r} is the reaction rate constant (h^{-1}); k_m is saturation constants (mg/ml); and C, G_2, G are concentrations of cellulose, cellobiose, and glucose (mg/ml), respectively. In addition, k_f and k_r are inactivation and reactivation rate constants (h^{-1}), respectively. SR is substrate reactivity and expressed as described in Eq (4).

3. Evaluation of model predictions

The model parameters were estimated using the AQUASIM by minimizing the sum of the squares of the weighted deviations ($\chi 2(m)$) between the experimental data and the model results. $\chi 2(m)$ is expressed as:

$$\chi 2(m) = \sum_{i=1}^{n} \left(\frac{X_{\exp,i} - X_i(m)}{\sigma_{\exp,i}}\right)^2 \quad (15)$$

In this equation $X_{\exp,i}$ is the i-th measurement, X_i (m) is the calculated value of the model variable corresponding to the i-th measurement, $\sigma_{\exp,i}$ is standard deviation, m is the model parameter and n is the number of data points. AQUASIM performs a minimization of the sum of squares with the restriction $m_{\min,i} \le m \le m_{\max,i}$, where $m_{\min,i}$ and $m_{\max,i}$ are the minimum and maximum of the constant variable m. The numerical values of the parameters were obtained by fitting the model to experimental data taken from the literature.

4. Results and Discussion

In this work, experimental results of enzymatic hydrolysis of cellulosic material under different conditions from four literatures are modeled. The conditions in the literatures are as follows:

1- Enzymatic hydrolysis of insoluble cellulose with initial substrate concentrations, 10, 20, 30, 40, 50, 75 g/l and cellulase concentration, 0.98 g/l; thereafter, initial substrate concentration, 50 g/l and cellulase concentrations, 0.49, 0.71, 0.98 g/l at 50°C, pH 4.5, in Fan and Lee study (Fan and Lee 1983).

2- Enzymatic hydrolysis of cellulose was prepared by a chemical treatment of cotton stalks, with initial substrate concentration, 40 g/l and cellulase concentration, 10 g/l; initial substrate concentration, 40 g/l and cellulase concentration, 25 g/l; initial substrate concentration, 80 g/l and cellulase concentration, 40 g/l; at 50°C, pH 4.5, in Gusakov et al study (Gusakov, Sinitsyn et al. 1985).

3- Enzymatic hydrolysis of noncrystalline cellulose (NCC) with initial substrate concentration, 11.5 g/l with 1 and 3 FPU/g-glucan cellulase loading at 50°C, pH 4.5 in Peri et al study (Peri, Karra et al. 2007).

4- Enzymatic hydrolysis of pretreated creeping wild ryegrass with initial substrate concentration, 42.5 g/l with 5 and 150 FPU/g-glucan enzyme loading at 50°C, pH 5 in Zheng et al study. Cellulase activity of 90 FPU/ml corresponding to 54 mg protein/ml (Zheng, Pan et al. 2009).

The main products of enzymatic hydrolysis of cellulosic material by cellulases are glucose and cellobiose. Therefore, the experimental results that were obtained from the literature containing glucose, cellobiose, and cellulose concentrations are evaluated by solving differential equations using AQUASIM software. The model parameters were estimated by minimizing the sum of the squares of the weighted deviations between measurements and calculated model results. The estimated parameters from the two models are shown in Table 1-2.

Table 1

Table 2

The comparisons between the experimental and predicted results using two models for prediction of glucose, cellobiose and cellulose concentrations versus time have been depicted in Fig. (2-7).

Fig. 2(a) shows the comparison of measured and predicted concentration versus time with initial substrate concentration 50 g/l and cellulase concentration, 0.98 g/l. Also, Fig. 2 demonstrate comparison of measured and predicted reducing sugar concentration at different initial cellulose concentration (10, 20, 30, 40, 50, 75 g/l) and cellulase concentration, 0.98 g/l (Fig. 2(b)); and at different initial cellulase concentration (0.49, 0.71, 0.98 g/l) and initial substrate concentration 50 g/l (Fig. 2(c)), in Fan and Lee study(Fan and Lee 1983).

Fig. 2

As can be seen, reducing sugar concentration is dependent on hydrolysis time, initial cellulose concentration and cellulase concentration. Sum of the squares of the weighted deviations ($\chi 2(m)$) have been calculated to be 80.52 and 69.39 for model 1 and 2, respectively. As predicted and illustrated in Fig. 2(c), the experimental results, as well as the model predictions both, indicate an increase in the reducing sugar production rates with increasing enzyme concentration. However, the hydrolysis rate did not increase at high enzyme concentration level because of saturation of enzyme on the available surface of cellulose.

Comparison between the predicted and experimental result of enzymatic hydrolysis of cellulose with condition base on Gusakov, Sinitsyn et al study(Gusakov, Sinitsyn et al. 1985) is illustrated in Fig. 3.

Fig. 3

Sum of the squares of the weighted deviations have been computed to be 57.810 and 38.42 for model 1 and 2, respectively. The simulation results represent that both models could successfully predict time course production of glucose, cellobiose, and cellulose during enzymatic hydrolysis.

Also, kinetic profiles of glucose, cellobiose, cellulose during enzymatic hydrolysis in Peri, Karra et al study(Peri, Karra et al. 2007) and Zheng, Y., et al. study (Zheng, Pan et al. 2009) are depicted in Fig. 4 and Fig. 5, respectively.

Fig. 4

Fig. 5

As can be seen, hydrolysis rate of cellulose is dependent on hydrolysis time, and cellulase concentration. Hydrolysis rate increase because of increasing enzyme concentration, this behavior is demonstrated in Fig 4(a, b) and Fig 5(a, b). However, the hydrolysis rate did not increase at high enzyme concentration level due to saturation of enzyme on the available surface of cellulose.

Sum of the squares of the weighted deviations have been calculated to be 11.65 and 5.49 for model 1 and 2 in Fig. 4, respectively. In addition, $\chi 2(m)$ values were 84.11 and 79.16 for model 1 and 2 in Fig. 5, respectively.

It can be observed that both model prediction fits well with the experimental values. However, comparison of $\chi 2(m)$ values of two models shows that the model proposed in this work is more accurate than the previously reported model in all the cases studied.

5. Conclusion

In the present study, a comparative study was performed between two models used for prediction of enzymatic hydrolysis of cellulosic material. One model was taken from the literature and another one was developed in this work from the basis of the substrate reactivity and enzyme inactivation. The simulation results indicate that the model proposed in this work could successfully be used to predict the time course of glucose, cellobiose, and cellulose during enzymatic hydrolysis. Moreover, the predictions by the model proposed in this work was more accurate to the experimental data reported in the literature when it is compared with previously reported models.

Authors' contributions

Hanieh Shokrkar: Conceptualization, Methodology, Data analysis and writing; Sirous Ebrahimi: Methodology

Conflict of interest

Conflict of Interest The authors declare no conflict of interest.

Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

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Table Captions:

Table 1. Estimate values of the parameters based on kinetic model from the literature

Table 2. Estimate values of the parameters based on proposed kinetic model

Figures Captions:

Fig. 1. Reaction scheme for modeling cellulose hydrolysis (a), literature model; (b), our model

Fig. 2. Comparison between predicted and experimental result of glucose, cellobiose and cellulose during enzymatic hydrolysis of cellulose with (a), initial substrate concentration, 50 g/l and cellulase concentration, 0.98 g/l; (b), different initial cellulose concentration (10, 20, 30, 40, 50, 75 g/l) and cellulase concentration, 0.98 g/l; (c), different initial enzyme concentration (0.49, 0.71, 0.98 g/l) and initial substrate concentration, 50 g/l. Solid and dashed lines show the simulation results of the previous model and our modified model, respectively.

Fig. 3. Comparison between predicted and experimental result of glucose, cellobiose, cellulose during enzymatic hydrolysis of cellulose (a), initial substrate concentration 40 g/l and concentration of cellulase, 10 g/l; (b), initial substrate concentration 40 g/l and concentration of cellulase, 25 g/l; (c), initial substrate concentration 80 g/l and concentration of cellulase, 40 g/l; 50°C; pH 4.5. Solid and dashed lines show the simulation results of the previous model and our modified model, respectively.

Fig. 4. Comparison between predicted and experimental result of glucose; cellobiose, cellulose during enzymatic hydrolysis of cellulose with (a), 1 FPU/g-glucan enzyme loading; (b), 3FPU/g-glucan enzyme loading. Solid and dashed lines show the simulation results of the previous model and our modified model, respectively.

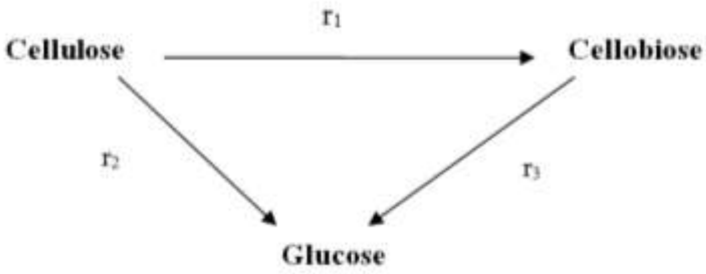
Fig. 5. Comparison between predicted and experimental result of glucose, cellobiose, cellulose during enzymatic hydrolysis of cellulose with (a), 5 FPU/g-glucan; (b), 150 FPU/g-glucan enzymes loading, Solid and dashed lines show the simulation results of the previous model and our modified model, respectively.

Table 1. Estimate values of the parameters based on kinetic model from the literature

Parameters	Estimate values(Fan and Lee 1983)	Estimate values(Gusakov, Sinitsyn et al. 1985)	Estimate values (Peri, Karra et al. 2007)	Estimate values(Zheng, Pan et al. 2009)
k_{1IG2} (mg/ml)	0.04	0.04	0.04	0.04
k_{1IG} (mg/ml)	0.10	0.10	0.10	0.10
k_{1r} (ml/(mg h))	7.62	0.294	160.2	16.5
k_{2IG2} (mg/ml)	132.50	132.50	132.50	132.50
k_{2IG} (mg/ml)	0.01	0.01	0.01	0.01
k_{2r} (ml/(mg h))	1.248	0.168	99.60	7.08
k_{3M} (mg/ml)	25.50	25.50	25.50	25.50
k_{3IG} (mg/ml)	2.10	2.10	2.10	2.10
k_{3r} (h^{-1})	1.626	0.204	312.60	267.60
$\chi 2(m)$	80.52	57.810	11.65	84.11

Table 2. Estimate values of the parameters based on proposed kinetic model

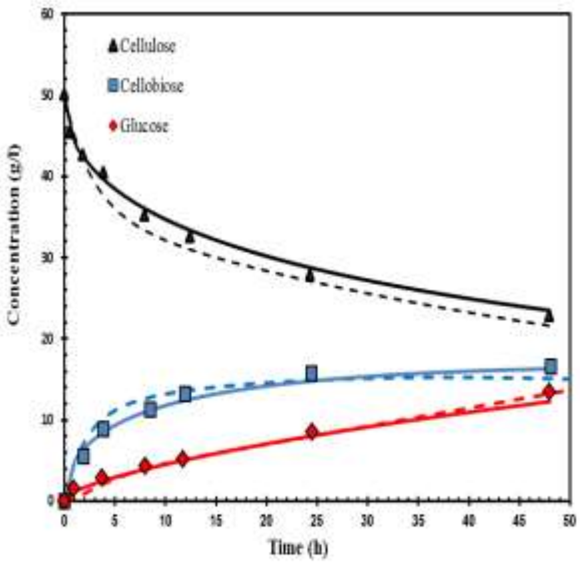
Parameters	Estimate values(Fan and Lee 1983)	Estimate values(Gusakov, Sinitsyn et al. 1985)	Estimate values(Peri, Karra et al. 2007)	Estimate values(Zheng, Pan et al. 2009)
k_r (h^{-1})	0.033	0.033	0.033	0.033
k_f (h^{-1})	0.251	0.251	0.251	0.251
k_{1r} (ml/(mg h))	10.86	0.36	612	42.30
K_m (mg/ml)	38.638	38.638	38.638	38.638
k_{2r} (h^{-1})	7.02	0.36	1320.60	852.60
$\chi 2(m)$	69.39	38.42	5.49	79.16



(a)

(b)

Fig. 1. Reaction scheme for modeling cellulose hydrolysis (a), literature model; (b), our model



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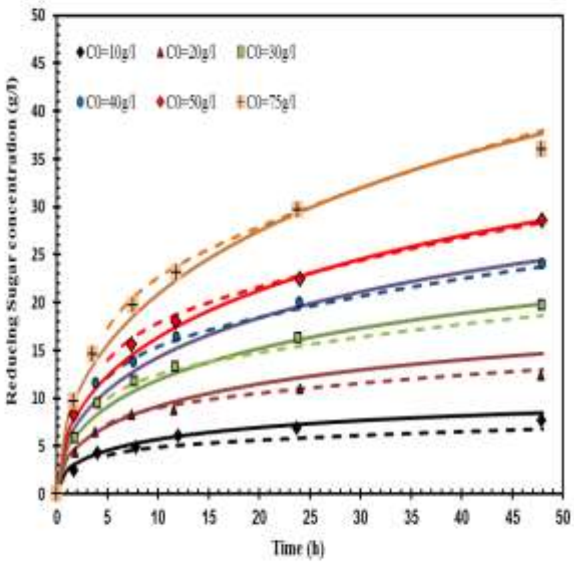

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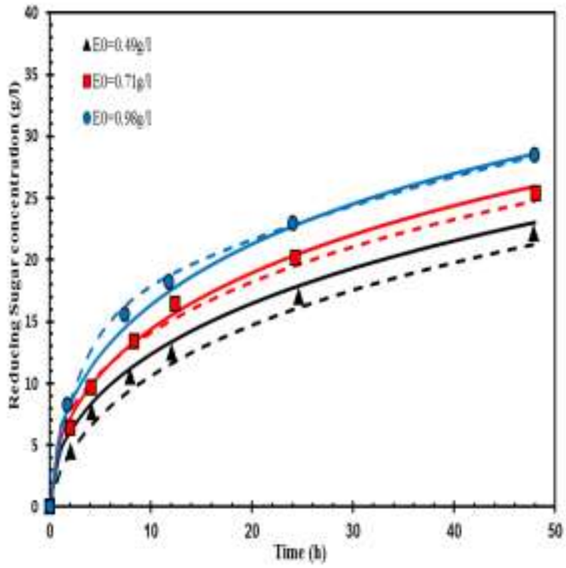

(c)

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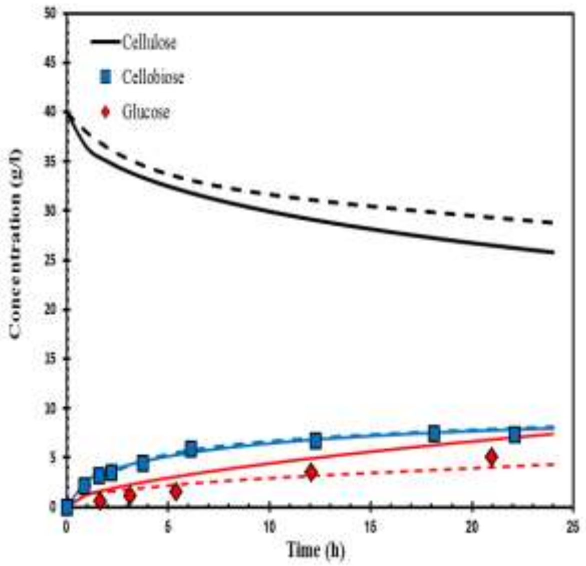
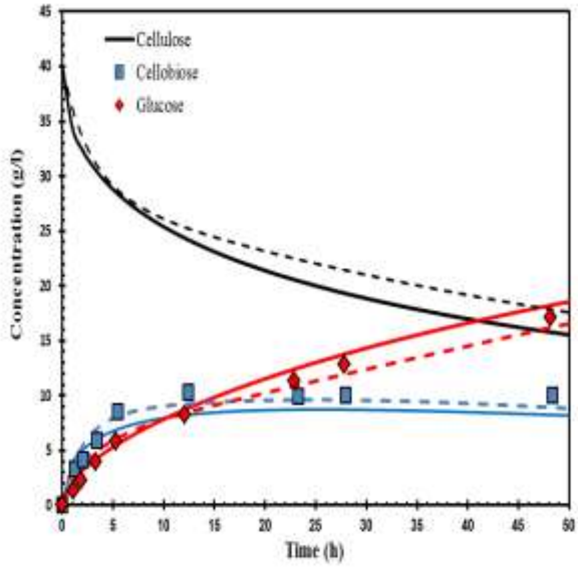
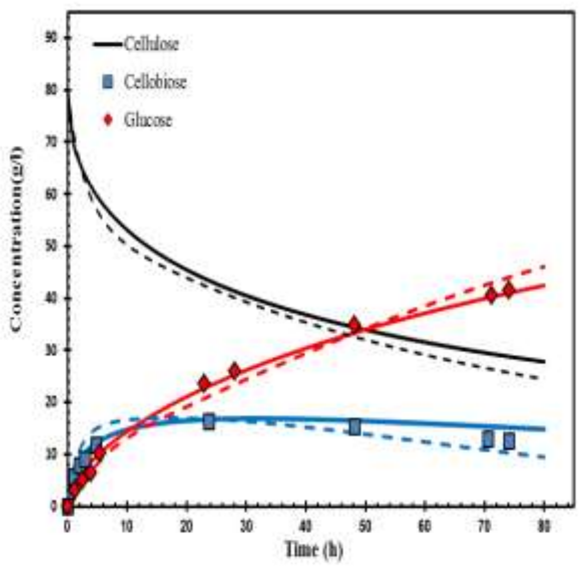




(a) (b)



(c)

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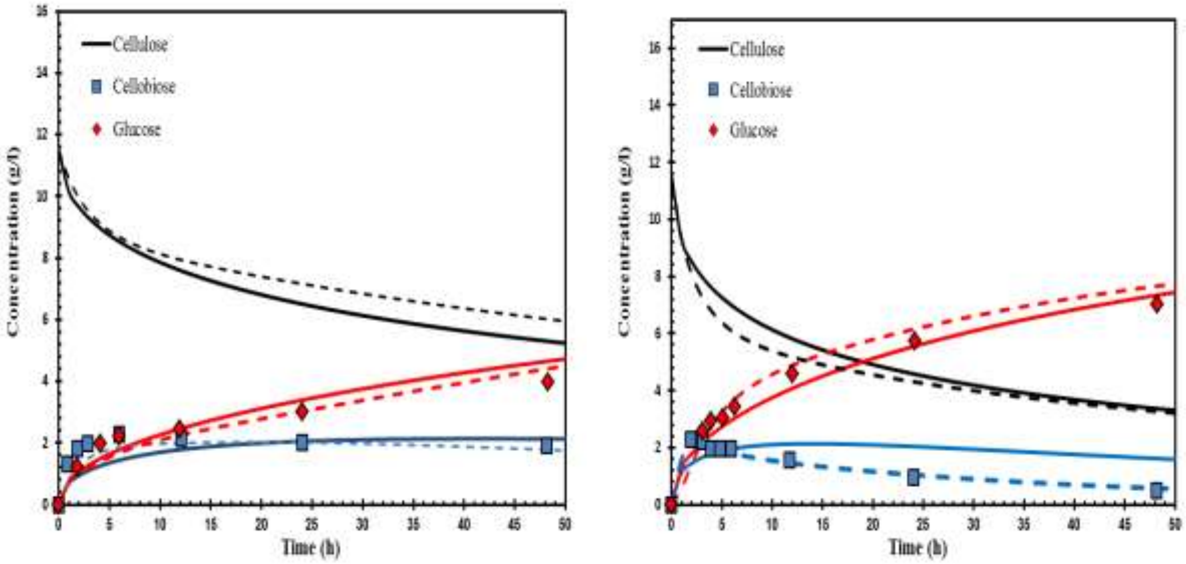

(a) (b)

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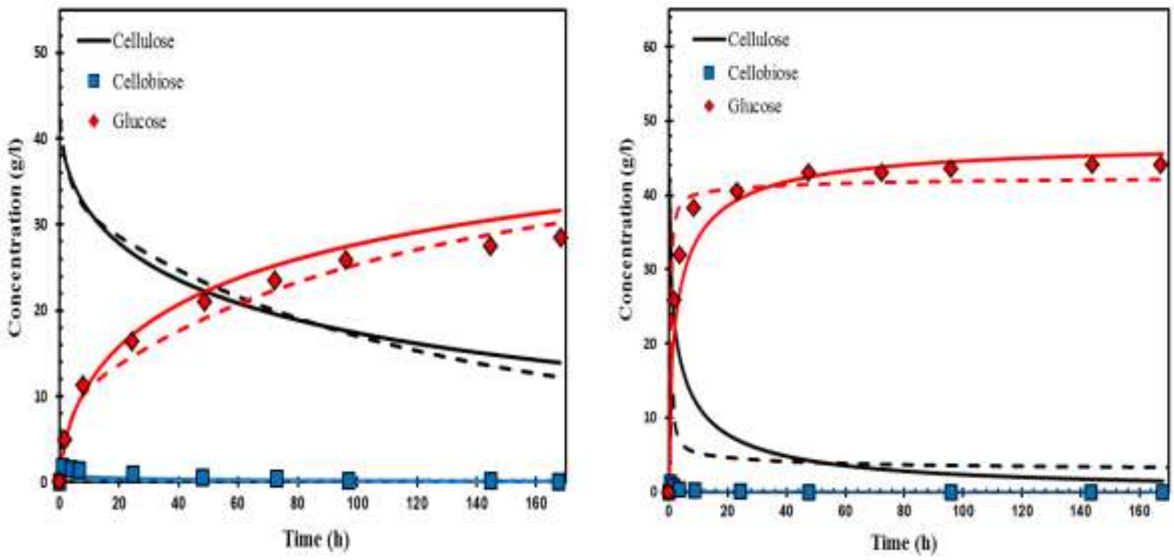

(a) (b)

Fig. 5. Comparison between predicted and experimental result of glucose, cellobiose, cellulose during enzymatic hydrolysis of cellulose with (a), 5 FPU/g-glucan; (b), 150 FPU/g-glucan enzymes loading, Solid and dashed lines show the simulation results of the previous model and our modified model, respectively.

Figures

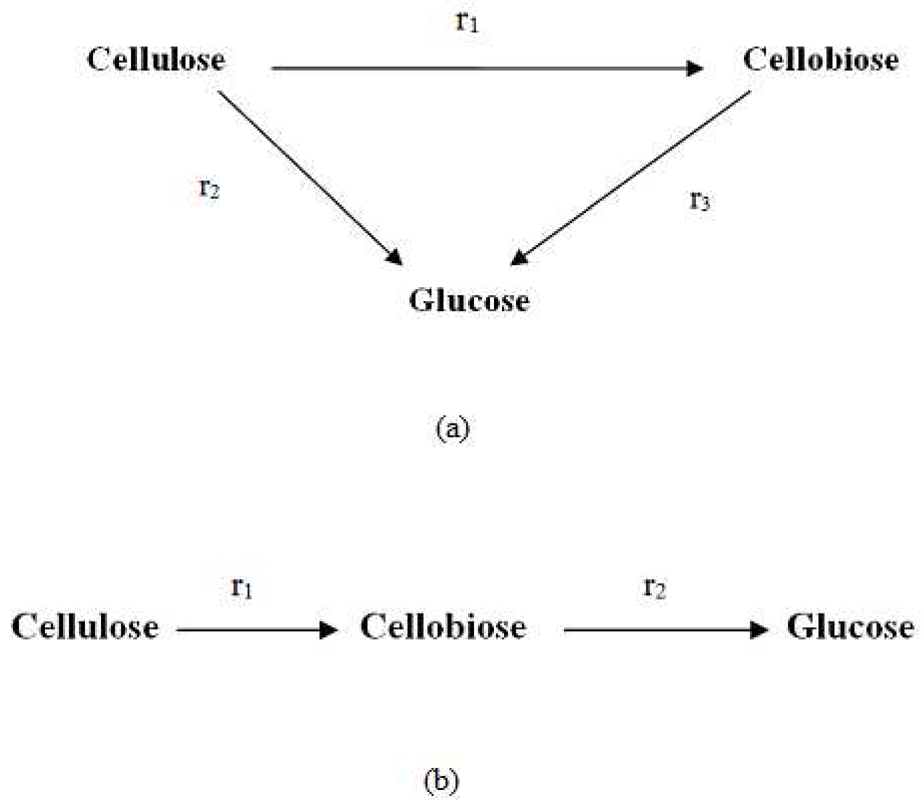

Figure 1

Reaction scheme for modeling cellulose hydrolysis (a), literature model; (b), our model

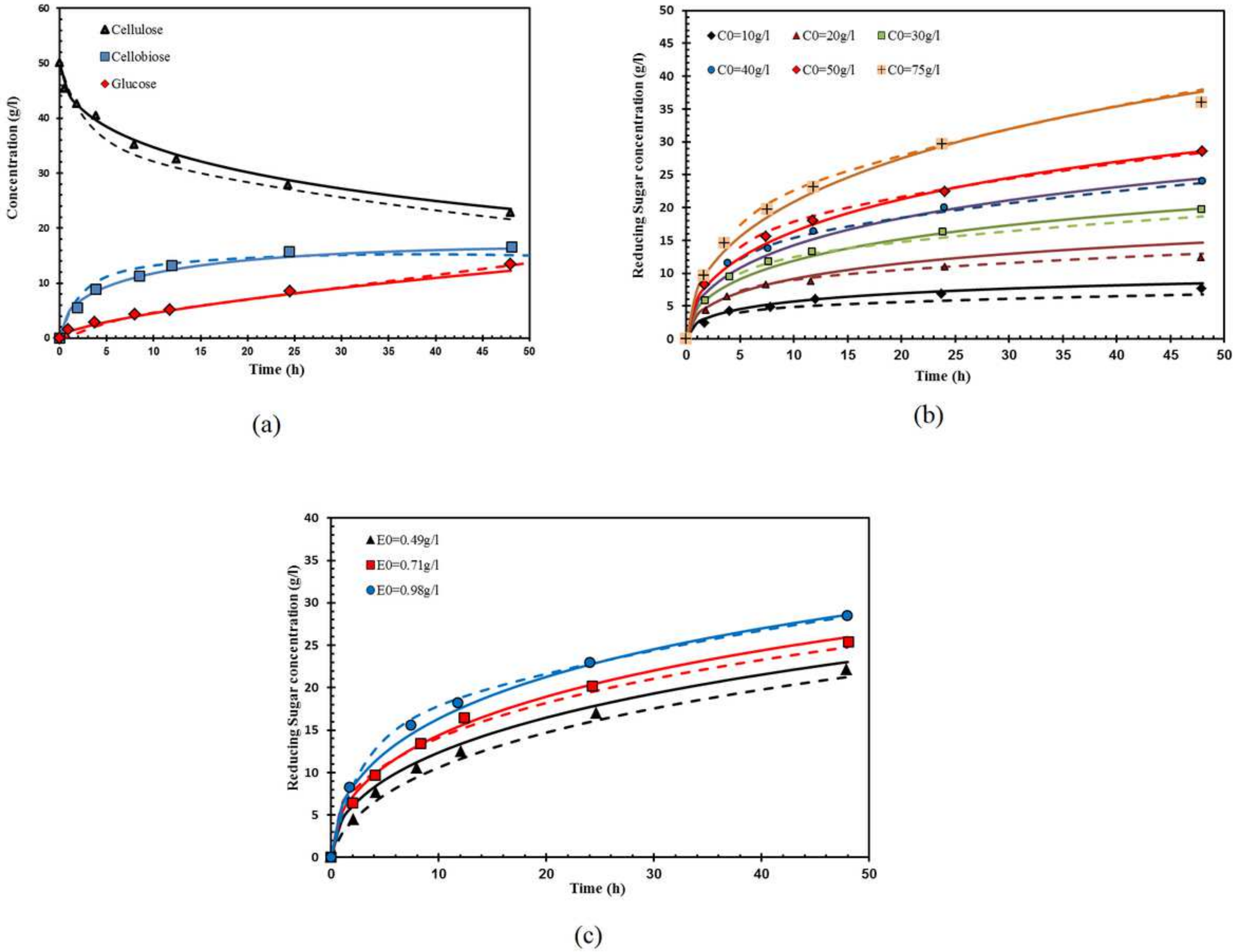

Figure 2

Comparison between predicted and experimental result of glucose, cellobiose and cellulose during enzymatic hydrolysis of cellulose with (a), initial substrate concentration, 50 g/l and cellulase concentration, 0.98 g/l; (b), different initial cellulose concentration (10, 20, 30, 40, 50, 75 g/l) and cellulase concentration, 0.98 g/l; (c), different initial enzyme concentration (0.49, 0.71, 0.98 g/l) and initial substrate concentration, 50 g/l. Solid and dashed lines show the simulation results of the previous model and our modified model, respectively.

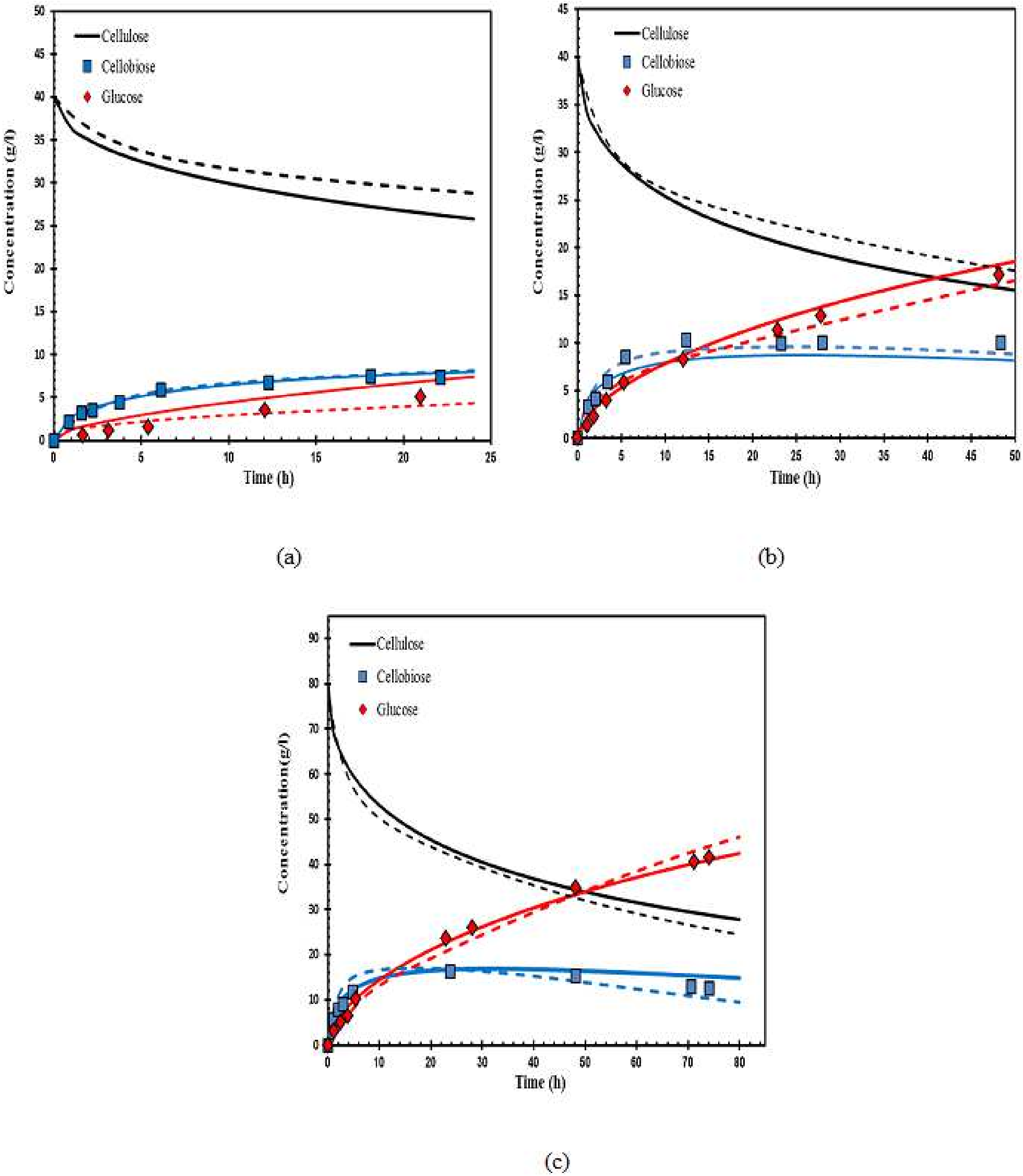

Figure 3

Comparison between predicted and experimental result of glucose, cellobiose, cellulose during enzymatic hydrolysis of cellulose (a), initial substrate concentration 40 g/l and concentration of cellulase, 10 g/l; (b), initial substrate concentration 40 g/l and concentration of cellulase, 25 g/l; (c), initial substrate concentration 80 g/l and concentration of cellulase, 40 g/l; 50°C; pH 4.5. Solid and dashed lines show the simulation results of the previous model and our modified model, respectively.

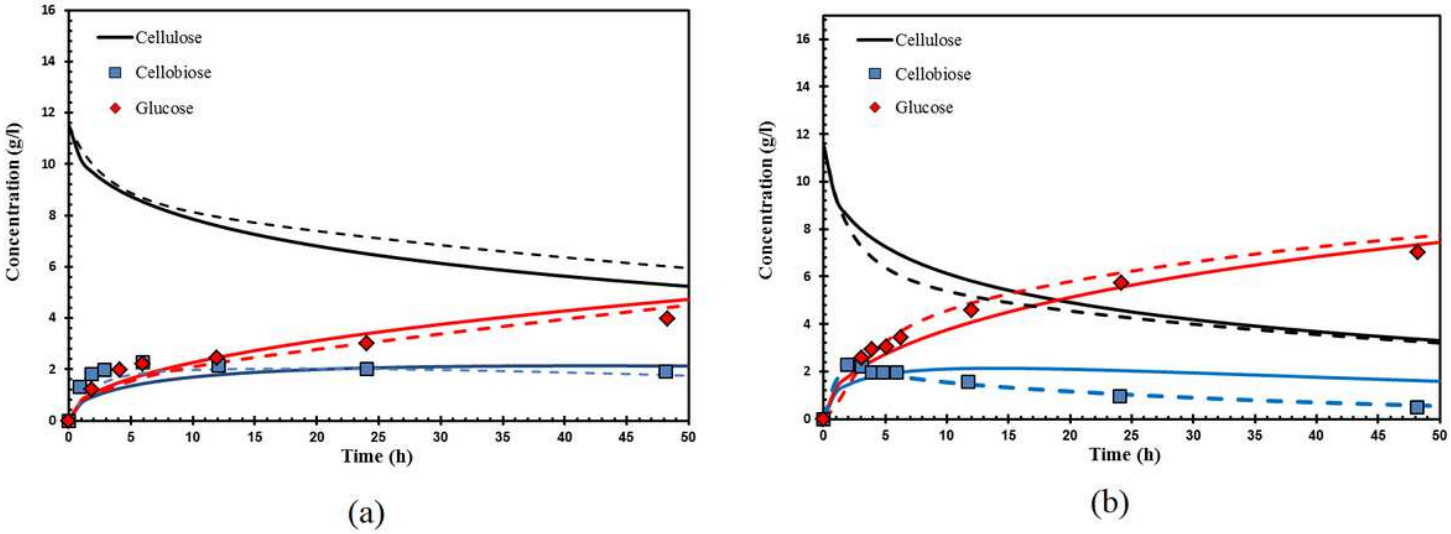

Figure 4

Comparison between predicted and experimental result of glucose; cellobiose, cellulose during enzymatic hydrolysis of cellulose with (a), 1 FPU/g-glucan enzyme loading; (b), 3FPU/g-glucan enzyme loading. Solid and dashed lines show the simulation results of the previous model and our modified model, respectively.

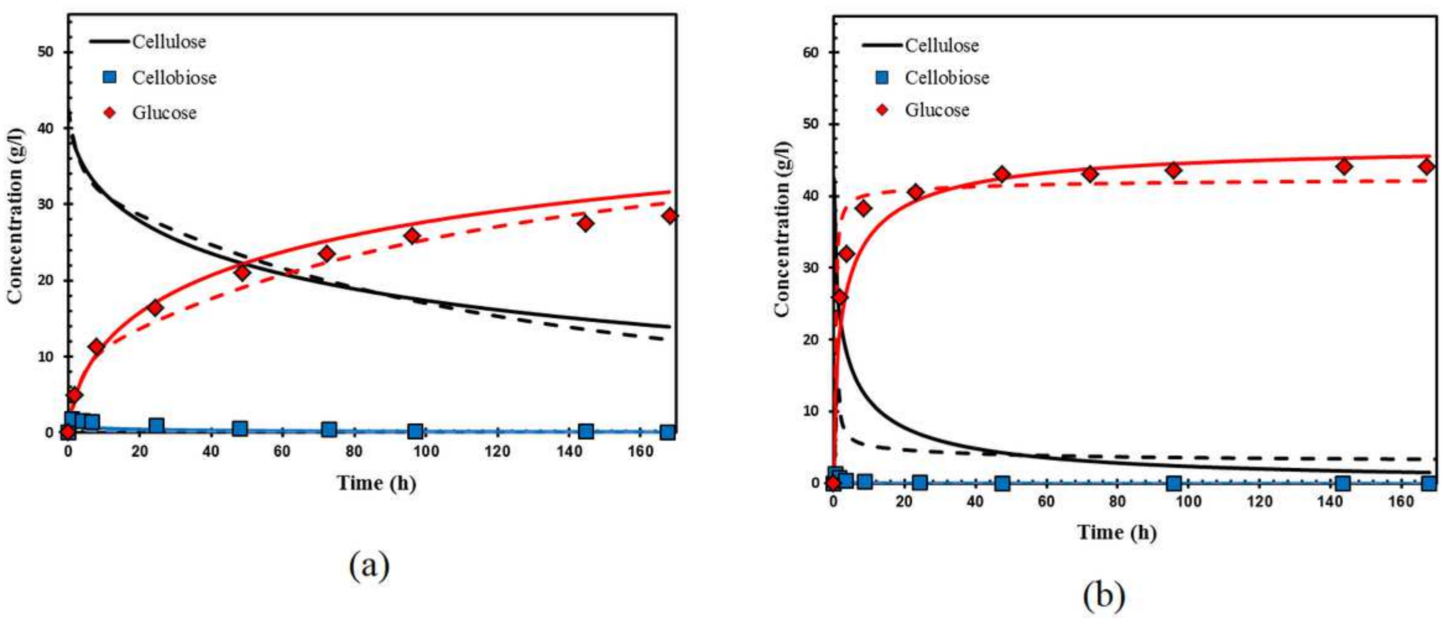

Figure 5

Comparison between predicted and experimental result of glucose, cellobiose, cellulose during enzymatic hydrolysis of cellulose with (a), 5 FPU/g-glucan; (b), 150 FPU/g-glucan enzymes loading, Solid and dashed lines show the simulation results of the previous model and our modified model, respectively.