Energy Conversion and Management 141 (2017) 236–243

Contents lists available at ScienceDirect

Energy Conversion and Management

journal homepage: www.elsevier.com/locate/enconman

Kinetic modeling and dynamic analysis of simultaneous saccharification and fermentation of cellulose to bioethanol

Jalil Shadbahr, Faisal Khan *, Yan Zhang

Department of Process Engineering, Memorial University of Newfoundland, St. John's, NL A1B 3X5, Canada

ARTICLE INFO

Article history:
Available online 28 August 2016

Keywords:
Simultaneous saccharification and fermentation
Enzyme loading
Bioethanol
Glucose
Mannose
Ethanol yield

ABSTRACT

Kinetic modeling and dynamic analysis of the simultaneous saccharification and fermentation (SSF) of cellulose to ethanol was carried out in this study to determine the key reaction kinetics parameters and product inhibition features of the process. To obtain the more reliable kinetic parameters which can be applied for a wide range of operating conditions, batch SSF experiments were carried out at three enzyme loadings (10, 15 and 20 FPU/g cellulose) and two levels of initial concentrations of fermentable sugars (glucose and mannose). Results indicated that the maximum ethanol yield and concentration were achieved at high level of sugar concentrations with intermediate enzyme loading (15 FPU/g cellulose). Dynamic analysis of the acquired experimental results revealed that cellulase inhibition by cellobiose plays the most important role at high level of enzyme loading and low level of initial sugar concentrations. The inhibition of glucose becomes significant when high concentrations of sugars were present in the feedstock. Experimental results of SSF process also reveal that an efficient mixing between the phases helps to improve the ethanol yield significantly.

1. Introduction

Ethanol produced from lignocellulosic biomass, the most abundantly available raw material on Earth has been considered as one of the most attractive and promising renewable energy sources [1]. Lignocellulosic material, obtained as a by-product of the agriculture/forestry industries or energy crops, is mainly composed of cellulose, hemicellulose, and lignin, among which cellulose and hemicellulose are digestible by microorganisms for energy [2]. Due to the complexity of the lignocellulosic macromolecular structure, biochemical conversion of lignocellulosic biomass consists of four processing steps: (1) pretreatment to liberate cellulose and hemicellulose. The main purpose of this step is to disorganize the crystalline structure of macro- and micro fibrils to release the polymer chains of cellulose and hemi-cellulose [3,4]; (2) enzymatic hydrolysis of polysaccharides; (3) fermentation of monomeric sugars and (4) ethanol recovery and dehydration [5–7]. The current technology of lignocellulosic ethanol does not support the cost-efficient production, preventing its commercialization [8]. Exploration of cost-reduction strategy is essential for the commercialization of lignocellulosic ethanol.

Cost-competitive technology can be developed by improving the performance of enzymatic hydrolysis and fermentation, the key processing steps in lignocellulosic ethanol. The reasons are twofold. Firstly cellulase, the enzyme used in hydrolysis of cellulose contributes significantly to the cost of the bioethanol production, accounting for 20–30% of the total cost [9,10]. Secondly, the cost of the downstream ethanol distillation is directly bound up with the ethanol concentration attainable from the fermentation of monomeric sugars. Ethanol concentration higher than 40 g/L is prerequisite to make the distillation process economically feasible [11]. Therefore, reducing the cost of lignocellulosic ethanol can be achieved by optimizing the hydrolysis and fermentation processes so that maximum ethanol concentration and yield are attainable at relative lower enzyme consumption.

Extensive research has demonstrated that SSF, the simultaneous saccharification (hydrolysis) of cellulose to fermentable sugars and fermentation of sugars to ethanol, helps to achieve higher ethanol productivity by reducing the inhibitory impact of converted sugars [12–15]. Nonetheless, SSF process is exceptionally complex and its performance (reaction conversion, final ethanol yield and concentration) is greatly influenced by many factors such as the type of lignocellulosic feedstock, the substrate concentration, the type and amount of cellulolytic enzymes and microorganisms, solution pH and reaction temperature [16,17]. The optimal pH (≈5.0) and reaction temperature (37 °C) [18,19] of SSF process

* Corresponding author.
E-mail address: fikhan@mun.ca (F. Khan).

http://dx.doi.org/10.1016/j.enconman.2016.08.025
0196-8904/

Nomenclature

$[B]$	concentration of cellobiose (g/L)
$[C]$	concentration of cellulose (g/L)
$[E]$	concentration of ethanol (g/L)
$[enzc]$	cellulase activity concentration (FPU/g cellulose)
$[enzg]$	β-glucosidase activity concentration (U/g cellulose)
$[G]$	concentration of glucose (g/L)
k_1	maximum specific rate of cellulose hydrolysis to cellobiose (h^{-1})
k'_1	lumped specific rate of cellulose hydrolysis to cellobiose (h^{-1})
k_2	specific rate of cellobiose hydrolysis to glucose (g/U h)
K_{eq}	cellulase adsorption saturation constant (FPU/g cellulose)
K_E	ethanol inhibition constant for the microorganism (g/L)
K_G	glucose saturation constant for the microorganism (g/L)
K_L	constant for β-glucosidase adsorption to lignin (L/g)
K_m	cellobiose saturation constant for β-glucosidase (g/L)
K_{1B}	inhibition constant of cellulase by cellobiose (g/L)
K_{1E}	inhibition constant of cellulase by ethanol (g/L)
K_{1G}	inhibition constant of cellulase by glucose (g/L)
K_{2G}	inhibition constant of β-glucosidase by glucose (g/L)
$[L]$	concentration of lignin (g/L)
$[M]$	concentration of mannose (g/L)
m_s	specific rate of substrate consumption for maintenance requirements (h^{-1})
r_G	volumetric rate of glucose consumption (g/L h)
r_M	volumetric rate of mannose consumption (g/L h)
r_X	volumetric rate of cell mass production (g/L h)
r_1	volumetric rate of cellulose hydrolysis to cellobiose (g/L h)
r_2	volumetric rate of cellobiose hydrolysis to glucose (g/L h)
t	time (h)
$[X]$	concentration of cell mass (g/L)
Y_{XG}	yield coefficient of cell mass from glucose (g/g)
λ	rate of decrease in cellulose specific surface area (h^{-1})
μ_m	maximum specific growth rate of the microorganism (h^{-1})

turned out to be quite rigid because a compromise between optimal pH and temperatures of the cellulolytic enzymes and the yeast is needed [16,20]. Dissimilarly, determination of the optimal substrate concentration and enzyme loading is not straightforward [5,21,22]. Usually, a high substrate concentration has to be used in the SSF process to obtain high ethanol concentration and yield [23–25]. However, high substrate concentration causes substrate inhibition, which substantially lowers the rate of the hydrolysis and metabolism of yeast [24]. Increasing the dosage of enzymes, to a certain extent, helps to increase the conversion rate of substrate, and hence improve the final ethanol yield and concentration. But high enzyme consumption significantly increases the operating cost [26]. Finding an optimum combination of substrate concentration and enzyme/microorganism loading for a specific feedstock is challenging in this regard.

Kinetic modeling of cellulose bioconversion is an important tool in predicting the rates of enzymatic hydrolysis and fermentation as well as the dynamic features of the process [27,28]. Kinetic modeling of SSF process is an influential step toward industrialization of bioethanol production from lignocellulosic biomass due to the fact that establishing the concepts of production process with emphasizing on the experimental data is not sufficient and it costs plenty of time and resources. Moreover, proper kinetic model and reliable model parameters are indispensable for optimizing the performance of SSF process. Although a number of kinetic models have been developed over the past years for SSF process [29–32], these models were usually tuned for specific substrates over a relatively narrow range of operating conditions. In this study, kinetic model of a batch SSF process was developed to incorporate the variations of substrate composition and enzyme loading. Obviously hydrolysate fraction of the pretreated biomass could be diluted in different ratios, which could lead to varied initial sugars concentration in the SSF feedstock. Evaluating kinetic model and kinetic parameters to variation of sugars concentration helps to obtain the more reliable kinetic parameters which can be applied for a wide range of operating conditions and biomass compositions. Therefore, in the current study, kinetic model parameters were estimated by fitting the models to experimental data obtained from a wide range of operating conditions. Dynamic characteristics and rate limiting causes of the SSF process were analyzed through the interactive influence of the initial concentrations of fermentable sugars and enzyme loading on the bioconversion of cellulose to ethanol.

2. Kinetic modeling

Simultaneous enzymatic hydrolysis and fermentation of cellulose is a complex multistep process and interactions between enzymes with solid substrate as well as the product inhibition mechanism are not fully understood. A modified mathematical model based on those reported by Philippidis et al. [12,31,32] and Pettersson et al. [33] were used in this study to quantify the enzymatic hydrolysis and sugar fermentation. The kinetic model assumes that cellulase hydrolyzes cellulose to cellobiose with negligible formation of glucose through the cooperation of endo- and exoglucanases and β-glucosidase converts one of cellobiose (342.29 g/mol) to two moles of glucose (180.16 g/mol). In addition, fermentation of mannose, a C-2 epimer of glucose which is usually present in a pretreated softwood substrate is also taken into account. One mole of glucose or mannose (180.16 g/mol) will be fermented to two moles of ethanol (46.06 g/mol) and two moles of carbon dioxide (44.01 g/mol). The reaction network for the biochemical conversion of cellulose is supposed to follow the route listed below:

The rates of reactions listed in Fig. 1 are expressed by Eqs. (1)–(5).

$$r_1 = \frac{k'_1[C]e^{-\lambda t}}{1 + [B]/K_{1B} + [G]/K_{1G}}\left(\frac{K_{1E}}{K_{1E} + [E]}\right)$$
$$\text{where } k'_1 = \frac{k_1[enzc]}{K_{eq} + [enzc]} \quad (1)$$

$$\text{Cellulose(C)} \xrightarrow{r_1} \text{Cellobiose(B)} \xrightarrow{r_2} \text{Glucose(G)} \xrightarrow{r_X, r_G} \text{Ethanol(E) \& Cellmass(X)}$$

$$\text{Mannose(M)} \xrightarrow{r_X, r_M} \text{Ethanol(E) \& Cellmass(X)}$$

Fig. 1. Reaction network of SSF process.

$$r_2 = \frac{k_2[enzg][B]}{K_M(1+[G]/K_{2G})+[B]}(1-K_L[L]) \quad (2)$$

$$r_X = \mu_m[X]\left(\frac{[G]+[M]}{K_G+[G]+[M]}\right)\left(\frac{K_E}{K_E+[E]}\right) \quad (3)$$

$$r_G = \frac{[G]}{[G]+[M]}\left(\frac{r_X}{Y_{XG}}+m_s[X]\right) \quad (4)$$

$$r_M = \frac{[M]}{[G]+[M]}\left(\frac{r_X}{Y_{XG}}+m_s[X]\right) \quad (5)$$

The mass balance for the key components in the SSF process can be described by the equations listed below:

$$\frac{d[C]}{dt} = -r_1 \quad (6)$$

$$\frac{d[B]}{dt} = 1.056r_1 - r_2 \quad (7)$$

$$\frac{d[G]}{dt} = 1.053r_2 - r_G \quad (8)$$

$$\frac{d[M]}{dt} = -r_M \quad (9)$$

$$\frac{d[X]}{dt} = r_X \quad (10)$$

$$\frac{d[E]}{dt} = 0.511(r_G + r_M) \quad (11)$$

In this study, ethanol yield with respect to total sugars in the media, including initial glucose and mannose and potential sugar which is convertible from cellulose by hydrolysis reaction, was used to evaluate the SSF performance. Total amount of sugars can be calculated based on the stoichiometry of the components involved in the process as illustrated below:

$$\text{Total sugars} = [G]_0 + [M]_0 + 1.111[C]_0 \quad (12)$$

where $[G]_0$, $[M]_0$ and $[C]_0$ are the initial concentrations of glucose, mannose and cellulose respectively. The theoretical maximum ethanol that can be achieved is calculated based on the total sugars in media and is defined as:

$$\text{Theoretical maximum ethanol} = 0.511\left([G]_0 + [M]_0 + 1.111[C]_0\right) \quad (13)$$

The constant 0.511 is the conversion factor of glucose to ethanol extracted from stoichiometry of the reaction. Based on the theoretical maximum ethanol produced from SSF process, the ethanol yield can be calculated by the following equation:

$$\text{Ethanol yield } (\%) = \frac{[E]_f - [E]_0}{0.511\left([G]_0 + [M]_0 + 1.111[C]_0\right)} \quad (14)$$

where $[E]_0$ and $[E]_f$ represent the initial and final concentration of ethanol.

3. Materials and methods

3.1. Feedstock

Feedstock for batch SSF process in this research includes extra pure microcrystalline cellulose, ACS grade glucose and 99% mannose purchased from Fisher Scientific. To investigate the influence of the initial sugar concentrations on the final ethanol concentration and yield, two levels of sugar concentration were used in the experiment. At the first level concentrations of 5 g/L and 4.5 g/L were used for glucose and mannose, respectively. For the second level of the experiments, glucose and mannose concentrations were increased to 10 g/L and 9 g/L, respectively. The amount of insoluble cellulose was fixed at 5% (w/v) for all the experiments and fresh ultrapure water was used for all steps of the experiments.

3.2. Enzymes

The commercial enzyme cellulase from *Trichoderma reesei* (ATCC 26921) supplemented with β-Glucosidase from almonds was purchased from Sigma-Aldrich. Filter paper units (FPU) were calculated using Santos et al. [34]. The amount of enzymes added to the reactants provides the activities of 10, 15, and 20 FPU/g cellulose. The activity for the β-Glucosidase was fixed at 30 U/g cellulose for all experiments.

3.3. Yeast preparation

Inoculum was prepared on the agar plate from the *Saccharomyces cerevisiae* demo plate purchased from the VWR Canada under the sterile condition, and then stored at 4 °C. The YPD broth from the HIMEDIA was used for the preparation of YPD solution with the concentrations of 10, 20, and 20 g/L for yeast extract, peptone, and dextrose, respectively. The YPD solution then sterilized in autoclave for 30 min at the pressure of 15 psi and temperature of 121 °C. When the temperature of YPD solution reached room temperature, the cells were added to solution and placed in the rotary shaker and incubated at 30 °C with the speed of 200 rpm for growing. After 24 h, grown cells were centrifuged for 10 min at 4500 rpm centrifuge to separate cells from the YPD solution. The separated cells were washed and centrifuged twice by ultrapure water and then stored in fridge at 4 °C for use.

3.4. SSF experiments

The SSF experiments were carried out in a 250 mL jacketed flask (Bellco, US) with an active volume of 100 mL. The reaction temperature was controlled by a Julabo FP 50 heated/refrigerated circulator (Allentown, PA, US). Experiments were conducted at 37 °C and pH of 5.0 for 96 h. During SSF experiment, solution pH was monitored using an Accumet AB 15 plus pH meter and adjusted by 1 M NaOH solution. For agitation, a baffled magnetic stirrer was used to provide the agitation at the speed of 350 rpm. In addition to the reactants, enzymes, and yeast, three chemical components were also added to reactor supplement the reaction as nutrients with the following final concentrations: $(NH_4)_2HPO_4$: 0.5 g/L, $MgSO_4.7H_2O$: 0.025 g/L, and Yeast Extract: 1.0 g/L. The samples were taken at 2, 4, 8, 12, 18, 24, 36, 48, 60, 72, 84, and 96 h for analysis. The summary of the SSF experimental conditions are given in Table 1.

Exp. #3 and Exp. #4 were carried out twice to examine the repeatability of the experimental methodology. It is confirmed that experiment results are duplicable and the ethanol yields under the conditions of Exp. #3 and Exp. #4 were achieved with ±2%.

3.5. Analytical method

Analysis of the samples was performed by HPLC system for the concentration of ethanol, glucose, cellobiose, and mannose. All the samples were taken in duplicate and after centrifuge and filtration by 0.2 μm sterile filter stored in a freezer for further analysis. Samples were analyzed using Agilent Hi-Plex H and Agilent Hi-Plex Pb columns by Dionex Ultimate 3000 HPLC system equipped with a Refractive Index detector (RefractorMax 520). Temperatures of the RI detector and HPLC column were set to 55 °C and 50 °C, respectively. 0.005 M sulfuric acid solution and ultrapure water,

Table 1
Initial sugar concentrations, enzyme and yeast loadings for SSF experiment.

Exp. #	Glucose concentration (g/L)	Mannose concentration (g/L)	Cellulase (FPU/g cellulose)	β-Glucosidase (U/g cellulose)	Yeast g dry cell/L
1	5	4.5	10	30	5
2	10	9			
3	5	4.5	15		
4	10	9			
5	5	4.5	20		
6	10	9			

Note: The amount of cellulose substrate was fixed at 5% (w/v) for all the experiments.

with the flowrate of 0.7 mL/min, were used as the mobile phases for Agilent Hi-Plex H and Hi-Plex Pb columns respectively.

3.6. Numerical method

The values of inhibition parameters K_m, K_{1B}, K_{1E}, K_{1G} and K_{2G}, the enzyme parameter K_L as well as the microorganism parameters K_E, K_G, m_s, and Y_{XG} were determined through a number of specific experiments by Philippidis et al. and were used directly in this study as the operating conditions of SSF experiment in this work are quite close to those reported by Philippidis et al. [12,31,32].

The remaining kinetic parameters (k_1, k_2, K_{eq}, λ and μ_m) were determined by minimizing the differences between experimental data and predicted amount at the same time. An error function F(p), defined as the sum of square deviations of the calculated concentration profiles from the experimentally measured curves, is used as the objective function to obtain the best-fit values of kinetic parameters.

$$F(p) = \min \sum_{i=1}^{n} \sum_{j=1}^{m} \left[c_{i,j}^{\exp} - c_{i,j}^{\mathrm{bmod}} \right]^2 \quad (15)$$

where $c_{i,j}^{\exp}$ and $c_{i,j}^{\mathrm{bmod}}$ are the measured and model predicted concentrations of component i at sampling point j. The best–fit kinetic parameters were determined by minimizing the scalar function F(p) using "fmincon", a constraint nonlinear optimization solver from MATLAB R2014b. Model predicted concentrations of sugars and ethanol, $c_{i,j}^{\mathrm{bmod}}$ were obtained by solving the initial problem ODEs (listed in Eqs. (6)–(11)) by ode15 s, a stiff ODE solver which uses the backward differentiation formula (BDF, also known as Gear's method).

4. Results and discussion

4.1. Kinetic parameters

Experimental measurements from Exp. #1 - Exp. #4 were used to determine the kinetic parameters and two sets of kinetic parameters were finally obtained with respect to the different initial sugar concentrations. The best-fit values of the kinetic parameters are listed in Table 2. Fig. 2 illustrates the comparison between model predictions and the measured experimental results of ethanol concentration from Exp. #1 - Exp. #4. Results from Table 2 indicated that cellulase adsorption saturation constant, K_{eq}, converged to a uniform value regardless of the variation of operating conditions. Higher initial sugar concentration resulted in strong product inhibition of enzymatic hydrolysis, leading to smaller values of k_1, λ and k_2. Meanwhile, higher initial sugar concentration accelerated the growth rate of microorganisms due to the presence of more nutrients, reflected from the convergence of a higher value of μ_m.

Table 2
Estimated kinetic parameters at different levels of sugar concentrations.

Tuned parameters		Low sugar level (Exp. #1, #3 & #5)	High sugar level (Exp. #2, #4 & #6)
k_1	h^{-1}	0.165–0.256	0.043–0.074
λ	h^{-1}	0.058–0.064	0.019–0.039
K_{eq}	FPU/g	117.90	117.81
k_2	g/U h	0.24–0.33	0.19–0.20
μ_m	h^{-1}	0.18–0.21	0.39–0.40

Other parameters[a]

K_{1B}	g/L	5.85	K_M	g/L	10.56
K_{1E}	g/L	50.35	K_G	g/L	3.73×10^{-5}
K_{1G}	g/L	53.16	K_L	L/g	0.0053
K_{2G}	g/L	0.62	m_s		0
K_E	g/L	50	Y_{XG}	g/g	0.113

[a] The values of these parameters come from Pettersson et al. [33].

To verify the validity of the derived kinetic parameters, the kinetic parameter values listed in Table 2 were used to simulate the ethanol concentration obtained from the batch SSF process under the operating conditions of Exp. #5 and Exp. #6 with the highest cellulose loading being used.

The comparison of the simulation results and the corresponding experimental data is shown in Fig. 3, from which a very good agreement between the model predictions and experimental results is observed.

The validity of the derived parameters is also proved by comparing them with those determined by other work under similar operating conditions. The values of the parameters listed in Table 2 are quite consistent with those reported by Pettersson et al., van Zyl et al., and Philippidis, et al. [30,32,33].

4.2. Dynamic characteristics of the SSF process

The dynamic features of the SSF process can be analyzed from the measured concentration profiles of glucose, mannose, cellobiose and ethanol illustrated in Fig. 4. In each case, glucose and mannose present in the feedstock were quickly converted to ethanol, accompanied by dramatic variations in the concentrations of glucose, mannose and ethanol within the first 2 h. After that, the concentrations of glucose and mannose decreased very slowly whereas the concentration of ethanol ascended gradually. Concentration of cellobiose, an intermediate product converted from cellulose by enzymatic hydrolysis, increased quickly to peak values in the first 2 h and then declined gradually till the end of the experiments.

Experimental results of the SSF process indicated that initial concentrations of fermentable sugars (glucose and mannose) have great impact on ethanol concentration. As seen from Fig. 4c and d, increasing the glucose concentration from 5 to 10 g/L and mannose from 4.5 to 9 g/L in the feedstock led to an escalation of ethanol concentration from 2.5 to 4.1 g/L (Exp. #1, #3 & #5) to about 6.5–7.6 g/L (Exp. #2, #4 & #6) after 2 h of SSF experiment. The highest ethanol concentration was obtained from Exp. #4 with the high level sugar concentration in the feedstock and intermediate enzyme loading (15 FPU/g cellulose). It is clearly seen from Fig. 4 that high concentration of sugars in the feedstock led to a strong inhibition effect on hydrolysis and fermentation when high enzyme loading (Exp. #6) was applied, under which a final ethanol concentration of 10.49 g/L was reached.

4.3. Product inhibition on enzymatic hydrolysis

Cellulase inhibition by hydrolysis products (cellobiose and glucose) has long been known. It is widely reported that cellobiose was the stronger inhibitor of cellobiose formation (reaction r_1)

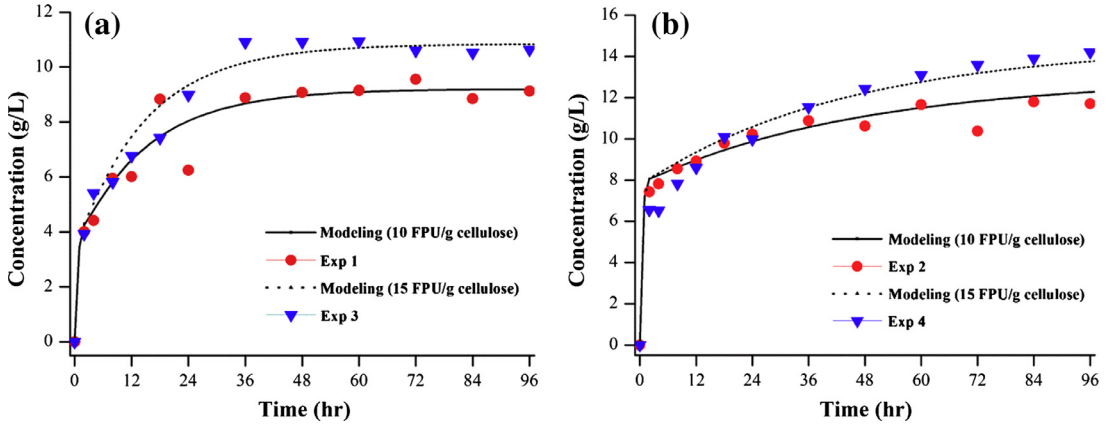

Fig. 2. Kinetic modeling and experimental ethanol concentration at different initial sugar concentration: (a) Glucose: 5 g/L and Mannose 4.5 g/L; and (b) Glucose: 10 g/L and Mannose 9 g/L.

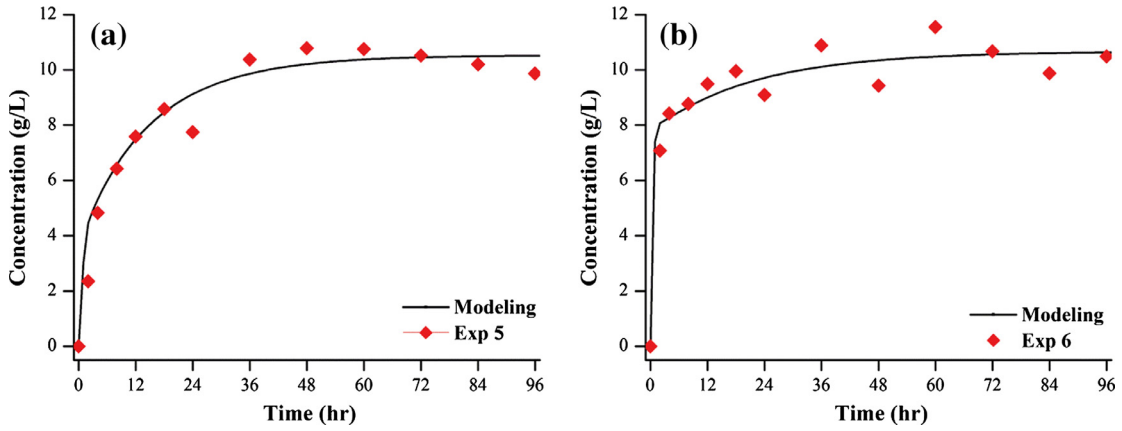

Fig. 3. Comparison of model prediction and experimental ethanol concentrations at enzyme loading of 20 FPU/g cellulose.

and glucose inhibition in reaction r_2 should be greater than cellobiose inhibition [12,35]. Experimental results from this study proved the strong inhibition of cellobiose and glucose on enzymatic hydrolysis. As seen from Table 3, the combination of the highest enzyme loading and high initial sugar concentration (Exp. #6) provides very low ethanol yield and ethanol concentration. Similar inhibition effect can also be observed from the experiments using low level of initial sugars, both ethanol yield and final ethanol concentration obtained from Exp. #5 are much lower than those from Exp. #3.

The product inhibition mechanism can be better understood by investigating the reaction rates of enzymatic hydrolysis (r_1, and r_2) based on different initial concentrations of sugars. At low level of initial sugars, the highest reaction rate of r_1 and r_2 occurred in Exp. #3 as seen from Fig. 5a, followed by those from Exp. # 5. The reaction rates of r_1 and r_2 are the lowest from Exp. #1. Reaction rates obtained at different enzyme loadings demonstrate that increasing enzyme loading from 10 to 15 FPU/g cellulose accelerated the conversion rate of cellulose (r_1), and relative higher amount of cellobiose produced from r_1 is the main cause for the attainment of higher r_2 and the subsequent conversion of glucose. However, further increase of enzyme loading to 20 FPU/g cellulose resulted in the accumulation of cellobiose in the substrate, which strongly inhibited the cellulase activity, leading to reduced reaction rates in r_1 and r_2 from Exp. #5. These results clearly indicate that cellobiose inhibition is dominant during the SSF process when low concentrations of sugars were used in the feedstock. In order to overcome the impact of cellobiose inhibition at high level of enzyme loading, changing the mode of the SSF reaction from batch to fed-batch seems to be promising. Gradually adding the enzyme into the reaction media ensures that there is fresh enzyme available any time for hydrolysing the remaining cellulose in reactor. Another solution to resolve the inhibition impact is implementing the continuous design instead of batch mode.

In case of high initial concentrations of sugars being used, inhibition of glucose became significant, which is reflected from the much slower reaction rates (r_1 and r_2) obtained from Exps. #2, #4 and #6 (Fig. 5b). Likewise, reaction rates of r_1 and r_2 are the highest from Exp. #4 when intermediate level of enzyme loading (15 FPU/g cellulose) was employed, followed by those from Exp. #2 (lowest enzyme loading, 10 FPU/g cellulose) and Exp. #6 (highest enzyme loading, 20FPU/g cellulose). The lowest reaction rates of r_1 and r_2 from Exp. #6 proved the strongest inhibition effects of both cellobiose and glucose. The higher concentrations of cellobiose and glucose in Exp. #6, according to Ishmayana et al. [36], expose the yeast to high osmotic stress, influences on fermentation performance of the yeast and reduces the amount of produced ethanol. These results clearly Fig. 5 also reveals that in the certain dosage of enzyme loading, glucose inhibition causes the significant decrease in r_1 and r_2. Comparing the reaction rates of Exp. #1 and Exp. #2 for instance highlights the significant inhibition impact of glucose at higher initial sugar level.

For SSF process with fixed substrate and yeast loading, the interplay between the enzyme loading and initial concentration

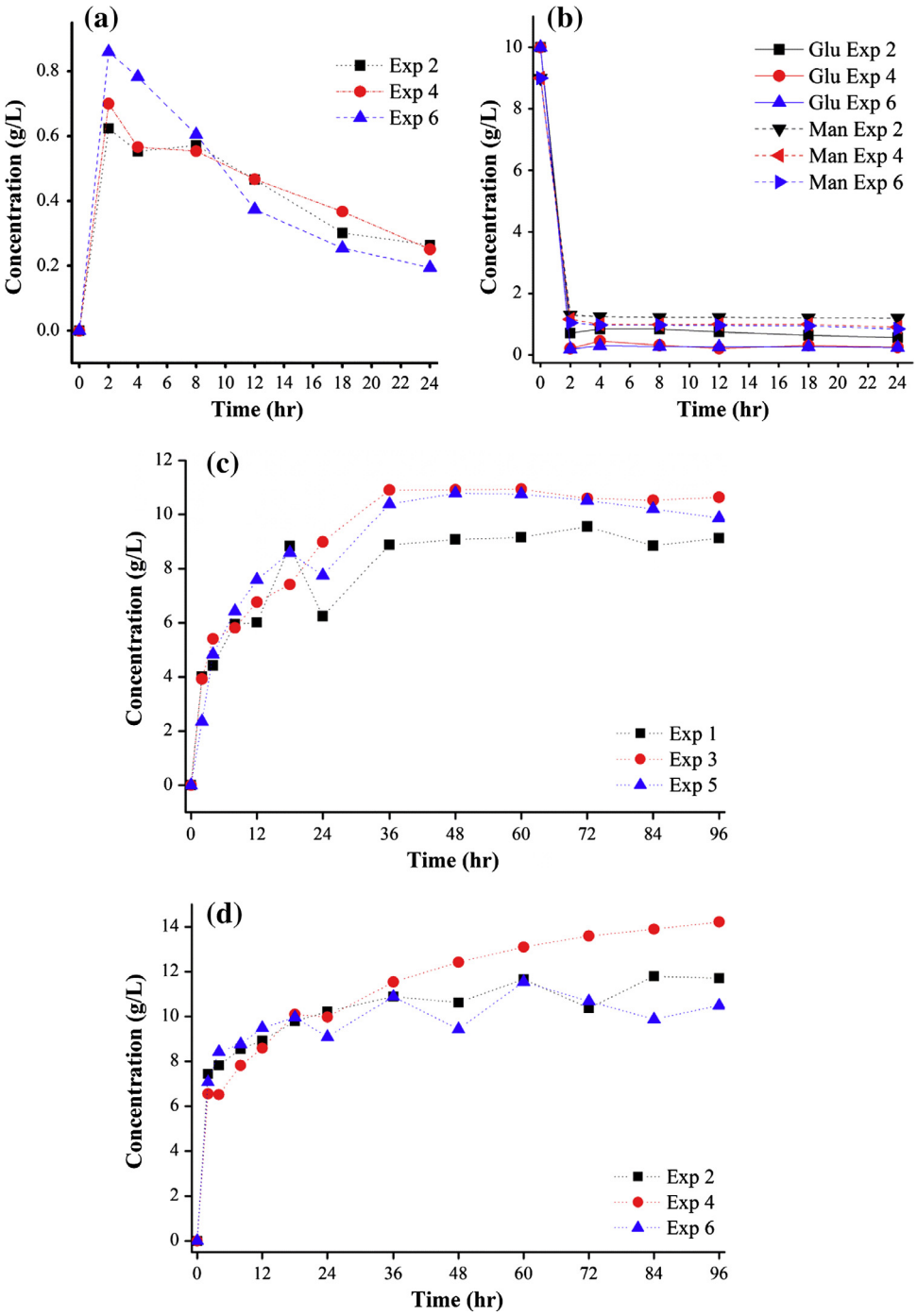

Fig. 4. Concentration profiles of cellobiose (a), sugars (b), and ethanol (c and d) for SSF experiments.

Table 3
Final ethanol yield and concentration from SSF with different operating conditions.

Exp. #	Ethanol yield (%)	Ethanol concentration (g/L)
1	27.45	9.13
2	30.72	11.70
3	31.98	10.63
4	37.32	14.22
5	29.69	9.87
6	27.54	10.49

of fermentable sugars is obvious. With lower initial concentration of sugars, the enhancement of ethanol yield and concentration is easily attainable by employing higher enzyme loading. However, due to the strong inhibitory effect of glucose, high enzyme loading results in a significant decrease in ethanol yield and concentration when the feedstock contains very high concentration of fermentable sugars. This provides useful information with respect to the optimization of SSF process. Depending on the substrate and sugar concentration in the feedstock of SSF, enzyme loading should be selected strategically.

The kinetic model and the acquired kinetic parameters are able to help the future studies regarding the optimization of SSF process. The five kinetic parameters were tuned in different conditions of the SSF reaction to evaluate the response of the system to various reaction conditions, therefore optimization of the SSF process

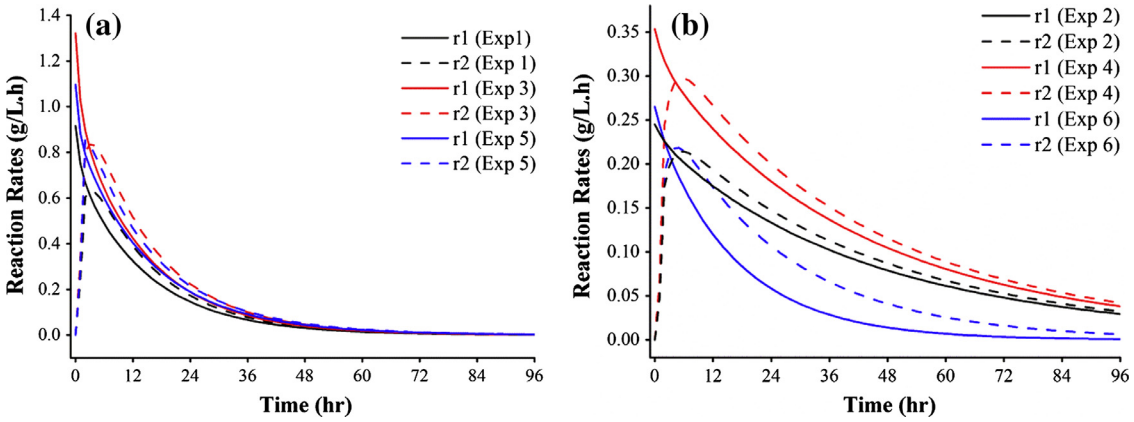

Fig. 5. Comparison of dynamic reactions rates for simultaneous enzymatic hydrolysis and fermentation.

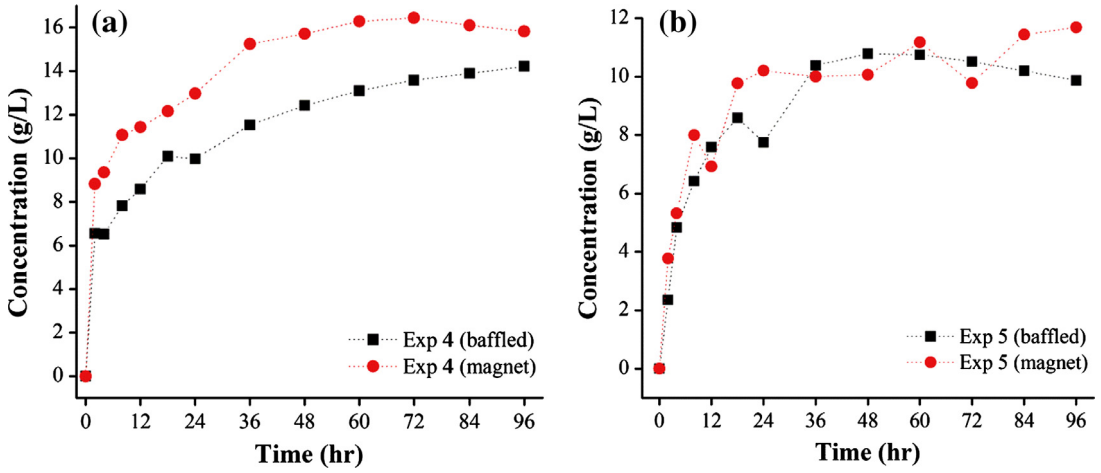

Fig. 6. Comparison of the ethanol concentration in (a) Exp. #4 and (b) Exp. # 5 by using baffled stirrer and magnet stirrer.

in a wider range of sugars concentration and enzyme loading would be possible through this model for further studies.

4.4. Impact of agitation

Inhomogeneity caused by inadequate mixing when working with high water insoluble solid content has been previously addressed in several ways. Several research articles reported that purely increasing the agitation speed does not have significant influence on final ethanol yield. In this work, two agitation modes were used to evaluate the impact of agitation on final ethanol concentration and yield. Two additional batch SSF runs were performed by implementing a magnet stirrer with the speed of 600 rpm instead of the baffled stirrer with the speed of 350 rpm while keeping other operating conditions the same as Exp. #4 and Exp. #5. The effect of agitation mode on the ethanol concentration is presented in Fig. 6.

Results from Fig. 6 reveal that magnet stirrer helps to enhance the ethanol yield for Exp. #4 from 37.32% to 41.53% and for Exp. #5 from 29.69% to 35.15%. Comparing the results of Exp. #4 and Exp. #5 reveals that stirring is an influential parameter that must be taken into account. Increasing the agitation rate in case of high solids loading significantly improves the SSF efficiency [37] and as it can be seen from Fig. 6, in both cases efficient agitation rate enhances the final ethanol concentration. The lower efficiency of the baffled stirrer might be due to incomplete mixing or even the formation of some blind spots in the reactor. These disadvantages led to insufficient interaction between enzyme and cellulose, as well as yeast and sugars. It causes diminish in efficiency of the system and in general decreases the ethanol yield.

5. Conclusion

Variations in the enzyme loading and initial sugar concentration lead to different product inhibition mechanism of batch SSF process. At low sugar concentrations, main cellulase inhibitory is caused by cellobiose. However, at high initial sugar concentrations, inhibition effects from cellobiose and glucose are both important. These inhibition effects are more remarkable in the batch media of process due to the accumulation of the end-products of the hydrolysis process. Moreover, at relative low enzyme loading, adding fermentable sugars to the reaction media diminishes the glucose inhibitory impacts and increases the final ethanol yield and concentration.

Results from this study also demonstrated that initial sugar concentration has significant influence on the reaction rate and rate constants. Higher initial sugar concentration resulted in strong product inhibition of enzymatic hydrolysis, leading to smaller values of k_1, λ and k_2. Meanwhile, higher initial sugar concentration accelerated the growth rate of microorganisms due to the presence of more nutrients, reflected from the convergence of a higher value of μ_m.

Finally, results from batch SSF experiments with two agitation methods reveal that a better mixing between the solid substrate and liquid mixture helps to improve the ethanol yield significantly.

Acknowledgement

Support of this research from NSERC Discovery Grant and Newfoundland Centre for Forest Science and Innovation are gratefully acknowledged. We would also like to thank Dr. Kelly Hawboldt for sharing with us her laboratory facilities.

References

[1] Wagner H, Kaltschmitt M. Biochemical and thermochemical conversion of wood to ethanol – simulation and analysis of difference processes. Biomass Convers Biorefinery 2013;3:87–102.
[2] Watanabe TE. In: Faraco V, editor. Lignocellulose conversion: enzymatic and microbial tools for bioethanol production: introduction: potential of cellulosic ethanol. Berlin: Springer; 2013. p. 1–20.
[3] Conde-Mejia C, Jimenez-Gutierrez A, El-Halwagi M. A comparison of pretreatment methods for bioethanol production from lignocellulosic materials. Process Saf Environ Prot 2012;90:189–202.
[4] Shadbahr J, Zhang Y, Khan F. Life cycle assessment of bioethanol production from woodchips with modifications in the pretreatment process. Appl Biochem Biotechnol 2015;175(2):1080–91.
[5] Balat M, Balat H, Öz C. Progress in bioethanol processing. Prog Energy Combust Sci 2008;34:551–73.
[6] Van Dyk JS, Pletschke BI. A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes factors affecting enzymes, conversion and synergy. Biotechnol Adv 2012;30 (6):1458–80.
[7] Jönsson LJ, Alriksson B, Nilvebrant N-O. Bioconversion of lignocellulose: inhibitors and detoxification. Biotechnol Biofuels 2013;6:16.
[8] Srirangan K, Akawi L, Mon-Young M, Chou CP. Towards sustainable production of clean energy carriers from biomass resources. Appl Energy 2012;100:172–86.
[9] Klein-Marcuschamer D, Oleskowicz-Popiel P, Simmons BA, Blanch HW. The challenge of enzyme cost in the production of lignocellulosic biofuels. Biotechnol Bioeng 2012;109:1083–7.
[10] Geraili A, Sharma P, Romagnoli JA. Technology analysis of integrated biorefineries through process simulation and hybrid optimization. Energy 2014;73:145–59.
[11] Koppram R, Tomás-Pejó E, Xiros C, Olsson L. Lignocellulosic ethanol production at high-gravity: challenges and perspectives. Trends Biotechnol 2014;32:46–53.
[12] Philippidis GP, Smith TK, Wyman CE. Study of the enzymatic hydrolysis of cellulose for production of fuel ethanol by the simultaneous saccharification and fermentation process. Biotechnol Bioeng 1993;41:846–53.
[13] Rass-Hansen J, Falsig H, Jørgensen B, Christensen CH. Prospective bioethanol: fuel or feedstock? J Chem Technol Biotechnol 2007;82:329–33.
[14] Hoyer K, Galbe M, Zacchi G. Production of fuel ethanol from softwood by simultaneous saccharification and fermentation at high dry matter content. J Chem Technol Biotechnol 2009;84:570–7.
[15] Sanchez ÓJ, Cardona CA. Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour Technol 2008;99:5270–95.
[16] Vasquez MP, Da Silva JNC, De Souza MB, Pereira N. Enzymatic hydrolysis optimization to ethanol production by simultaneous saccharification and fermentation. Appl Biochem Biotechnol 2007;136–140:141–54.
[17] Lopez-Linares JC, Romero I, Cara C, Ruiz E, Castro E, Moya M. Experimental study on ethanol production from hydrothermal pretreated rapeseed straw by simultaneous saccharification and fermentation. J Chem Technol Biotechnol 2014;89:104–10.
[18] Zhu M, Li P, Gong X, Wang J. A comparison of the production of ethanol between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using unpretreated cassava pulp and enzyme cocktail. Biosci Biotechnol Biochem 2012;76(4):671–8.
[19] Hoyer K, Galbe M, Zacchi G. Effects of enzyme feeding strategy on ethanol yield in fed-batch simultaneous saccharification and fermentation of spruce at high dry matter. Biotechnol Biofuels 2010;3(14):1–11.
[20] Liu K, Lin X, Yue J, Li X, Fang X, Zhu M, et al. High concentration ethanol production from corncob residues by fed-batch strategy. Bioresour Technol 2010;101:4952–8.
[21] Stenberg K, Bollok M, Reczey K, Galbe M, Zacchi G. Effect of substrate and cellulase concentration on simultaneous saccharification and fermentation of steam-pretreated softwood for ethanol production. Biotechnol Bioeng 2000;68 (2):204–10.
[22] Neves MA, Kimura T, Shimizu N, Nakajima M. State of the art and future trends of bioethanol production. Dynam Biochem. Process Biotechnol Mol Biol 2007;1:1–14.
[23] Cheung SW, Anderson BC. Laboratory investigation of ethanol production from municipal primary wastewater solids. Bioresour Technol 1997;59:81–96.
[24] Huang X, Penner MH. Apparent substrate inhibition of the Trichoderma reesei cellulase system. J Agric Food Chem 1991;39:2096–100.
[25] Valjamae P, Pettersson G, Johansson G. Mechanism of substrate inhibition in cellulose synergistic degradation. Eur J Biochem 2001;268:4520–6.
[26] Sun Y, Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 2002;83:1–11.
[27] Morales-Rodriguez R, Gernaey KV, Meyer AS, Sin GA. Mathematical model for simultaneous saccharification and cofermentation (SSCF) of C6 and C5 Sugars. Chin J Chem Eng 2011;19(2):185–91.
[28] Sasikumar E, Viruthagiri T. Simultaneous saccharification and fermentation (SSF) of sugarcane bagasse – kinetics and modeling. Int J Chem Biomol Eng 2010;3(2):57–64.
[29] Shin D, Yoo A, Kim SW, Yang DR. Cybernetic modeling of simultaneous saccharification and fermentation for ethanol production from steam-exploded wood with Brettanomyces custersii. J Microbiol Biotechnol 2006;16(9):1355–61.
[30] van Zyl JM, van Rensburg E, van Zyl WH, Harms TM, Lynd LR. A kinetic model for simultaneous saccharification and fermentation of Avicel with Saccharomyces cerevisiae. Biotechnol Bioeng 2011;108:924–33.
[31] Philippidis GP, Spindler DD, Wyman CE. Mathematical modeling of cellulose conversion to ethanol by the simultaneous saccharification and fermentation process. Appl Biochem Biotechnol 1992;34:543–56.
[32] Philippidis GP, Hatzis C. Biochemical engineering analysis of critical process factors in the biomass to ethanol technology. Biotechnol Prog 1997;13 (3):222–31.
[33] Pettersson PO, Eklund R, Zacchi G. Modeling simultaneous saccharification and fermentation of softwood. Appl Biochem Biotechnol 2002;98–100:733–46.
[34] Santos ALF, Kawase KYF, Coelho GLV. Enzymatic saccharification of lignocellulosic materials after treatment with supercritical carbon dioxide. J Supercrit Fluids 2011;56:277–82.
[35] Kent R, Kurašin M, Teugjas H, Väljamäe P. Strong cellulase inhibitors from the hydrothermal pretreatment of wheat straw. Biotechnol Biofuels 2013;6:135.
[36] Ishmayana S, Learmonth RP, Kennedy UJ. Fermentation performance of the yeast Saccharomyces cerevisiae in media with high sugar concentration. In: Proceedings of the 2nd international seminar on chemistry. p. 379–85.
[37] Kadic A, Palmqvist B, Lidén G. Effects of agitation on particle-size distribution and enzymatic hydrolysis of pretreated spruce and giant reed. Biotechnol Biofuels 2014;7(77):1–10.