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Resource-Efficient Technologies 2 (2016) S96–S104

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Research paper

Optimal control of enzymatic hydrolysis of lignocellulosic biomass

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Received 20 June 2016; received in revised form 4 November 2016; accepted 7 November 2016

Available online 19 December 2016

Abstract

Cellulose hydrolysis is a key step in lignocellulosic ethanol production. At present, commercial production of lignocellulosic ethanol is limited due to the long hydrolysis times and requirement of large quantity of expensive enzymes. Therefore, reduction of the enzyme consumption as well as hydrolysis time is crucial and model based optimisation methods can be used for the same. A semi-mechanistic model with cellobiose, glucose, and xylose inhibition with Arrhenius based relationship between temperature and kinetic parameters and thermal deactivation of enzymes was used for the present study. Optimal control problem with temperature as control variable was formulated after considering two different objective functions. For the objective of glucose concentration maximisation at final batch time, the benefit of implementing optimal control increased with reducing batch times. For the batch time of 24 hours, the final glucose concentration increased by 3.2%. For the objective of batch time minimisation, the reduction of batch time was 5.8% and it was observed for a target glucose concentration of 45 g/kg of cellulose. The use of optimal control can reduce the enzyme requirement up to 77.8% of endoglucanase and exoglucanase for glucose maximisation and 22.2% for batch time minimisation. The above results show the usefulness of optimal temperature control in increasing the glucose concentration, and reducing the batch time without increasing the enzyme used.

Keywords: Enzymatic hydrolysis; Optimal control; Temperature; Batch time; Glucose concentration

1. Introduction

In lignocellulosic ethanol production, the hydrolysis of lignocellulose is important because it decides the amount of glucose that is offered for fermentation [1]. The polymeric sugars like cellulose and hemicellulose are converted to their corresponding monomers by chemical, physicochemical and biological methods. Chemical methods utilise either an acid or an alkali whereas, physico-chemical methods utilise high temperature, high pressure along with a chemical reagent [2]. These methods are energy intensive and are prone to produce degradation products which are not desirable. Therefore, partial degradation of cellulose, hemicellulose and lignin is done by the chemical or physicochemical methods which expose the cellulosic substrate for further hydrolysis under mild conditions by using enzymes like cellulase [3].

The enzymatic hydrolysis of cellulose is the result of synergistic action of multiple enzyme components having different mechanisms of action. These enzymes are found commonly in fungal species like *Penicillium verruculosum, Trichoderma reesei, Aspergillus niger, Sporotrichum Thermophile* [4–7]. Hydrolytic enzymes are also available as commercial preparations like Celluclast, Cellic CTec2, Speczyme CP, Novozyme 188, Cytolase CL, and Accellerase [8–12]. The components of cellulase are endoglucanases, exoglucanases and β-glucosidases. The fraction of each of these components in a given enzyme mixture is dependent on the source of the enzyme. The endoglucanases bind to the cellulose and exposes the reducing and non-reducing ends resulting in the formation of cellooligomers. The exoglucanases bind to the reducing and non-reducing ends of the cellooligomers converting the same to cellobiose. The final component that acts is the β-glucosidase which converts cellobiose to glucose [13]. The insufficient quantity of β-glucosidases in the enzyme mixture leads to accumulation of cellobiose which inhibits the hydrolysis reactions. Apart from cellobiose, the glucose, cellooligomers, and xylose also inhibit the hydrolysis reaction. Lignin reduces the enzyme available for hydrolysis by non-productive adsorption. In addition to the quantity of enzyme, maintaining optimal operating conditions like temperature and pH is also important. The typical operating temperature for cellulose hydrolysis ranges between 40 and 55 °C and pH

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http://dx.doi.org/10.1016/j.reffit.2016.11.006

Table 1
Solutions proposed and their drawbacks in cellulose hydrolysis process.

S. no.	Challenges	Solutions	Drawbacks
1.	Cellobiose accumulation and inhibition	1. Additional supplementation of β-glucosidase in hydrolysis 2. Engineered yeast that can produce β-glucosidase for SSF [9]	Increase in the total cost due to additional supplementation
2.	Glucose inhibition	1. Simultaneous Saccharification and Fermentation (SSF) 2. Glucose tolerant enzymes [16]	In case of SSF, • Ethanol inhibition • Difference in optimum temperature for hydrolysis and fermentation [17]
3.	Lignin adsorption	Adding proteins or surfactants [18]	Cost of additional protein/surfactant
4.	Degradation products form pretreatment	1. Detoxification [19] 2. Less calcitrant feedstock and mild conditions for pretreatment [19]	1. Cost associated with additional processing step [19] 2. Poor sugar yield in pretreatment [19]
5.	Low solid loading	Continuous feeding of substrate and/or enzyme in a fed-batch reactor	With increase in the substrate content, the amount of glucose inhibition is also higher
6.	Enzyme cost	1. Recycling the enzymes by readsorption 2. Using engineered enzymes with higher efficiency [20] 3. Improving the efficiency of the process by optimisation [21].	Recycling by readsorption is not suitable for β-glucosidase

ranges from 4.5 to 5.5. The enzymes are susceptible to degradation upon exposure to high temperature, and mixing speed [14,15]. The condition in which the enzyme starts degrading is dependent on the source of enzyme.

Different strategies were proposed in the literature to overcome the challenges in enzymatic hydrolysis of lignocellulose. Table 1 list some of these strategies along with their drawbacks. The solutions suggested in Table 1 recommend modifying either the enzyme source or the process to overcome the above mentioned challenges.

While improvements in enzymes and process development are expensive and long term solutions, optimising the process operations to improve economic feasibility can develop a short term solution. Moreover, process optimisation is anyway essential for commercial operation of the plant. Considering the complexity and nonlinearity of the process, model based optimisation using systematic models is more appropriate than through heuristic strategies. The focus of this work is to increase the glucose yield and reduce the batch time by using optimal temperature control. Previously, studies on dynamic optimisation by controlling feeding strategies of substrate, and enzyme for fed-batch reactor were done. These studies have demonstrated the increase in solid loading up to 20% in fed-batch reactor [22]. Owing to the higher chances of contamination at longer batch times, and inhibition by accumulated glucose in fed-batch reactors, the present study is done for batch reactor. Among the parameters for batch hydrolysis of cellulose, since temperature is more sensitive as well as controllable, temperature is chosen as the control variable for the present study [23].

The paper is organised as follows. The process of enzymatic hydrolysis of cellulose is explained in section 2. The kinetic modelling of cellulose hydrolysis is presented in section 3. Section 4 explains the optimal control problem formulation. The results of maximisation of glucose concentration and minimisation of hydrolysis time studies are explained in section 5. Section 6 concludes the paper.

2. Enzymatic hydrolysis process

Lignocellulosic biomass is generally pretreated where the objective is to disrupt the cellular matrix to make the cellulose accessible to enzymes. The pretreated biomass is then sent for enzymatic hydrolysis. The enzymatic hydrolysis of cellulose is a heterogeneous reaction; therefore, the first step in cellulose hydrolysis is the binding of the enzymes to the substrate by adsorption. The bound fraction of endoglucanase and exoglucanase converts cellulose to cellobiose. On the contrary, the unbounded fraction of β-glucosidase converts cellobiose to glucose. This implies that the bound fraction of endoglucanase and exoglucanase plays a major role in cellobiose formation whereas, the free fraction of β-glucosidase is crucial in glucose formation. In addition to cellulose, the lignin present in the substrate can also bind to the hydrolytic enzymes and reduce the amount of the same available for cellulose hydrolysis. The adsorption step is followed by hydrolysis reaction which is inhibited by hydrolysis products like cellobiose, and glucose as well as degradation products from pretreatment like xylose, furfural. Apart from non-productive adsorption due to lignin and inhibition by products of hydrolysis and degradation products of pretreatment, the amount of enzyme also reduces due to deactivation by temperature. The deactivation temperature for a given enzyme is dependent on the source of the enzyme. Therefore, only the enzyme available after deactivation takes part in adsorption as well as hydrolysis reaction. Representing enzymatic hydrolysis of cellulose by a kinetic model is essential to study the performance of the hydrolysis process and improving the same. The kinetic model of cellulose hydrolysis is explained in section 3.

3. Kinetic modelling of cellulose hydrolysis

The semi-mechanistic model developed by Kadam et al. [8] is used in this study. This model is chosen because it is less complex than a mechanistic model and more reliable than an empirical model.

In this section, the modelling of cellulose hydrolysis is explained in three parts, namely, adsorption of enzymes to cellulose, hydrolytic breakdown of cellulose, and thermal deactivation of enzymes.

3.1. Enzyme adsorption

Since cellulose is an insoluble substrate, enzymatic hydrolysis of cellulose is a heterogeneous reaction. The endoglucanase, exoglucanase, and β-glucosidase are fed as liquids. The enzymes fed should bind to the surface of the solid substrate for cellulose hydrolysis to happen. In this model, the mixture of endoglucanase and exoglucanase is represented by E_1 and β-glucosidase is represented by E_2. The amount of enzymes bound to the substrate is calculated by Langmuir type adsorption isotherm and is given by Eq. (1).

$$E_{iB} = \frac{E_{imax} K_{iad} E_{iF} S}{1 + K_{iad} E_{iF}} \tag{1}$$

where, E_{iB} (i = 1,2) is the bound concentration of enzyme (g/kg), E_{imax} is the maximum mass of enzyme that can absorb onto a unit mass of substrate (g protein/g cellulose), K_{iad} is the dissociation constant for the enzyme adsorption, E_{iF} is the concentration of free enzyme in solution (g/kg cellulose), S is the substrate concentration (g/kg).

3.2. Hydrolysis of cellulose

Unlike some earlier models where the cellulose was assumed to be of the amorphous and crystalline regions, this model assumes the substrate to be uniform. However, the decrease in the reactivity of the substrate as the reaction proceeds is captured by the substrate reactivity parameter given by Eq. (2).

$$Rs = \alpha \frac{S}{S_0} \tag{2}$$

where, S refers to the substrate concentration at time t and S_0 refers to the initial substrate concentration. Kadam et al. [8] represented cellulose hydrolysis by three reactions, cellulose to cellobiose (r_1), cellulose to glucose (r_2), and cellobiose to glucose. Among r_1, r_2, and r_3, r_3 is represented by Michaelis–Menten kinetics with competitive inhibition by glucose and xylose. All the three reactions are inhibited by cellobiose, glucose and xylose. The rate equations for cellulose to cellobiose (r_1), cellulose to glucose (r_2), and cellobiose to glucose are given by Eqs. (3), (4), and (5) respectively.

$$r_1 = \frac{k_{1r} E_{1B} R_s S}{1 + \frac{G_2}{K_{1IG2}} + \frac{G}{K_{1IG}} + \frac{X}{K_{1IX}}} \tag{3}$$

$$r_2 = \frac{k_{2r} (E_{1B} + E_{2B}) R_s S}{1 + \frac{G_2}{K_{2IG2}} + \frac{G}{K_{2IG}} + \frac{X}{K_{2IX}}} \tag{4}$$

$$r_3 = \frac{k_{3r} E_{2F} G_2}{K_{3M}\left[1 + \frac{G}{K_{3IG}} + \frac{X}{K_{3IX}}\right] + G_2} \tag{5}$$

where, E_{1B} (g/g) is bound concentration of endoglucanase and exoglucanase, E_{2B} (g/g) is the bound concentration of β-glucosidase, and k_{ir} (i = 1,2,3) is the reaction rate constant (kg/g.h). G, G_2, S, X are concentrations (g/kg) of glucose, cellobiose, substrate (cellulose) and xylose respectively. K_{iIG} (i = 1,2,3) are the inhibition constants for glucose (g/kg), K_{iIG2} (i = 1,2) are the inhibition constants for cellobiose (g/kg), K_{iIX} (i = 1,2,3) are the inhibition constants for xylose (g/kg), and K_{3M} is the substrate (cellobiose) saturation constant (g/kg). The mass balance for cellulose, cellobiose and glucose are given by Eqs. (6), (7), and (8).

$$\frac{dS}{dt} = -r_1 - r_2 \tag{6}$$

$$\frac{dG_2}{dt} = 1.056 r_1 - r_3 \tag{7}$$

$$\frac{dG}{dt} = 1.111 r_2 + 1.053 r_3 \tag{8}$$

3.3. Enzyme deactivation kinetics

Kadam et al. [8] explained the relationship between relationship between temperature and reaction kinetics by Arrhenius relationship and the model predicts sugar yield up to a temperature of 50 °C. Above 50 °C, the model prediction was higher than the actual yield. Kadam et al. [8] have concluded the inaccuracy in prediction of sugar yield above 50 °C should be due to deactivation of the hydrolytic enzymes [8]. The hydrolytic enzymes degrade due to mixing speed and temperature [15,24,25]. In this study, the mechanical deactivation of enzymes due to mixing is assumed to be negligible. However, in an optimal control problem, a dynamic temperature profile will be maintained and hence the effect of temperature with and without deactivation is crucial. The thermal deactivation kinetics was adapted from Caminal et al. [14]. Caminal et al. [14] defined the rate of decrease of enzymatic hydrolysis by Eq. (9) and the rate of decrease in the available enzyme is given by Eq. (10) [14].

$$\frac{r}{r_0} = \exp(-k_d t) \tag{9}$$

$$\frac{E}{E_0} = \exp(-k_d t) \tag{10}$$

where r is the rate of reaction at time t, r_0 is the initial rate of reaction, k_d is the deactivation constant, E is the amount of enzyme available after degradation at time t, and E_0 is the amount of enzyme added initially. In Eqs. (9) and (10), k_d is a function of temperature. Therefore, the value of k_d was calculated for different temperatures from the available values of r and r_0 in Caminal et al. [14] and the relationship between k_d and temperature is given by Eq. (3).

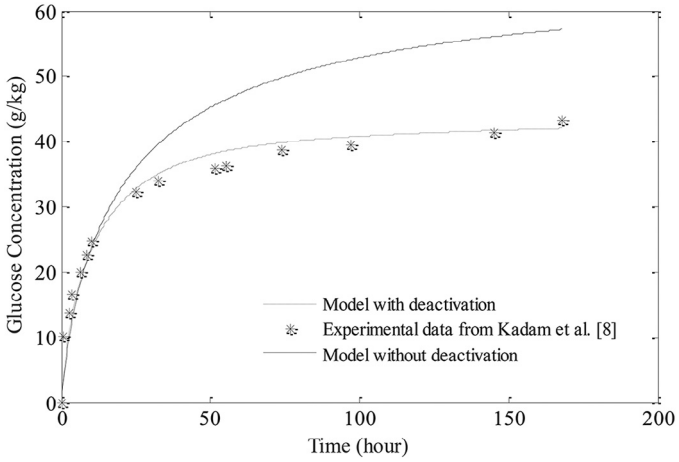

Fig. 1. Comparison of glucose concentration during enzymatic hydrolysis batch process from experiment and model with and without consideration of thermal deactivation at 55 °C.

$$k_d = 10^{-65}\exp(0.4465 * T) \tag{11}$$

Eq. (11) was derived using the data given in Caminal et al. [14]. Caminal et al. [14] and Kadam et al. [8] have used different enzymes and hence the parameters appearing in the Eq. (11) were estimated by fitting the glucose profiles at 55 °C from Kadam et al. [8]. The final equations used for deactivation kinetics are

$$\mathrm{E1T} = \mathrm{E1T0} * \exp(-\mathrm{k_{d1}} * \mathrm{t}) \tag{12}$$

$$\mathrm{k_{d1}} = 10^{-20} * (\exp(0.1316 * \mathrm{T})) \tag{13}$$

$$\mathrm{E2T} = \mathrm{E2T0} * \exp(-\mathrm{k_{d2}} * \mathrm{t}) \tag{14}$$

$$\mathrm{k_{d2}} = 10^{-65} * \exp(0.4480 * \mathrm{T}) \tag{15}$$

Where E1T refers to the total concentration of cellulase at time t, E2T refers to the total concentration of β-glucosidase at time t, $E1T_0$ is the initial concentration of cellulase, and $E2T_0$ is the initial concentration of β-glucosidase. T refers to temperature and t refers to time. On comparing Eqs. (13) and (15), it is evident that the E2T is more sensitive to temperature than E1T. This is in agreement with the results of Calderson et al. [26] which say that Novozyme188 (E2T) is more sensitive to temperature than Celluclast (E1T) [26]. The model prediction with and without deactivation and experimental data is shown in Fig. 1 for temperatures 55 °C. It can be seen in Fig. 1 that after modification, the model is also able to predict for temperatures up to 55 °C.

4. Optimal control problem formulation

Control theory determines the time dependent (dynamic) profile of the decision variable. The problem formulation of a typical optimal control problem is given by,

$$Max(J) = h(x(t_f), t_f) + \int_{t_0}^{t_f} g(x(t), u(t), t)\,dt \tag{16}$$

Subject to:

$$\dot{x} = a(x(t), u(t), t), \tag{17}$$

$$x(t_0) = x_0 \tag{18}$$

where, J is the performance index, $h(x(t_f), t_f)$ is the terminal cost, and $g(x(t), u(t), t)$ is the integral cost.

Few studies on application of control theory are available for cellulose hydrolysis. Hodge et al. [22] used control theory for finding the optimal control strategies for a fed batch reactor with feeding strategies of substrate and enzyme as the control variables [12]. Tai et al. [27] studied biomass feeding strategies for fed batch model [17]. Similarly, Rodriguez et al. [21] used closed loop PI controller and studied the feeding strategies of insoluble solids, endo and exoglucanases, and β-glucosidase. Even though these studies were conducted for higher solid loading, the hydrolysis times were too long. For example, Rodriguez et al. [21] and Hodge et al. [22] have conducted hydrolysis for up to 288 hours. However, longer batch times will be difficult to maintain in reality due to higher operational cost and susceptibility to bacterial contamination like lactobacillus producing lactic acid consuming glucose especially for continuous and fed-batch systems [28]. Therefore, this work focuses on optimal control studies carried out for batch reactor with reduced batch times. Temperature based optimal control has been used for other enzyme catalysed processes like hydrolysis of penicillin to 6-Amino Penicillanic Acid (6-APA) [29]. For a batch reactor, the substrate and enzymes are fed initially and are not fed during the reaction and hence cannot be chosen as a control variable. However, temperature can be changed during the reaction and has significant impact on the reaction rates. Therefore, temperature is chosen as the control variable for this study. The proposed optimal control problem provides the optimal temperature profile for the enzymatic hydrolysis reactor. In practice, the reactor temperature will be maintained at the optimal level through heating and cooling mechanism, either in the form of a jacket surrounding the reactor or a coil immersed in the reactor. The optimal temperature profile recommended in this work will be given as a dynamic set-point to the closed loop controller. These closed loop controllers are often PI (proportional-integral) or PID (proportional-integral-derivative) controllers. These controllers will control the hot and cold fluid flow rates (manipulated variables) to ensure that the actual temperature is following the desired trajectory. In this paper, the optimal control problem is formulated by Pontryagins's Maximum principle and solved by steepest ascent of Hamiltonian [30]. The problem formulation for maximisation of glucose and minimisation of hydrolysis time is explained in the subsequent sections.

4.1. Maximisation of glucose concentration

The objective function for the optimal control problem solved in this case is to find the temperature profile that will maximise the glucose concentration for a given batch time. The modified version of the model proposed by Kadam et al. [8] with thermal deactivation kinetics was used.

Objective function:

$$Max\left(G\left(t_f\right)\right) \tag{19}$$

Subject to Eqs. (6)–(8) explained in section 3.2.

The above formulation was solved using the method of steepest ascent and more details on this method are given in the Appendix.

4.2. Minimisation of hydrolysis time

One of the major bottlenecks of enzymatic hydrolysis is the slow rate of hydrolysis and hence longer batch times. Therefore, minimisation of batch time was considered as the second objective of the optimal control problem. Since the original problem is formulated in time domain, the model needs to be reformulated to solve the minimum time problem. We used the method proposed by Benavides et al. [31] to reformulate the problem and making glucose concentration as the independent variable. The reformulated model is given by Eqs. (20)–(23).

$$\frac{dS}{dG} = \frac{-r_1 - r_2}{1.111r_2 + 1.053r_3} \tag{20}$$

$$\frac{dG_2}{dG} = \frac{1.056r_1 - r_3}{1.111r_2 + 1.053r_3} \tag{21}$$

$$\frac{dG}{dG} = \frac{1.111r_2 + 1.053r_3}{1.111r_2 + 1.053r_3} \tag{22}$$

$$\frac{dt}{dG} = \frac{1}{1.111r_2 + 1.053r_3} \tag{23}$$

5. Results and discussion

The optimal control problems formulated as explained in section 4 were solved with an initial cellulose concentration of 60 g/kg and all other background sugars were assumed to be zero. As the mixing issues arise after 15% solid loading, the mass transfer limitations due to solid loading are assumed to be negligible [22]. An enzyme loading of 45 mg protein/g cellulose of E_1 and 4.16 mg/g cellulose of E_2 were considered for all the optimal control problems solved. All control problems were solved for different initial guesses to address the issue of non-convexity.

5.1. Maximisation of glucose concentration

Initially, the maximisation of glucose problem was solved for 168 hours since Kadam et al. [8] had developed the model for 168 hours. Similarly, the temperature for base case was chosen as 318 K since the actual model was developed using experimental data at the same temperature. It was noticed that the glucose concentration at final time over the base case increased only by 0.28%. Moreover, 93% of the substrate was already converted for a batch time of 168 hours. However, a batch time of 168 hours is too long and often not acceptable for commercial applications. While larger batch times allow more conversion, smaller batch times enable multiple batches to be operated in a given time span, which may effectively provide more overall profit. Many experimental studies on enzymatic hydrolysis are also performed for 72, 48, and 24 hours. Therefore, control studies were performed for batch times of 72, 48, and 24 hours as well.

Table 2
Results for maximisation of glucose concentration for different batch times.

S. no.	Batch time (hours)	Glucose yield for 318 K	Glucose yield with optimal control profile	% improvement in the yield
1.	168	56.3	56.4	0.28
2.	72	49.6	50.2	1.21
3.	48	45.3	46.0	1.46
4.	24	36.8	38.0	3.21

The glucose concentration increased by 1.21, 1.46, and 3.21% for 72, 48, and 24 hours respectively (Table 2). This behaviour showed that using optimal temperature profile was more beneficial for lower batch times than higher batch times. The glucose concentration with and without optimal temperature control for a batch time of 24 hours is given in Fig. 2. Fig. 3 shows that the optimal temperature profile initially started as high as 333.4 K. However, the temperature started decreasing immediately and reached up to 320.7 K. This can be explained based on the temperature dependency of cellulose hydrolysis. The initial increase in temperature corresponds to increase in hydrolysis rate corresponding to the Arrhenius relationship between the temperature and kinetic parameter. However, as mentioned in section 3.1, the enzymes are susceptible to degradation by higher temperature and hence decrease in temperature in the later stages.

In the absence of optimal temperature profile, the concentration of glucose can also be increased by increasing the enzyme concentration. Therefore, simulations were carried out where the final concentrations achieved using optimal control were achieved instead by increasing the enzyme loading and keeping the temperature constant at 318 K. Table 3 shows the

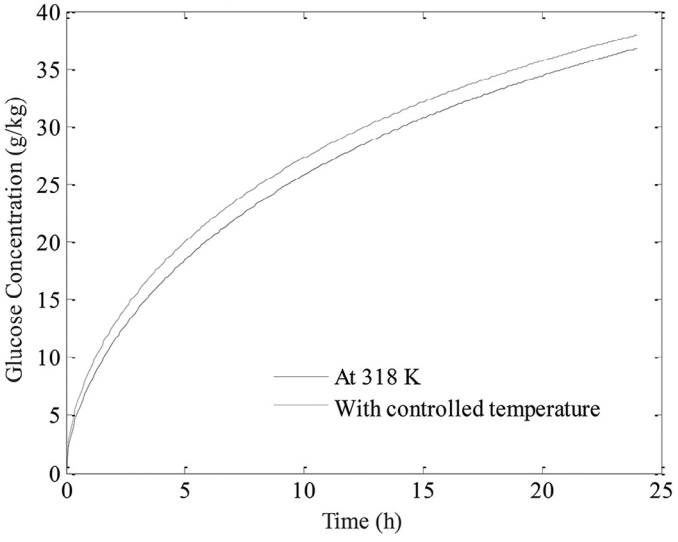

Fig. 2. Glucose concentration for maximisation of glucose problem for 24 hours.

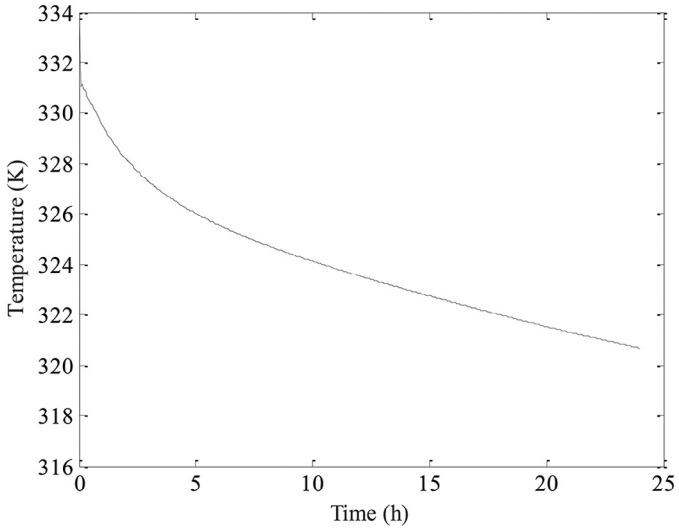

Fig. 3. Control profile for maximisation of glucose problem for 24 hours.

Table 3
Percentage increase in enzymes required for maximisation of glucose concentration in absence of optimal control.

S. no.	Batch time	Either E_1 or E_2		Both E_1 and E_2
		Endoglucanase and Exoglucanase (E_1)	β-glucosidase (E_2)	
1.	24	77.78	19	13
2.	48	31.1	14.2	10
3.	72	30	17.31	10
4.	168	11.11	7.21	7

Table 4
Minimisation of hydrolysis time for different glucose concentrations.

S. no.	Target glucose concentration (g/kg)	Initial temp (°C)	Time without control (h)	Time with control (h)	% Reduction in time	Average optimal temperature (K)
1.	56.27	45	168	165.7	1.36	321.3
2.	50.643	45	80.41	77.71	3.35	323.2
3.	45	45	46.68	43.98	5.78	324.3

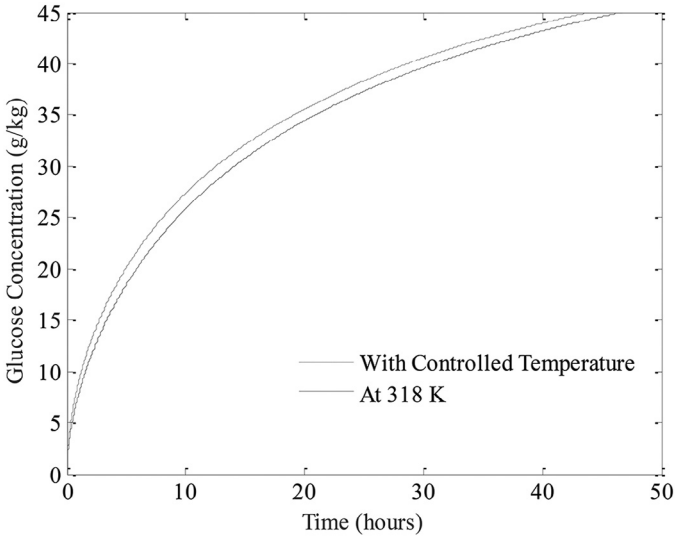

Fig. 4. Glucose concentrations for minimisation of hydrolysis time problem to reach glucose concentration of 45 g/kg cellulose.

percentage increase in endoglucanase and exoglucanase or β-glucosidase required to achieve the glucose concentrations mentioned in Table 2 for fixed batch times. Among the two enzyme mixtures, the requirement of E_1 is relatively higher than E_2. This could be because, addition of E_1 results in formation of cellobiose and glucose through r_1 and r_2 whereas, E_2 produces glucose through r_2 and r_3. Considering the fact that endoglucanase and exoglucanase mixtures as well as β-glucosidase are expensive, instead of using either of these enzymes, equal percentage of both can also be used. The percentage of additional enzyme required in absence of optimal control was reduced when both enzymes were used instead of using one of them. This reduction could be due to the synergism between the enzymes. These results demonstrate the use of optimal control in improving the process efficiency without increasing the enzyme required.

5.2. Minimisation of hydrolysis batch time

In base case simulation, 56.27 g/kg of glucose was formed in 168 hours. By using optimal temperature profile obtained by the method explained in section 4.2, the batch time was reduced by 1.36% for the glucose concentration of 56.27 g/kg. Such a minor reduction was expected since the batch time of 168 hours is very high and hence the scope for improving batch time was limited. Therefore, the batch time minimisation problem was solved for reduced concentrations of 50.643 and 45 g/kg which were 90 and 80% of the above concentration, respectively. For these cases, the hydrolysis time was reduced by 3.35 and 5.78 % for 50.643 and 45 g/kg (Table 4). The glucose profile for a target concentration of 45 g/kg cellulose with and without control is depicted in Fig. 4. Similar to the maximisation of glucose case, the increased enzyme loading to achieve same results as that of optimal control problem was estimated. For a target glucose concentration of 45 g/kg, enzyme loading of endoglucanase and exoglucanase should be increased by 28% or β-glucosidase by 12.5%. The additional enzyme requirements in absence of control for all the target glucose concentrations are listed in Table 5. Therefore, apart from the reduction in batch time this can also be viewed as a means of reducing the enzyme consumption. The temperature profile for glucose

Table 5
Percentage increase in enzymes required for batch time minimisation in absence of optimal control.

S. no.	Target concentration	Either E_1 or E_2		Both E_1 and E_2
		Endoglucanase and exoglucanase (E_1)	β-glucosidase (E_2)	
1.	45	28	12.5	8.3
2.	50.643	13.33	7.21	5.3
3.	56.27	7.33	4.33	2.8

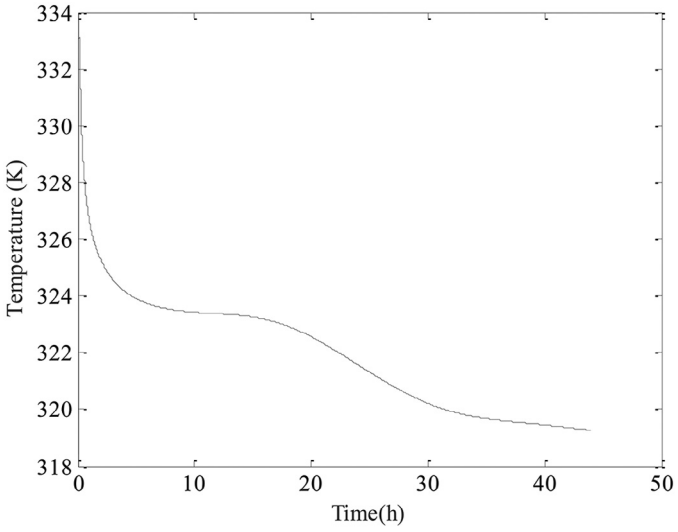

Fig. 5. Temperature profile for minimisation of hydrolysis time problem to reach glucose concentration of 45 g/kg cellulose.

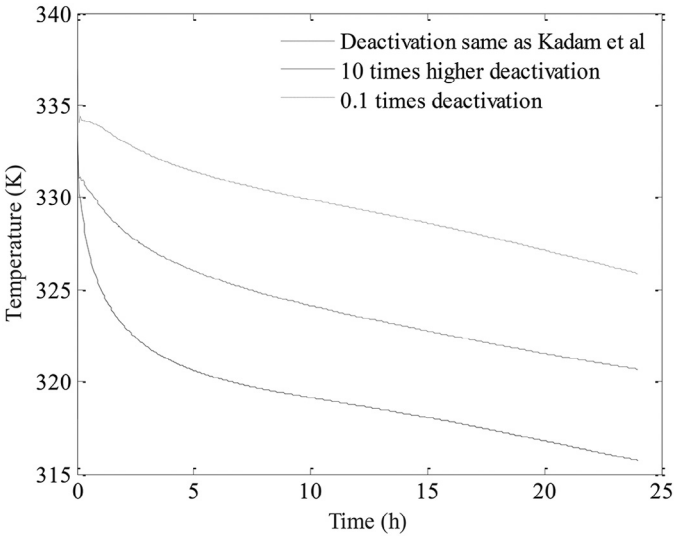

Fig. 7. Control profile for different rates of deactivation.

concentration of 45 g/kg is shown in Fig. 5. The temperature reached a maximum of 333.1 initially and then decreased up to 319.3 K. The initial increase in temperature was due to the increase in rate constants with respect to temperature but it was followed by decrease in temperature due to thermal deactivation of enzymes. It can also be noted that the average optimal temperature increases with decrease in the target concentration. This is because the reduction in the target concentration corresponds to a reduced the batch time. Therefore, maintaining a higher temperature is more beneficial for shorter batches than longer ones.

Fig. 6 shows the bound, free, degraded enzymes for a target glucose concentration of 45 g/kg. It can be seen from Fig. 6 that the enzymes degrade during the initial stages (first 20 hours) of the reaction due to the high temperature and then degradation becomes stable for the next few hours due to the reduction in the temperature below 323 K where the enzyme degradation is not significant. However, the increase in the degraded enzyme in the later stages can be attributed to the degradation due to reaction time.

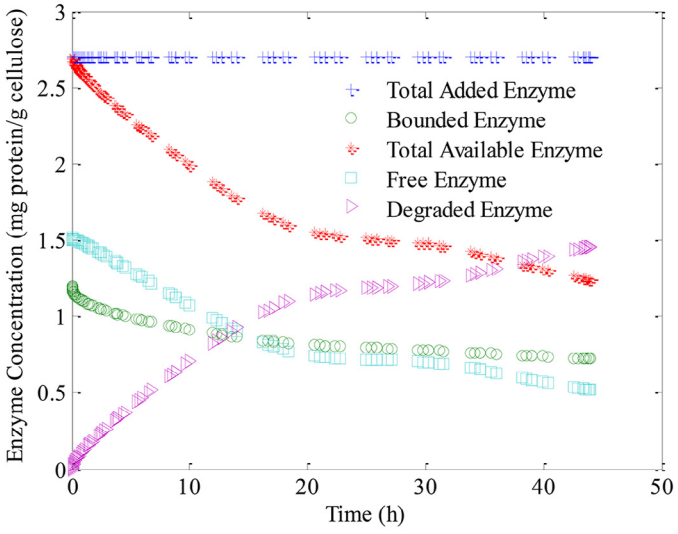

Fig. 6. Enzyme profiles for a target glucose concentration of 45 g/kg cellulose.

5.3. *Effect of rate of deactivation*

Since rate of deactivation as well as the deactivation temperature is dependent on the source of enzyme, the impact of rate of deactivation on control profile was studied for deactivation rate ten times higher and lower than the above case. Comparing the control profiles of different rates of deactivation, it is observed that both the maximum temperature and the rate of decrease in temperature also decrease with batch time (Fig. 7). For a batch time of 24 hours, the glucose concentration increased by 1% for 10 times deactivation and 8% for 0.1 times deactivation. From the glucose concentration and the nature of temperature profiles, it is evident that using temperature based control is more suitable for enzymes that are less susceptible to thermal degradation and in that case, it is better to operate at a constant but lower temperature. In case of minimum time problem, the reduction in batch time was only 0.3% for 10 times deactivation whereas it was 5% for 0.1 times deactivation. In this case also, the benefit of optimal control is less for 10 times deactivation. The costate equations, necessary conditions were derived with k_{d1} and k_{d2} values corresponding to 10 times higher and 10 times lower deactivation.

6. Conclusion

The optimal temperature control was applied for maximisation of glucose concentration and minimisation of enzymatic hydrolysis time of lignocellulosic biomass. The glucose yield improved by 3.21% for batch time of 24 hours and corresponding enzyme savings was 77.74% for endoglucanase and exoglucanase or 19% for β-glucosidase. Similarly, the batch time was reduced by 5.78% for a target glucose concentration of 45 g/kg cellulose and corresponding enzyme savings

by 44.4% for endoglucanase and exoglucanase or 2.8% for β-glucosidase. Moreover, the comparison of control profiles suggests that temperature based optimal control was more suitable for enzymes that are less susceptible to thermal degradation. This approach can further be extended to other reactor configurations like fed-batch and control variables like solid loading, substrate and enzyme feeding rates.

Acknowledgement

This work has been funded by the Department of Science and Technology (DST), Government of India through grant SSB/S3/CE/015/2013.

Appendix

In order to solve the maximisation of glucose concentration problem using Maximum principle, the Hamiltonian is formulated as:

$$H = p_1 * (-r_1 - r_2) + p_2 * (1.056 * r_1 - r_3) + p_3 * (1.111 * r_2 + 1.053 * r_3). \quad (24)$$

In Eq. (23), p_1, p_2, and p_3 are adjoint variables, r_1, r_2, and r_3 are explained in Eqs. (3)–(5). The adjoint variables are calculated by differentiating the Hamiltonian (Eq. (23)) with respect to the state variables as given in Eq. (24).

$$\frac{dp}{dt} = -\frac{dH}{dx} \quad (25)$$

The necessary condition was derived by differentiating the Hamiltonian with respect to the control variable. In this case, the control variable is temperature and hence the Hamiltonian was differentiated with respect to temperature.

The above formulated problem was solved by the method of steepest descent/ascent. The solution terminated when the difference between two consecutive objective function values was negligible.

For minimisation of hydrolysis batch time, there is one additional state equation for time comparing to the maximisation of glucose case. The Hamiltonian for this problem is formulated as given in Eq. (26).

$$H = (1) + p_1\left(\frac{-r_1 - r_2}{1.111r_2 + 1.053r_3}\right) + p_2\left(\frac{1.056r_1 - r_3}{-r_1 - r_2}\right) + p_3\left(\frac{1.111r_2 + 1.053r_3}{1.111r_2 + 1.053r_3}\right) + p4\left(\frac{1}{1.111r_2 + 1.053r_3}\right) \quad (26)$$

The adjoint equations and the necessary condition were derived similar to the maximisation of glucose case.

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