Optimal control in fed-batch reactor for the cellobiose hydrolysis

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ABSTRACT. Glucose can be obtained from cellulose through enzymatic hydrolysis by the enzymes of the celullase complex. Cellobiose hydrolysis by cellobiase exhibits substrate and product inhibition, which reduces the reaction's performance. The researches available in the literature on this subject were made in batch reactors; a study concerning the possibility on the use of another type of reactor has not yet been made. The aim of this work is to analyze the use of a fed-batch reactor to this enzymatic reaction. Feed policy was determined using the optimal control theory, where substrate conversion and final product concentration were maximized. Simulated results were compared with experimental data obtained by Calsavara *et al.* (1999) in a batch reactor and indicated that substrate inhibition overcomes product inhibition. The fed-batch operation was advantageous in some situations.

Key words: optimal control, fed-batch reactor, optimization, cellobiose, Pontryagin's maximum principle, enzymatic inhibition.

RESUMO. Controle ótimo em reator batelada alimentada para a hidrólise enzimática da celobiose. A glicose pode ser obtida através da hidrólise enzimática pelas enzimas do complexo celulase. a hidrólise da celobiose pela celobiase exibe inibição pelo substrato e pelo produto, que reduzem a performance da reação. os trabalhos sobre esta hidrólise enzimática disponíveis na literatura foram conduzidos em reatores batelada, portanto um estudo sobre a possibilidade de utilização de outro tipo de reator ainda não foi realizado para esta hidrólise. o objetivo deste trabalho é analisar o uso de um reator batelada alimentada para esta reação enzimática. a política de alimentação foi determinada utilizando a teoria de controle ótimo, onde a conversão do substrato e a concentração do produto final foram maximizadas. os resultados simulados foram comparados com dados experimentais obtidos por calsavara *et al.* (1999) em um reator batelada e indicaram que a inibição pelo substrato se sobrepõe a inibição pelo produto. a operação em batelada alimentada mostrou-se vantajosa em algumas situações.

Palavras-chave: controle ótimo, reator batelada alimentada, otimização, celobiose, princípio máximo de Pontryagin, inibição enzimática.

Introduction

Cellulosic wastes have become important as alternative energy source and raw material for industries. Glucose can be obtained from cellulose through enzymatic hydrolysis by the enzymes of the cellulase complex. In the cellulose hydrolysis, the cellobiase plays an essential role in cellobiose hydrolysis, which is an intermediate stage of this reaction.

The cellobiose hydrolysis by cellobiase yielding glucose has also been described with substrate and product inhibition kinetics (Woodward and Wohlpart, 1982; Grous *et al.*, 1985; Dekker, 1986). A greater advantage of the cellulosic wastes could be taken if the inhibition effects were reduced. Researches on this subject available in the literature were made in batch reactors, so a study concerning the possibility of using another type of reactor has not yet been done.

The aim of this work is to investigate the use of a fed-batch reactor, where the substrate feed policy will be obtained through optimal control theory. The results will be compared to the experimental data of Calsavara *et al.* (1999), obtained in a batch reactor. Optimal control has been extensively used to optimize the substrate feed policy on fermentative process (Cuthrell and Biegler, 1989; Levien, 1992;

Shukla and Pushpavanam, 1998; Costa *et al.*, 1999) but, just a very few cover fed-batch enzymatic reactions with inhibition kinetics (Waghmare and Lim, 1981; Sengupta and Modak, 2001).

Material and methods

Experimental data

The cellobiose hydrolysis reaction occurs through cellobiase enzyme (EC 3.2.1.21) yielding two molecules of glucose. Calsavara *et al.* (1999) made conversion tests with this reaction in a batch reactor with different initial substrate concentrations, using a commercial enzyme (Novozym 188). These experimental data will be used for comparison purposes with the optimal fed-batch reator.

Pontryagin's maximum principle

The formulation of an optimal control problem can be written as

minimize the index performance

$$J = \int_{t_0}^{t_f} F(\mathbf{x},\mathbf{u},t)dt + G\left(\mathbf{x}\left(t_f\right),\mathbf{u}\left(t_f\right),t_f\right) \qquad (1)$$

subject to the equality constraints,

$$\frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x},\mathbf{u},t), \qquad \begin{matrix}\mathbf{x} \in \Re^n \\ \mathbf{u} \in \Re^m\end{matrix} \qquad (2)$$

the initial conditions,

$$\mathbf{x}(t_0) = \mathbf{x}_0 \qquad (3)$$

and to the inequality constrains

$$\mathbf{u}_{\min} \le \mathbf{u} \le \mathbf{u}_{\max} \qquad (4)$$

Pontryagin's Maximum Principle is one of the available methods to solve optimal control problem (Wozny and Li, 2000). It states that the optimal control that minimizes the index performance, *J*, must minimize the Hamiltonian *H*, defined as

$$H = \sum_{i=1}^{n} \lambda_i f_i \qquad (5)$$

and λ is the adjoint variables vector. These variables are defined by the ordinary differential equations system,

$$\frac{d\boldsymbol{\lambda}}{dt} = -\frac{\partial H}{\partial \mathbf{x}}, \qquad \boldsymbol{\lambda} \in \Re^n \qquad (6)$$

and by the terminal conditions according to the *trasnsversality boundary conditions*

$$-\boldsymbol{\lambda}^T\left(t_f\right)\delta\,\mathbf{x}\left(t_f\right) + H\left(t_f\right)\delta t_f = 0 \qquad (7)$$

Another condition that must be obeyed to assure that Hamiltonian is minimum is

$$\boldsymbol{\varphi} = \frac{\partial H}{\partial \mathbf{u}} = 0 \qquad (8)$$

When the problem is nonlinear in the control variables, Equation 8 provides the optimal operation policy for the control variables **u**. On the other hand, if the problem is linear in the control variables, the solution of this problems results in a bang-bang control strategy, then,

$$\begin{matrix}\mathbf{u} = \mathbf{u}_{\min} & \textit{when} & \boldsymbol{\varphi} > 0 \\ \mathbf{u} = \mathbf{u}_{\max} & \textit{when} & \boldsymbol{\varphi} < 0\end{matrix} \qquad (9)$$

Algorithm

The problem defined by the equations (2) and (6) with initial (equation 3) and terminal (equation 7) conditions is a typical two-boundary-value-problem (TBVP) and it is usually hard to solve. The following algorithm was used to solve the optimal control problem (Ramirez, 1994):

1. A constant control is assumed as u = u_{max};
2. The state equations are integrated forward in time until the final time is reached;
3. The adjoint equations are integrated backward in time from the final time to the initial time. The new value of the manipulated variable is calculated based upon the sign of ϕ (equation (9)).
4. The control policy of step 3 is implemented by integrating the states as in the step 2. Steps 2 and 3 are repeated until the control policy converges to the optimal control.

Based on the above algorithm, a computational program was written in Fortran 90. Runge-Kutta method, through the routine IVMRK, was used to integrate ordinary differential equations systems.

Formulation of the optimal control problem

The behavior of a fed-batch reactor can be described as

$$\frac{dx_1}{dt} = D(x_{1F} - x_1) - r \qquad x_1(0) = x_{10} \qquad (10)$$

$$\frac{dx_2}{dt} = 2r - Dx_2 \qquad x_2(0) = x_{20} \qquad (11)$$

$$\frac{dx_3}{dt} = Dx_3 \qquad x_3(0) = x_{30} \tag{12}$$

where x_1 is the substrate concentration, x_2 is the product concentration, x_3 is the reactor volume, x_{1F} is the feed substrate concentration and D is the dilution rate. Taking into account the noncompetitive inhibition by the substrate and the competitive inhibition by the product (Corazza *et al.*, 2002), the reaction rate *r* will be

$$r = \frac{x_1 . V_{\max}}{x_1\left(1+\frac{x_1}{ks}\right) + km\left(1+\frac{x_1}{ks}+\frac{x_2}{ki}\right)} \tag{13}$$

where *ki* is the product inhibition constant, *km* is the Michaelis-Menten constant, *ks* is the substrate inhibition constant and V_{max} is the maximum reaction rate. The values of these parameters determined previously (Corazza *et al.*, 2002) at 50ºC are shown in Table 1.

Table 1. Values of kinetic parameters at 50ºC ⋆.

x_{10} [g/L]	20.0	2.0	2.0
x_{20} [g/L]	0	0	1.8
ki [g/L]	0.4919	2.0406	1.2288
km [g/L]		0.8687	
ks [g/L]		17.7085	
$V_{\max}$ [g/L.min]		0.04752	

⋆km, ks and Vmax values are the same for different x10 and x20.

In order to optimize the feed substrate policy that maximizes the final product concentration and the substrate conversion, the performance index was defined as

$$J = -x_2(tf) - X(tf) \tag{14}$$

where *X(tf)* is the substrate conversion at the specified final time,

$$X(tf) = \frac{x_{10}.x_{30} + x_{1F}\left(x_3(tf) - x_{30}\right) - x_1(tf).x_3(tf)}{x_{10}.x_{30} + x_{1F}\left(x_3(tf) - x_{30}\right)} \tag{15}$$

and where $x_2(tf)$ is the product concentration at the specified final time *tf*.

The feed-substrate concentration x_{1F} was fixed and the dilution rate D was the manipulated variable. This latter is defined as

$$D = \frac{F}{V} \tag{16}$$

where F is the volumetric flow and V is the reactor volume. In our task, D was bounded by

$D_{min} = 0.0\ min^{-1}$ and $D_{max} = 0.005\ min^{-1}$.

Application of the pontryagin's maximum principle

According to equation (5), Hamiltonian is

$$H = \lambda_1\left(D(x_{1F} - x_1) - r\right) + \lambda_2\left(2r - Dx_2\right) + \lambda_3 Dx_3 \tag{17}$$

where **x** is the state variables vector and **λ** is the adjoint variables vector. Differential equations of these variables are defined by (equation 6)

$$\begin{aligned}\frac{d\lambda_1}{dt} = \lambda_1 &\left(-D - \frac{V_{\max}}{x_1\left(1+\frac{x_1}{ks}\right)} + \frac{x_1 V_{\max}\left(1+\frac{2x_1}{ks}+\frac{km}{ks}\right)}{\left(x_1\left(1+\frac{x_1}{ks}\right) + km\left(1+\frac{x_1}{ks}+\frac{x_2}{ki}\right)\right)^2} \right) + \\ \lambda_2 &\left(2\frac{V_{\max}}{x_1\left(1+\frac{x_1}{ks}\right) + km\left(1+\frac{x_1}{ks}+\frac{x_2}{ki}\right)} - \frac{2x_1 V_{\max}\left(1+\frac{2x_1}{ks}+\frac{km}{ki}\right)}{\left(x_1\left(1+\frac{x_1}{ks}\right) + km\left(1+\frac{x_1}{ks}+\frac{x_2}{ki}\right)\right)^2} \right)\end{aligned} \tag{18}$$

$$\frac{d\lambda_2}{dt} = \frac{\lambda_1 x_1 V_{\max} km}{\left(x_1\left(1+\frac{x_1}{ks}\right) + km\left(1+\frac{x_1}{ks}+\frac{x_2}{ki}\right)\right)^2 ki} + \lambda_2\left(-2\frac{x_1 V_{\max} km}{\left(x_1\left(1+\frac{x_1}{ks}\right) + km\left(1+\frac{x_1}{ks}+\frac{x_2}{ki}\right)\right)^2 ki} - D \right) \tag{19}$$

$$\frac{d\lambda_3}{dt} = \lambda_3 D \quad (20)$$

and the terminal conditions (equation 7),

$$\lambda_1(tf) = \frac{x_3(tf)}{x_{10}.x_{30} + x_{1F}\left(x_3(tf) - x_{30}\right)} \tag{21}$$

$$\lambda_2(tf) = -1 \tag{22}$$

$$\lambda_3(tf) = \frac{-\left(x_{1F} - x_1(tf)\right)}{x_{10}.x_{30} + x_{1F}\left(x_3(tf) - x_{30}\right)} + \frac{\left(x_{10}.x_{30} + x_{1F}\left(x_3(tf) - x_{30}\right) - x_1(tf).x_3(tf)\right).x_{1F}}{\left(x_{10}.x_{30} + x_{1F}\left(x_3(tf) - x_{30}\right)\right)^2} \tag{23}$$

Since the Hamiltonian is linear in the manipulated variable and,

$$\frac{\partial H}{\partial D} = \phi = \lambda_1\left(x_{1F} - x_1\right) - \lambda_2 x_2 + \lambda_3 x_3 \tag{24}$$

the solution is obtained by

$$\begin{aligned} D &= D_{\min} \quad when \quad \phi > 0 \\ D &= D_{\max} \quad when \quad \phi < 0 \end{aligned} \tag{25}$$

which it is known as bang-bang control. Then, D assumes constant values and since the volume is not constant, the volumetric flow will not be constant. But, there is also the possibility that ϕ remains equal to zero over a finite period of time. In this case, the solution is a singular arc.

An important constraint is the maximum volume of the reactor. In all cases, initial volume was 2L and the maximum volume of the reactor was 4L. Then, once the maximum volume is achieved, flow rate and consequently, the dilution rate will be equal to zero until the end of the reaction.

Results

Following the algorithm (section 2.3), the feed substrate policy was determined for several conditions. In any condition, the solution obtained was not the singular arc, leading to a bang-bang control, where the manipulated variable could only assume the boundaries values, D_{min} or D_{max}. For all cases, only during a finite period of time, D assumed D_{max}. This time interval begins in t_1 and finishes in t_2. In all cases, maximum volume of the reactor is achieved at t_2. Table 2 shows the assumed conditions, the studied x_{1F} values and t_1 and t_2 to each circumstance. For x_{1F} values out of range of the values shown in Table 2, the manipulated variable assumed $D_{mín}$ all the time.

Table 2. Reaction conditions, studied x_{1F} range and the feed period of time.

x_{10} [g/L]	x_{20} [g/L]	x_{1F} [g/L]	tf [min]	t_1 [min]	t_2 [min]
20.0	0	1.0	3000	10	400
		3.0		10	300
		5.0		10	250
		8.0		10	190
		10.0		10	160
		15.0		10	80
2.0	0	0.2	300	10	100
		0.5		10	70
		1.0		10	40
		1.3		10	30
		1.5		10	20
2.0	1.8	0.2	600	10	130
		0.5		10	80
		1.0		10	40
		1.3		10	20

Besides the dilution rate (D) remains in constant values, D_{min} or D_{max}, the same does not happen with the flow rate (F), because volume (V) is not constant (equation 5). This can be seen in Figure 1. It shows the policy for dilution rate and flow rate to x_{10} = 20g/L with x_{1F} = 3g/L, x_{1F} = 10g/L and x_{1F} = 18g/L.

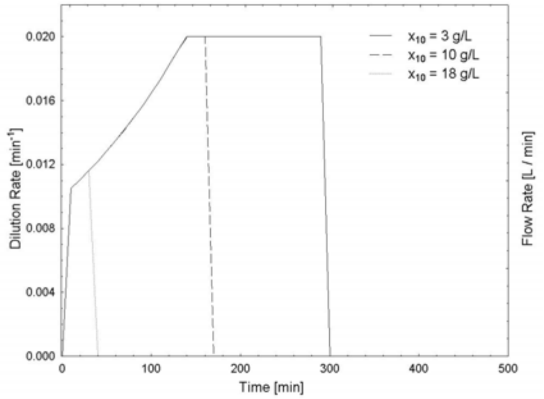

Figure 1. Dilution rate and Flow rate policies with x_{10} = 20 g/L and x_{1F} = 3 g/L.

For comparison purposes, final times and initial concentrations were taken from conversion tests realized by Calsavara *et al.* (1999) at 50°C.

Figure 2 shows the results obtained of conversion *versus* batch time with several feed substrate concentration and batch experimental data from Calsavara *et al.* (1999) to x_{10} = 20g/L.

Conversion curves and batch experimental data (Calsavara *et al.*, 1999) to x_{10} = 2g/L and x_{10} = 2g/L with x_{20} = 1.8g/L are in the Figures 3 and 4 respectively.

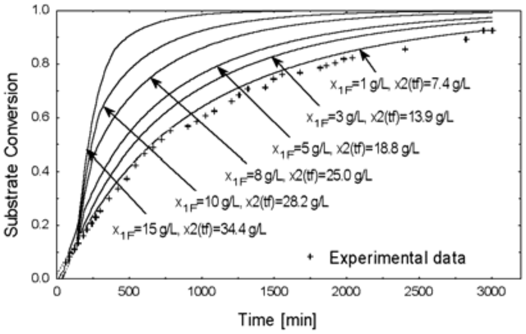

Figure 2. Substrate conversion *versus* time with x_{10} = 20g/L.

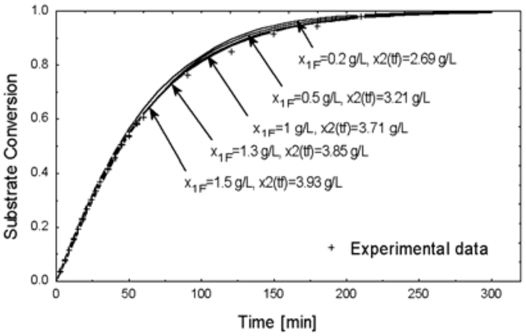

Figure 3. Substrate conversion *versus* time with x_{10} = 2g/L.

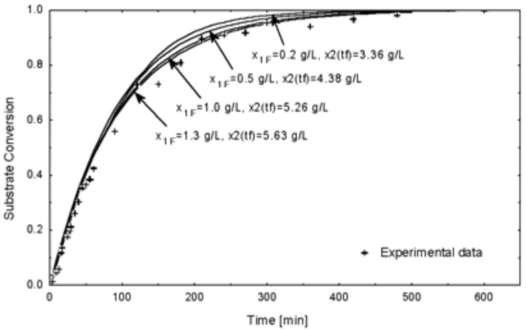

Figure 4. Substrate conversion versus time with x10 = 2g/L and x20 = 1.8g/L.

Discussion

For a substrate initial concentration of 20g/L (Figure 2), all curves in the fed-batch reactor presented a better performance than experimental data obtained in the batch reactor. High substrate feed concentration decreases only product concentration, which means product inhibition effects decrease, while low substrate feed concentration decreases product and substrate concentration in the reactional medium. The curves with high feed substrate concentration presented a little or no difference compared with batch operation. On the other hand, for feed-substrate concentration up to 5g/L, conversion near 1.0 was achieved in a period of time shorter than to the final time with experimental data. Thus, we can say that substrate inhibition overcomes product one, due to the performance of the conversion curves with lower x_{1F}.

In the other conditions (Figures 4 and 5), fed-batch operation does not offer any advantage in relation to batch reactor. Feed substrate concentrations should be too high for the initial substrate concentration, not decreasing the substrate inhibition effects.

A comparison between Figures 3 and 4 confirms the verification that substrate inhibition overcomes product one. The only difference between the two conditions is that in Figure 4 there is an initial product concentration of 1.8g/L, increasing product inhibition. In spite of this, substrate feed does not reduce product inhibition effects, so this is substrate inhibition prevailing.

Final product concentrations obtained are always lower than experimental data. They are shown in Table 3, while final product concentration values obtained experimentally (Calsavara *et al.*, 1999) were for x_{10} = 20g/L, 37.128g/L, for x_{10} = 2g/L, 3.92g/L and for x_{10} = 2g/L with x_{20} = 1.8g/L, 5.80g/L. This is due to the dilution effect on the substrate and product concentrations. For x_{10} = 20g/L, feed decreases inhibition effects and final time of reaction. On the other hand, the final product concentration is lower. The final product concentration and batch time should be compensated to each purpose.

Conclusion

Substrate feed policy of a fed-batch reactor was optimized in the enzymatic hydrolysis of cellobiose, through Pontryagin's Maximum Principle. The solution of the problem was a bang-bang control, where the dilution rate assumed bounded values, D_{min} or D_{max}. It is noteworthy that, in spite of the constant dilution rate, the volumetric flow was not constant, since the volume did not remain constant. The results were compared with experimental data obtained by Calsavara *et al.* (1999) in a batch reactor.

For a substrate initial concentration of 20g/L, fed-batch operation is advantageous only with low feed substrate concentration. Batch time obtained with fed-batch operation was lower but final product concentration was also lower, so it is necessary to compensate batch time and final product concentration to each purpose.

On the other hand, at low substrate initial concentration, the feed substrate concentration was too high and the fed-batch operation did not offer any advantage over the batch operation.

Optimal control theory can be applied in other enzymatic systems. The use of different kinds of reactors and operation conditions can be investigated through it, in order to minimize inhibition effects.

Table 3. Final product concentration obtained for every substrate initial concentration.

$x_1(0)=20$ g/L		$x_1(0)=2$ g/L		$x_1(0)=2$ g/L, $x_2(0)=1.8$ g/L	
X_{1F} (g/L)	$x_2(tf)$ (g/L)	x_{1F} (g/L)	$x_2(tf)$ (g/L)	x_{1F} (g/L)	$x_2(tf)$ (g/L)
1.0	7.41	0.2	2.69	0.2	3.36
3.0	13.94	0.5	3.21	0.5	4.38
5.0	18.84	1.0	3.71	1.0	5.26
8.0	25.03	1.3	3.85	1.3	5.63
10.0	28.24	1.5	3.93	-	-
15.0	34.40	-	-	-	-

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Nomenclature

D	dilution rate;
$D_{\min}$	minimum dilution rate;
$D_{\max}$	maximum dilution rate;
F	volumetric flow;
H	Hamiltonian;
J	performance index;
Ki	product inhibition constant;
Km	Michaelis-Menten constant;
Ks	substrate inhibition constant;
r	reaction rate;
t	time;
tf	final time;
$\boldsymbol{u}$	control variables vector;
u_{min}	control variables vector minimum value;
u_{max}	control variables vector maximum value;
V	reactor volume;
V_{max}	maximum reaction rate;
X	substrate conversion;
$\boldsymbol{x}$	state variables vector;.
x_0	initial conditions;
x_1	substrate concentration;
x_2	product concentration;
x_3	reaction volume;
x_{1F}	feed substrate concentration;
λ	adjoint variables vector.

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