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NON-LINEAR OPTIMUM EXPERIMENTAL DESIGN TO ESTIMATE KINETIC PARAMETERS OF THE ENZYMATIC HYDROLYSIS OF LIGNOCELLULOSE PROCESS

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Abstract

A non-linear optimum experimental design is implemented to estimate the kinetic parameters of the lignocellulose enzymatic hydrolysis process, mainly focused on reaction rate constants and activation energy parameters. The reaction is based on the mechanism of simultaneous consecutive enzymatic reactions of cellulose and hemicellulose in a batch mode. Mathematical model is presented as a set of Ordinary Differential Equations (ODE's); to produce a non-linear optimum experimental design a control variable that directly affects existent kinetic parameters of the system (temperature) is included to produce (non-linear) state variable profiles. The accuracy of parameter estimation is evaluated through a D-optimality criterion (Pronzato & Walter, 1988). A comparison between model prediction profiles and experimental data is performed.

Keywords: Control Variable; Enzymatic Hydrolysis; Non-linear Experiments; Optimality Criterion

INTRODUCTION

Recent demand on green technologies in order to mitigate environmental impact has brought about the interest in several processes, such as the enzymatic conversion of existent polysaccharides of lignocellulose to produce high sugar content syrups.

The aim of this study is to implement a non-linear optimum experimental design to estimate the enzymatic hydrolysis kinetic parameters of the lignocellulose in batch mode. In accordance with the non-linear experimental approach (Heidebrecht et al., 2011), the kinetic parameter estimation results more precise than when linear experiments are

performed. Hence, the advantages of the non-linear experimental design method to estimate kinetic parameters must be an important tool to control the process, even more when industrial manufacture (biorefinery) is desired.

METHODOLOGY

System description

Simultaneous cellulose and hemicellulose enzymatic hydrolysis of lignocellulose consist in placing an amount of pretreated material and a complex enzyme dosage (cellulase and xylanase complex) to interact for a certain period of time (72 hours) to produce high sugar content syrups, mainly hexoses and pentoses. In this study, corncob stocks are used to evaluate the conversion during the hydrolytic reaction; this material is previously pretreated by a thermo-chemical process to break down the rigid lignocelluloses cell wall and ease the enzyme interaction with the polymers of interest.

Mathematical model formulation

The enzymatic conversion of cellulose and hemicellulose from pretreated corncob stocks consists of coupled heterogeneous - homogeneous chain reaction that needs a sophisticated model to describe the process (Bansal et al., 2009). The model takes into account the following assumptions:

a) In terms of inhibition, glucose is used as surrogated of hexoses (glucose, mannose and galactose) and xylose and arabinose for pentoses.
b) The amount of enzyme adsorption onto substrate is evaluated experimentally following the Langmuir adsorption model (Kadam et al., 2004).
c) Enzyme complex works sinergically to break down the core polymer chains under the set conditions.
d) Substrate reactivity is closely related to cellulose and hemicellulose polymerization degree; in addition, it is restrained by the presence of lignin in the reaction media.

Modeling kinetic equations for each state are written as follows:

$$\frac{dS_1}{dt} = -\left[\frac{k_{1r}\exp\left(-E_{1a}/R*\left(1/T_1 - 1/T_2\right)\right)E_{1B}R_{S1}S_1}{1+\frac{Gn}{c1Ign}+\frac{G}{c1Ig}+\frac{(X+A)}{c1Ix}}\right] - \left[\frac{k_{2r}\exp\left(-E_{2a}/R*\left(1/T_1 - 1/T_2\right)\right)*(E_{1B}+E_{2B})R_{S1}S_1}{1+\frac{Gn}{c2Ign}+\frac{G}{c2Ig}+\frac{(X+A)}{c2Ix}}\right] \quad (1)$$

$$\frac{dGn}{dt} = +\left[\frac{k_{1r}\exp\left(-E_{1a}/R*\left(1/T_1 - 1/T_2\right)\right)E_{1B}R_{S1}S_1}{1+\frac{Gn}{c1Ign}+\frac{G}{c1Ig}+\frac{(X+A)}{c1Ix}}\right] - \left[\frac{k_{3r}\exp\left(-E_{3a}/R*\left(1/T_1 - 1/T_2\right)\right)E_{2F}Gn}{k_{3m}\left(1+\frac{G}{c3Ig}+\frac{(X+A)}{c3Ix}\right)+Gn}\right] \quad (2)$$

$$\frac{dG}{dt} = +\left[\frac{k_{2r}\exp\left(-E_{2a}/R*\left(1/T_1 - 1/T_2\right)\right)(E_{1B}+E_{2B})R_{S1}S_1}{1+\frac{Gn}{c2Ign}+\frac{G}{c2Ig}+\frac{(X+A)}{c2Ix}}\right] + \left[\frac{k_{3r}\exp\left(-E_{3a}/R*\left(1/T_1 - 1/T_2\right)\right)E_{2F}Gn}{k_{3m}\left(1+\frac{Gn}{c3Ign}+\frac{(X+A)}{c3Ix}\right)+Gn}\right] \quad (3)$$

$$\frac{dS_2}{dt} = -\left[\frac{k_{4r}\exp\left(-E_{4a}/R*\left(1/T_1 - 1/T_2\right)\right)E_{1B}R_{S2}S_2}{1+\frac{Xn}{c4Ign}+\frac{G}{c4Ig}+\frac{(X+A)}{c4Ix}}\right] - \left[\frac{k_{5r}\exp\left(-E_{5a}/R*\left(1/T_1 - 1/T_2\right)\right)(E_{1B}+E_{2B})R_{S2}S_2}{1+\frac{Xn}{c5Ign}+\frac{G}{c5Ig}+\frac{(X+A)}{c5Ix}}\right] \quad (4)$$

$$- \left[\frac{k_{6r}\exp\left(-E_{6a}/R*\left(1/T_1 - 1/T_2\right)\right)(E_{1B}+E_{2B})R_{S2}S_2}{1+\frac{Xn}{c6Ign}+\frac{G}{c6Ig}+\frac{(X+A)}{c6Ix}}\right]$$

$$\frac{dXn}{dt} = +\left[\frac{k_{4r}\exp\left(-E_{4a}/R*\left(1/T_1 - 1/T_2\right)\right)E_{1B}R_{S2}S_2}{1+\frac{Xn}{c4Ign}+\frac{G}{c4Ig}+\frac{(X+A)}{c4Ix}}\right] - \left[\frac{k_{7r}\exp\left(-E_{5a}/R*\left(1/T_1 - 1/T_2\right)\right)E_{2F}Xn}{k_{7m}\left(1+\frac{G}{c7Ig}+\frac{(X+A)}{c7Ix}\right)+Xn}\right] \quad (5)$$

$$- \left[\frac{k_{8r}\exp\left(-E_{8a}/R*\left(1/T_1 - 1/T_2\right)\right)E_{2F}Xn}{k_{8m}\left(1+\frac{G}{c8Ig}+\frac{(X+A)}{c8Ix}\right)+Xn}\right]$$

$$\frac{dX}{dt} = +\left[\frac{k_{5r}\exp\left(-E_{5a}/R*\left(1/T_1 - 1/T_2\right)\right)(E_{1B}+E_{2B})R_{S2}S_2}{1+\frac{Xn}{c5Ign}+\frac{G}{c5Ig}+\frac{(X+A)}{c5Ix}}\right] + \left[\frac{k_{7r}\exp\left(-E_{7a}/R*\left(1/T_1 - 1/T_2\right)\right)E_{2F}Xn}{k_{7m}\left(1+\frac{G}{c7Ig}+\frac{(X+A)}{c7Ix}\right)+Xn}\right] \quad (6)$$

$$\frac{dAr}{dt} = +\left[\frac{k_{6r}\exp\left(-E_{6a}/R*\left(1/T_1 - 1/T_2\right)\right)(E_{1B}+E_{2B})R_{S2}S_2}{1+\frac{Xn}{c6Ign}+\frac{G}{c6Ig}+\frac{(X+A)}{c6Ix}}\right] + \left[\frac{k_{8r}\exp\left(-E_{8a}/R*\left(1/T_1 - 1/T_2\right)\right)E_{2F}Xn}{k_{8m}\left(1+\frac{G}{c8Ig}+\frac{(X+A)}{c8Ix}\right)+Xn}\right] \quad (7)$$

where $S_1, G_n, G, S_2, X_n, X, Ar$ are the cellulose, cellooligosaccharides, glucose, hemicellulose, xylooligosaccharides, xylose and arabinose concentrations, respectively. k_{ir} are the reaction rate constant, c_{iIg} are the inhibition constant for hexosas, c_{iIx} are the inhibition constant for pentosas, c_{iIgn} are the inhibition constant for cellooligosaccharides, c_{iIxn} are the inhibition constant for xylooligosaccharides, k_{im} are the substrate saturation

constants, R_{si} are the substrate reactivities, T is the temperature, E_{iF} and E_{iB} are the free and bound enzyme concentrations. R is the universal gas constant and E_{ia} represent the activation energies.

The non-linear optimum experimental design follows the algorithm suggested by Heidebrecht and collaborators (Heidebrecht et al., 2011), wherein the objective function comprises the sensivities of the measurement signal with respect to estimate parameters over the whole duration of the reaction.

RESULTS

The formulation approach was solved numerically by applying the orthogonal collocation on finite elements method. Three collocation points were chosen per finite element, and the finite elements ranged between 25 and 50. The discretized problem was implemented in GAMS, and CONOPT was used to solve it.

For the non-linear optimum experimental design, the kinetic parameters that are directly affected by the control variables (temperature) are the reaction rate constants and the activation energies; therefore a total of 16 parameters needed to be estimated ($k_{1r},...,k_{8r}$, $E_{1a},...,E_{8a}$).

To initialize the kinetic parameters in a non-linear optimum experimental design, a set of linear experiments were conducted at bioengineering laboratory facilities. Figure 1 depicts the obtained non-linear profiles for each state and control (temperature) variables after some iterative programming and experimental work. Products profiles (glucose, xylose and arabinose) present an exponential behavior during the first reaction time, while in the subsequent hours they started to decrease dramatically due to the presence of sugars in the system. Xylooligosaccharides remained almost constant during the hydrolysis as a consequence of the low xylanase activities. The control variable followed a trajectory located between the lower and upper bounds where the catalyst (enzyme complex) was capable to work.

For this particular case, the objective function value was 2.836 x 10^{-5} (determinant maximization). Table 1 summarizes parameter estimates obtained from the iterative process (simulation and experimental work); they represent the kinetic constant rates and activation energies for the set conditions, and are valid for the control variable trajectory.

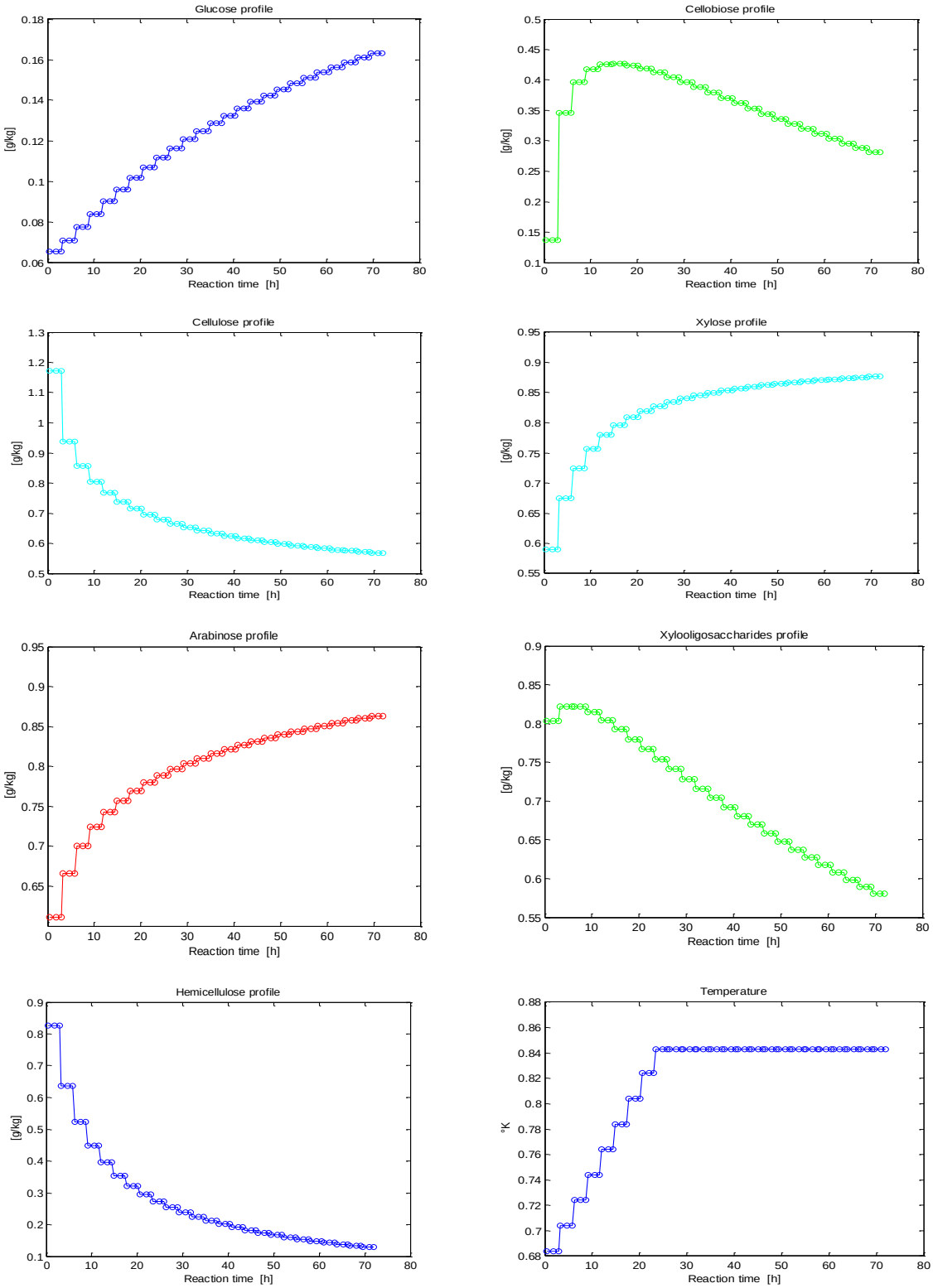

Figure 1: Non-linear optimum experiment profiles for the enzymatic hydrolysis of cellulose and hemicellulose polymers.

Table 1: Kinetic parameter estimates obtained from iterative process (simulation and experimental work)

Objective function value (determinant maximization): 2.836 x 10^{-5}					
k_{1r}	78.63	$g/mg.h$	E_{1a}	-5643	$cal/mole$
k_{2r}	942.20	$g/mg.h$	E_{2a}	-4882	$cal/mole$
k_{3r}	413.34	$1/h$	E_{3a}	-5597	$cal/mole$
k_{4r}	115.281	$g/mg.h$	E_{4a}	-4797	$cal/mole$
k_{5r}	322.39	$g/mg.h$	E_{5a}	-4312	$cal/mole$
k_{6r}	178.45	$g/mg.h$	E_{6a}	-3931	$cal/mole$
k_{7r}	436.0	$1/h$	E_{7a}	-5843	$cal/mole$
k_{8r}	559.09	$1/h$	E_{8a}	-5899	$cal/mole$

CONCLUSIONS

The programming of non-linear optimum experimental design to estimate kinetic parameters of the process described by enzymatic hydrolysis of lignocelluloses was addressed, devising a new approach to control the process and its economical implications due to the experimental work reduction. However, the non ideal control temperature could bring about some deviations from the optimal profiles, additionally the change of the enzymatic complex or other factors that could enhance the performance of the reaction have to be taken into account in future work.

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