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Modelling of amorphous cellulose depolymerisation by cellulases, parametric studies and optimisation

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ABSTRACT

Improved understanding of heterogeneous cellulose hydrolysis by cellulases is the basis for optimising enzymatic catalysis-based cellulosic biorefineries. A detailed mechanistic model is developed to describe the dynamic adsorption/desorption and synergistic chain-end scissions of cellulases (endoglucanase, exoglucanase, and β-glucosidase) upon amorphous cellulose. The model can predict evolutions of the chain lengths of insoluble cellulose polymers and production of soluble sugars during hydrolysis. Simultaneously, a modelling framework for uncertainty analysis is built based on a quasi-Monte-Carlo method and global sensitivity analysis, which can systematically identify key parameters, help refine the model and improve its identifiability. The model, initially comprising 27 parameters, is found to be over-parameterized with structural and practical identification problems under usual operating conditions (low enzyme loadings). The parameter estimation problem is therefore mathematically ill posed. The framework allows us, on the one hand, to identify a subset of 13 crucial parameters, of which more accurate confidence intervals are estimated using a given experimental dataset, and, on the other hand, to overcome the identification problems. The model's predictive capability is checked against an independent set of experimental data. Finally, the optimal composition of cellulases cocktail is obtained by model-based optimisation both for enzymatic hydrolysis and for the process of simultaneous saccharification and fermentation.

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1. Introduction

Enzymatic hydrolysis of cellulosic materials to produce reducing sugars has long been pursued for its potential for providing abundant food and energy resources. It is a multi-step process that takes place in a heterogeneous reaction system [1], in which insoluble cellulose is initially broken down at the solid-liquid interface (with enzyme adsorption/desorption) via the synergistic actions of endoglucanases ([EC 3.2.1.4]) and exoglucanases ([EC 3.2.1.91]). This initial degradation is accompanied by further liquid-phase hydrolysis of soluble intermediate products, i.e., short cellulose oligosaccharides and cellobiose, which are catalytically cleaved to produce glucose by the action of β-glucosidase ([EC 3.2.1.21]).

Mechanistic understanding of the overall hydrolysis system is certainly interesting for designing rational approaches for enzymatic hydrolysis and subsequent fermentation processes. However, the complexity of the system, which arises from the concerted action of several enzymes on a solid substrate/mixture in a heterogeneous system, makes experimental kinetic studies very difficult. Accordingly, although many models of enzymatic hydrolysis have been developed over the past decades, most of them are empirical correlations and data-driven and as a result are only applicable to specific cases/conditions [2–8]. Generally, they are: (1) simply lumping the different cellulolytic enzymes together as a single catalyst; (2) treating the cellulose mixture as a single bulk concentration; (3) simplifying the reaction system as a homogeneous one, i.e., without considering the enzyme adsorption onto and desorption from solid particles; (4) lacking analysis of model identifiability and parameter uncertainty [9]. These approaches are summarised in recent reviews of enzymatic hydrolysis of (ligno) cellulose [10,11].

Efforts to propose mechanistic models have been made to enhance understanding of the enzymatic hydrolysis of cellulose [12–24]. However, they mainly lack thorough parametric studies and experimental validation. Consequently, the predictability of the models, especially for extrapolations, is still in doubt. At the

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Nomenclature

C accessible binding sites on cellulose unoccupied by enzymes (mmol sites/L)
cov(θ) covariance matrix of estimated parameters
$D_{\theta_{1,\ldots,k}}$, D variances in model outputs associated with simultaneous changes in the parameters $\theta_{1,\ldots,p}$ and in all the parameters, respectively
diag diagonal elements of a matrix
DP(= *N*) initial polymerization degree of cellulose substrate
E = *en*, *ex*, *bg* E = englucanase, exoglucanse, beta-glucosidase, respectively
E' = *en*, *ex* E' = englucanase, exoglucanse
E_a activation energy (kJ/mol)
E_{load} enzyme loading (g/L)
$E_{l,f}$ concentration of free enzyme in liquid phase (g/L)
$E_{l,t}$ concentration of total enzymein liquid phase (g/L)
$E_{s,f}$ concentration of free enzyme in solid phase (g/L)
$E_{s,t}$ concentration of total enzyme in solid phase (g/L)
$\langle E^E \oplus G_i \rangle$ substrate-enzyme complex (g enzyme/L)
Fa fraction of accessible β-glucosidic bonds
G1, G2, G3 concentration of glucose, cellobiose, cellotriose (mmol/L), respectively
Gi concentration of cellulose polymer with polymerization degree i(mmol/L)
I_{GI^E}, $I_{G2^{E'}}$, $I_{G3^{E'}}$ inhibition constant of glucose, cellobiose, cellotriose (g/L), respectively
J(θ) sum of squares of residuals between simulations and measurements
K_{ad^E} adsorption equilibrium constant (L/mmol sites)
K_{dis^E} equilibrium constant of enzymatic hydrolysis (mmol β-glucosidic bonds/L)
K^E "apparent" reaction constant (103 mmol/(g enzyme × h))
k^{*E} "intrinsic" reaction constant (103 mmol/(g enzyme × h))
k_f adsorptionrate constant (L/(mmol sites × h))
k_r desorptionrate constant (h^{-1})
M^E enzyme molecular weight (g/mmol)
m, n, p number of model variables, data points, parameters, respectively
R_{e_i} relative values between first-order and total effect sensitivity indices
R(θ_i, θ_j) correlation coefficient between the estimated parameters θ_i and θ_j
r_{Gi} production rate of G_i (mmol/(L × h))
S_{θ_i}, $S_{\theta_i}{}^{tot}$ first-order, total effect sensitivity indices with respect to parameter i, respectively
W_i weighting matrix
$Y_{G_{123}}/G_N$ conversion of cellulose G_N to soluble sugars (% C/C)
$y_i, \hat{y}(t_i, \theta)$ measured variables, estimated variable values, respectively

Greek letters
α_t significance level for *t*-test
$2\alpha^E$ number of cellobiose lattice occupied by one molecule of enzyme (mmol sites/mmol enzyme)
θ, θ^{lb}, θ^{ub}, θ_0 parameter vector, lower and upper bounds, initial guesses, respectively
v confidence intervals of parameters at αt significance level
θ_{refer} reference values for corresponding parameters, cited from literature
λ^E first order deactivation constant of enzyme (1/h)
δ^E binding capacity of substrate (mmol sites/mmol β-glucosidic bonds)
$\sigma^2{}_j$ errorvariance of jth meansurement

same time, studies to investigate fundamental mechanisms of random hydrolysis (random chain scission) and processive hydrolysis (chain-end scission) of polymers have been carried out extensively using population balance modelling [25–30]. Population balance modelling involves tracking the numbers of entities and behaviour of a population of particles based on the analysis of the behaviour of single particles in local conditions [31,32]. The results provide clues to the underlying mechanisms of the enzymatic hydrolysis process of cellulose.

Following the above advances, this work develops a mechanistic depolymerisation (scission) model of enzymatic hydrolysis, taking into account the enzyme adsorption/desorption processes in this heterogeneous system. Furthermore, a systematic sensitivity analysis-based method is proposed for parametric studies, model reduction and verification with published experimental data. Finally, the model's predictive capability is checked against an independent set of data and model-based optimisation studies are presented.

2. Model development

2.1. Model assumptions

Model development is based on the following assumptions:

(1) There are several studies about how to modify and improve the various physical properties of cellulose as a substrate, such as particle size, fibre structure, accessibility, crystallinity index, and amorphicity index [24,33–39]. In this work, for simplification, the substrate is assumed to be completely amorphous (non-crystalline) pure cellulose and be well ground into a very fine powder.
(2) The enzymatic hydrolysis takes place in a well-stirred tank reactor. As a result, there is no mass transfer limitation during enzymatic hydrolysis.
(3) The binding probability of enzyme to one polymer molecule is proportional to the molecule's polymerization degree. In other words, the enzyme has equal accessibility to every β-glucosidic bond of the polymer [21,22].
(4) The quasi-steady state approximation holds for any intermediate complex, i.e.,$\langle E^E \oplus G_i \rangle E = en, ex, bg$.
(5) The loading of cellulases (i.e., total loading of the three core enzymes) is low. More specifically, the loading is no more than 15 mg/g-glucan and 750 mg/L. Accordingly, interaction/crowding effects between different kinds of enzymes are negligible during adsorption/desorption. The adsorption/desorption of each kind of enzyme is described separately and considered reversible.
(6) Cellulosic polymers with chain lengths over four are deemed to exist in solid phase and cannot dissolve into liquid. As shown by Fig. 1, solid particles ($DP \geq 4$) are depolymerized by endoglucanases and exoglucanases, while soluble shorter polymers (namely cellotriose and cellobiose) are exclusively cleaved by β-glucosidases. This is a reasonable assumption if one compares the enzyme specific activities between insoluble and soluble substrates. It has been found experimentally that both endoglucanases and exoglucanases have relatively low

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