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A multiscale three-zone reactive mixing model for engineering a scale separation in enzymatic hydrolysis of cellulose

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HIGHLIGHTS

- Multiscale three-zone mixing model for enzymatic hydrolysis of cellulose formulated.
- Convection (macromixing), diffusion (micromixing), solid adsorption, reactions.
- Macromixing gives higher initial hydrolysis rate, micromixing produces more glucose.
- Optimal mixing strategy with initial macromixing followed by micromixing devised.
- Model matches experiments showing 26% increase of glucose yield on optimal mixing.

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ABSTRACT

This multiscale three-zone reactive mixing model provides a theoretical framework for engineering a scale separation in batch enzymatic hydrolysis of cellulose to strategize significant leaps in glucose yields. Formulated using the Liapunov–Schmidt method of the classical bifurcation theory, our model explores the multiscale spatiotemporal dynamics between the fundamental processes of macromixing (convection) and micromixing (diffusion) of the enzymes (Endoglucanase, Exoglucanase, β-glucasidase) and reducing sugars, adsorption and desorption of enzymes on the solid cellulosic substrates, and the product-inhibited liquid and solid phase enzymatic reactions that depolymerize microcrystalline cellulose (Avicel). The model is validated for a range of substrate loadings (2–5%) using our experimental results for the two asymptotic cases of no mixing and continuous mixing, as well as for the macro/micro scale-separated optimal mixing strategy that increases the glucose yield by up to 26% by macromixing completely for an initial period followed by micromixing for the remaining duration of the hydrolysis.

1. Introduction

The production of cellulosic ethanol forms the central strand in the contemporary bioenergy narratives that are trying to address the connected global challenges of energy crisis and climate change. Of the three steps (pretreatment, enzymatic hydrolysis, microbial fermentation) that constitute the process of bioethanol production from lignocelluloses (Sun and Cheng, 2002), the enzymatic hydrolysis of long chain carbohydrates to glucose monomer and other short chain reducing sugars through polymer chain scission is the rate limiting step. Thus, successful attempts to enhance the hydrolysis rate and the reducing sugar yields help to reduce the bioethanol cost in the fuel market.

Two parallel approaches for increasing the hydrolysis yield appear in the literature: a biochemical kinetic route and a reactor engineering route. The former includes studies on shear (Reese and Ryu, 1980) and temperature (Andreaus et al., 1999) induced enzyme inactivation, shear induced substrate breakage and reaction surface enhancement (Palmqvist et al., 2011), production of thermally stable enzymes exhibiting higher substrate specificity and reduced product inhibition (Zhang et al., 2006; Sainz, 2009), optimization of enzyme composition (Berlin et al., 2006), rates of enzyme adsorption on cellulose surface (Zhang and Lynd, 2004) and enzyme deactivation (Howell and Mangat, 1978), and the effects of substrate and enzyme loading (Cara et al., 2007; Zhu et al., 2008), product inhibition (Ferchak and Pye, 1983), particle size (Yeh et al., 2010) and reagent addition (Ouyang et al., 2010) on the kinetics of hydrolysis. The reactor engineering route consists in using reactors with different shaking and/or mixing patterns (Ingesson et al., 2001; Roche et al., 2009; Kinnarinen et al., 2012; Lavenson et al., 2014), semi-batch reactors (Gupta et al., 2012), recycling enzymes (Xue et al., 2012), loading enzymes under static and agitated conditions

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(Taneda et al., 2012) and process integration (Lennartsson et al., 2012).

We follow the reactor engineering route for increasing the biofuel yield by asking some key contextual questions: (a) how does mixing affect the kinetics of enzymatic hydrolysis of cellulose in batch systems? (Gaikwad and Chakraborty, 2013); (b) what is the optimally mixed reactor configuration for enzymatic hydrolysis in continuous systems? (Chakraborty et al., 2010); (c) what is the optimal reactor mixing strategy for batch hydrolysis of cellulose? (Pal and Chakraborty, 2013); (d) what are the optimal mixing and temperature conditions for catalytic conversion of cellulose to biofuels in ionic liquid media? (Gaikwad and Chakraborty, 2014).

A unifying understanding of the answers to these questions may be attempted by exploring the fundamentals that underlie the process – the complex interlocked dynamics between fluid mixing, mass transfer and biochemical kinetics. This paper attempts to unravel the spatiotemporal effects of reactor mixing on the dynamics of cellulose hydrolysis by using a three-zone reactive mixing model that incorporates convection, diffusion, solid adsorption and reaction with product inhibition. Evidently, such a process governed by these fundamental phenomena is multiscale in nature, where the disparate length scales of convection, diffusion, adsorption, liquid and solid phase reaction import their individual complexities to the system. Our aim is to unlock their complexities and exploit their interdependent dynamics to engineer a strategy for enhancing product yields. This is what our multiscale three-zone reactive mixing model attempts to do, the three zones being representative of the three scales of the system, namely, macromixing, micromixing and solid phase reaction.

In order for our model to capture the fundamental phenomena at the disparate length and time scales intrinsic to this complex system, the model has been rigorously formulated from the fundamental Convection–Diffusion-Reaction equations using the Liapunov–Schmidt method of the classical bifurcation theory (Chakraborty and Balakotaiah, 2002). In the process of validating our multiscale three-zone reactive mixing model using our experimental data on optimal mixing strategy for batch enzymatic hydrolysis of cellulose (Pal and Chakraborty, 2013), we shall engineer a smart strategy of scale separating the reactor mixing to attain significant leaps in glucose yields for the production of cellulosic fuels. Our strategy of mixing at disparate length scales at various times during the evolving reaction process is not limited to cellulose hydrolysis alone; it lays the theoretical formalism of scale sliding for a range of multiscale product-inhibited reaction systems.

2. Methods

2.1. Materials

Microcrystalline cellulose (Avicel PH101 with average particle size 50 μm and particle density 0.6 g/cm^3), purchased from Sigma Aldrich Co. USA, is used as substrate for enzymatic hydrolysis. *Trichoderma viride* derived cellulase with an enzymatic activity of 1 U/mg of solid and containing all the three components, i.e., Endoglucanase, Exoglucanase and β-glucosidase, has been purchased from HIMEDIA Labs, Mumbai, India. A pH of 4.0–5.0 and a temperature of 40–50 °C optimize the enzymatic activity of the cellulase on celluloses. 0.1 M Sodium acetate buffer (pH 5.0) is used to maintain the pH of the reaction mixture.

2.2. Experimental methods

The batch reaction is performed with Avicel concentration of 2% (wt./vol.) and enzyme loading of 1 mg/20 mg of substrate at a pH of 5.0 (maintained using 0.1 M sodium acetate buffer) and temperature of 50 °C in a 100 ml conical flask with a reaction mixture of 10 ml, kept in an incubator-cum-shaker under aseptic conditions. We investigate the effects of initial mixing on enzymatic hydrolysis of cellulose by mixing the batch reactor at 150 rpm for initial mixing periods of 0 h (no mixing), 1, 2, 3, 4, 5, 6, 7, 8 and 72 h (continuous mixing). The solid loading is also varied from 2% to 5% with 1% increase at a time, keeping all other parameters constant.

2.3. Analytical methods

The glucose concentration is measured by GOD–POD kit obtained from Accurex Biomedical Pvt. Ltd., Mumbai, India. Glucose is first converted by glucose oxidase (GOD) to gluconic acid and hydrogen peroxide, following which the peroxide (POD) oxidatively couples with 4-aminoantipyrene and phenol in the presence of peroxidase to produce a red quinoeimine dye that absorbs light at 505 nm. The absorbance is directly proportional to the glucose concentration in the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2 \quad (1)$$

$$\text{H2O2} + 4 - \text{AAP} + \text{Phenol} \xrightarrow{\text{POD}} \text{Red quinoeimine dye} + H_2O \quad (2)$$

3. Theory

Fig. 1 schematizes how the insoluble cellulose particles are dispersed in the hydrolyzing medium in the reactor. Cellulose molecules with Degree of Polymerization (DP) of six or lower, known as reducing sugars, are soluble in the hydrolyzing medium while cellulose molecules with DP > 6 are insoluble in the hydrolyzing medium. The latter makes the enzymatic hydrolysis reactions of cellulose heterogeneous in nature. As shown in Fig. 1, this complex process of convection, molecular diffusion, solid–liquid adsorption and liquid–liquid and solid–liquid hydrolysis reaction can be separated into three representative zones in series for the ease of modelling and quantification. The three zones (as their names in Fig. 1 suggest) are representative of the three scales of the system, namely, macromixing or reactor scale mixing, micromixing or local mixing, and solid phase reaction.

In Fig. 1, Zone 1 represents the Macromixing Zone, where the three enzymes – Endoglucanase, Exoglucanase and β-glucasidase – and the soluble reducing sugars with DP $\leqslant$ 6 mix due to convection-dominated bulk mixing or macromixing. Diffusive mixing, though present in Zone 1, is negligible when compared to convective mixing. The dominance of macromixing (or convective mixing at large length scales) over micromixing (diffusive mixing at small length scales) makes the enzyme-catalyzed depolymerization of soluble sugars unlikely in Zone 1 since the reactions require the enzyme and the sugar molecules to come in molecular contact with each other, which can be affected by diffusion alone.

Macromixing or convective mixing in Zone 1 is followed by micromixing or diffusion-dominated molecular level mixing in Zone 2, where Endoglucanase and Exoglucanase come in molecular contact with the soluble sugars to further break them down to dimers, which, in turn, are reduced to the monomer glucose by the β-glucasidase. Thus, Zone 2 or the Micromixing Zone is where both diffusion and homogenous liquid phase reaction happen simultaneously. Needless to mention, convection is negligible as compared to diffusion in Zone 2 that is primarily located in and around the mass transfer boundary layer near the surface of the solid cellulose particles. It is in Zone 2 that the soluble enzymes get the easiest access to the soluble sugars freshly depolymerized from the solid substrate, thus triggering glucose formation as well as product inhibition.