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Kinetic modeling of multi-feed simultaneous saccharification and co-fermentation of pretreated birch to ethanol

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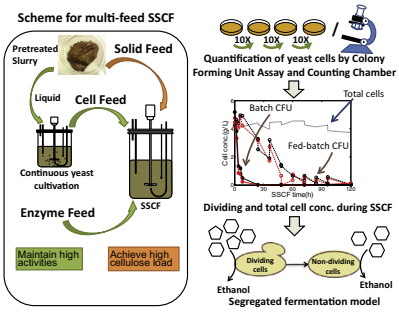
HIGHLIGHTS

- We modeled dynamic profiles of fed-batch SSCF with substrate, enzyme and cell feeds.
- We described cellulase adsorption on lignocellulose by pseudo-second order kinetics.
- A segregated model described fermentation at conditions that inhibited cell growth.
- The model predicted results in multi-feed SSCF with 10–15% steam-pretreated birch.
- Multi-feed improved the reproducibility of high gravity hydrolysis and fermentation.

GRAPHICAL ABSTRACT



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ABSTRACT

Fed-batch simultaneous saccharification and fermentation (SSF) is a feasible option for bioethanol production from lignocellulosic raw materials at high substrate concentrations. In this work, a segregated kinetic model was developed for simulation of fed-batch simultaneous saccharification and co-fermentation (SSCF) of steam-pretreated birch, using substrate, enzymes and cell feeds. The model takes into account the dynamics of the cellulase–cellulose system and the cell population during SSCF, and the effects of pre-cultivation of yeast cells on fermentation performance. The model was cross-validated against experiments using different feed schemes. It could predict fermentation performance and explain observed differences between measured total yeast cells and dividing cells very well. The reproducibility of the experiments and the cell viability were significantly better in fed-batch than in batch SSCF at 15% and 20% total WIS contents. The model can be used for simulation of fed-batch SSCF and optimization of feed profiles.

1. Introduction

Using renewable materials is the key to achieve sustainable production of the biofuel ethanol, and of other chemicals. Being abundant and relatively cheap, lignocellulosic biomass has greater supply potential and less competing uses compared to starch crops (Wyman, 1999). To overcome the recalcitrance of lignocellulosic biomass, physical and/or chemical pretreatment methods have been developed (Mosier et al., 2005). However, degradation products are also formed during thermal treatment, which are inhibitory to fermenting microorganisms and cellulase (Almeida et al., 2007; Kim et al., 2011; Klinke et al., 2004; Oliva et al., 2003; Palmqvist and Hähn-Hägerdal, 2000; Tengborg et al., 2001). In

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some lignocellulosic biomass, hemicellulosic sugars, especially xylose, comprise a relatively high fraction and are usually more readily released during pretreatment than glucose (Alvira et al., 2010; Jeffries, 1983; Mosier et al., 2005). Overcoming the effects of inhibitors, and utilizing both hexoses and pentoses are crucial factors for efficient ethanol production from lignocellulosic materials.

One option is to perform so called simultaneous saccharification and co-fermentation (SSCF), using genetically modified xylose consuming *Saccharomyces cerevisiae* strains. Fed-batch SSCF allows operation at high cumulative solid contents, and keeps the concentrations of inhibitors low when pretreated materials are gradually added to the bioreactor. The feed strategies have been developed from substrate feeding only, to enzyme and substrate feeding (Olofsson et al., 2010a,b), and to cell, enzyme and substrate feeding (Koppram and Olsson, 2014). Another important aspect is to prepare yeast cells with high inhibitor tolerance. Pre-cultivation and adaptation of yeast with diluted, pretreated hydrolysate have shown significant effects on the performance in SSCF and on the capacity of *in situ* detoxification (Alkasrawi et al., 2006; Liu, 2011).

It would be very useful to have a validated kinetic model capable of predicting SSCF performance over broader ranges of substrates and their concentrations, enzyme dosages, and process configurations, for analysis and optimization of ethanol production from lignocellulosic material. An SSCF process involves simultaneous hydrolysis and sugar co-utilization. Therefore, a complete SSCF model should describe at least enzymatic hydrolysis of cellulose, uptake of glucose and xylose by yeast, cell growth, product formation, and inhibition effects. However, the complexity and incomplete understanding of actions of enzymes and yeast cells in lignocellulosic media makes it difficult to formulate such models. Practical issues such as quantification of substrate residues and yeast cells during SSCF also pose challenges to the model development.

A frequently cited SSF model was developed by South et al. (1995), who used a Langmuir adsorption model plus a conversion-dependent reaction rate to describe enzymatic hydrolysis, and Monod kinetics to describe the fermentation process. Shao et al. (2009) further developed this model for fed-batch SSF by tracking the extents of hydrolysis of all substrate populations originating from discrete feeding events. Zhang et al. (2009) incorporated xylose co-fermentation, cell maintenance and cell death to simulate an SSCF process with low cell growth. Cell growth and product formation have usually been described by unstructured, unsegregated growth models, e.g. Monod kinetics and linear rate equations, which associate product yields with growth and maintenance of a single homogeneous cell population (Shao et al., 2009; Shen and Agblevor, 2010; South et al., 1995; van Zyl et al., 2011; Zhang et al., 2009).

In this work, a kinetic model was developed to recreate and evaluate a multi-feed SSCF, i.e., a fed-batch SSCF with substrate, yeast cell and enzyme feeding, of pretreated birch at varied solid contents, and to deal with some identified limitations of published models. Specifically, pseudo-second order adsorption kinetics and dynamic equilibrium of enzyme adsorption were used to reflect the changing properties of the enzyme-cellulose system, and to enable convenient adaptation of models for processes with different feed schemes. A segregated model with two cell populations was used to describe the co-fermentation process at very low average cell growth, where the two populations had distinctly different growth kinetics and were distinguished by the total cell number and the colony forming ability. The effect of precultivation of the yeast with hydrolysate was also included in the model by affecting the transformation rate from dividing cells to non-dividing cells.

2. Methods

2.1. Preparations of pretreated birch, enzymes and yeast

SO_2-catalyzed (1%, w/v) steam pretreated birch slurry was obtained from SEKAB (Örnsköldsvik, Sweden). The content of water insoluble solid (WIS) of the slurry was about 15% (w/w) and its composition is shown in Table 1. The slurry was centrifuged and the solids were collected and used for enzymatic hydrolysis and SSCF experiments. The supernatant was used for pre-cultivation of yeast. Cellic C-Tec II (Novozymes, Denmark), with capabilities of hydrolyzing both cellulose and hemicellulose, was used in all experiments. The measured cellulase activity of the Cellic C-Tec II preparation was 149 ± 6 FPU/g. The yeast *S. cerevisiae* KE6-12, a recombinant xylose-fermenting yeast developed by metabolic and evolutionary engineering, was used in all fermentation experiments (Koppram et al., 2013).

2.2. Enzymatic hydrolysis

Enzymatic hydrolysis was conducted in a 3.6 L fermentor (INFORS HT, Switzerland) with final working weight of 1.5 kg for batch and 1 kg for fed-batch experiments. All fermentors were autoclaved before use. The hydrolysis slurry was mixed with cell-free fermentation broth, collected by filtration of a yeast culture on birch hydrolysate. This was done to minimize the risk of estimating kinetics that might be invalid under actual SSCF conditions, by mimicking the SSCF broth as far as possible except for the absence of cells. Batch hydrolysis was carried out with 10% WIS (w/w, dry basis), 8.2 or 16.5 FPU/g WIS of enzyme dosage, and 125 μL/L vitahop (Betatech Gmbh, Schwabach, Germany) to avoid bacterial contamination. The hydrolysis was initiated by adding enzyme preparation to the fermentor, and continued at 35 °C, pH 5.0 and stirrer speed of 300 rpm for 120 h. Samples for WIS measurement and sugar analysis were taken at the reaction time of 0, 2, 4, 6, 8, 24, 48, 72, 96 and 120 h.

Fed-batch hydrolysis was conducted at 10% (w/w) and 20% (w/w) final accumulated total WIS additions, starting with 4.8% and 12.1% WIS, respectively. Enzyme preparations equivalent to 8.2 and 20 FPU/g final accumulated WIS were added at the beginning. Solid substrates were fed discretely at time points 24, 42, 54, and 64 h. The medium and operating conditions were the same as those in batch hydrolysis.

2.3. Semi-continuous yeast culture

Yeast cells for SSCF were produced and adapted in a three-step process as described previously (Olofsson et al., 2008). The inoculum was prepared for 24 h in shake flask and transferred to fermentors for aerobic batch culture, with defined minimal medium according to Verduyn et al. (1992). Feeding of minimal medium mixed with hydrolysate corresponding to the levels in the following SSCF experiment was started when ethanol produced during

Table 1
Composition of pretreated birch slurry[a].

Solid phase (% of WIS)		Liquid phase (g/L)	
Glucan	48.5	Glucose	9.2
Xylan	1.7	Xylose	59.1
Mannan	0.2	Mannose	6.7
Galactan	<0.02	Galactose	3.5
Arabinan	<0.02	Arabinose	1.7
		Acetic acid	18.3
		Furfural	3.7

[a] According to producer's analyses.