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Research paper

Kinetic model of cellulose degradation using simultaneous saccharification and fermentation

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ABSTRACT

Numerical analyses of energy production processes are important for practical applications. Here, we established a model of enzymatic hydrolysis and ethanol fermentation. The kinetic model of these reactions were represented by Langmuir adsorption, Michaelis-Menten, and Shuler models. In our model, ethanol fermentation and enzymatic hydrolysis models were fit to experimental data separately, and their model equations were then combined for fitting of experimental data, including the size of cellulose, amount of adsorbed protein, and decreases in glucose as the theoretical production values for SSF. From these kinetic models, the theoretical values for saccharification and fermentation were calculated, and optimal parameters were determined. Using these parameters, theoretical curves for simultaneous saccharification and fermentation were predicted. Additionally, we identified changes in the radius of cellulose and the concentrations of cellulose, cellobiose, glucose, and ethanol during saccharification and fermentation.

1. Introduction

Bioethanol produced from lignocellulosic biomass has potential applications as an alternative to fossil fuels. Lignocellulosic biomass contains three types of polymers, i.e., cellulose, hemicellulose, and lignin. Cellulose, which is the main component of plant cell walls, consists of D-glucose linked by β-1,4 glycoside bonds and has a tightly packed structure owing to strong hydrogen bonding [1]. In contrast, hemicellulose exhibits a complex carbohydrate structure containing multiple types of polymers, which vary among different species. Lignin is a phenylpropanoid polymer containing of p-coumaryl, coniferyl, and sinapyl alcohol. These phenylpropanoids are bound together through various types of linkages [1].

To obtain bioethanol from lignocellulosic biomass, the biomass is subjected to various processes, including pretreatment, enzymatic hydrolysis, and ethanol fermentation. Pretreatment is a deconstruction method involving physical and chemical pulverization. During pretreatment, the accessibility of cellulose is improved [2], and glucose can then be obtained from cellulose through enzymatic hydrolysis. Finally, during the ethanol production step, glucose can be converted to ethanol by fermentation using a variety of microorganisms [3].

In general, enzymatic hydrolysis and ethanol fermentation are performed separately. However, effective ethanol production can be achieved by carrying out enzymatic hydrolysis and ethanol fermentation in same reactor. In this method, enzymatic hydrolysis and fermentation is performed simultaneously (termed simultaneous saccharification and fermentation [SSF]) [4]. SSF is a cost-effective, simple method compared with separate hydrolysis and fermentation (SHF). Moreover, SSF may allow high levels of production owing to reduced end-product inhibition during enzymatic hydrolysis [5]. Therefore, numerous studies have been performed to establish SSF as a feasible method, using biomass (i.e., wood, herbaceous biomass) as the starting material for practical applications.

Numerical analyses based on common reactions are needed in order to evaluate the large amounts of data produced by studies of SSF and to improve the SSF process. In previous studies, empirical models have been established for various analytical purposes [6]. Enzymatic hydrolysis during SSF is complex and requires a variety of enzymes. For example, cellobiohydrolase and end-glucanase hydrolyze cellulose after its adsorption, and cellobiose is generated by cellobiohydrolase and converted to glucose by β-glucosidase. A previous study modeled this enzyme adsorption process using Langmuir adsorption [7–9]. Kadam et al. investigated enzymatic saccharification using the Langmuir model with xylose [7]. In

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another study, Langmuir adsorption was applied to enzymatic hydrolysis and fit well with the experimental result [9]. Thus, inactivation of adsorbed enzyme may cause a delay in the rate of enzymatic hydrolysis. For establishment of a more suitable model, in addition to the inactivation rate, it is also necessary to consider changes in the radius of cellulose over time. Importantly, Langmuir adsorption has also been used for analysis of adsorption of Avicell and biomass [10], and the Michaelis-Menten model has been used for analysis of cellulose degradation [11]. Moreover, Kadam et al. used a combined model involving Langmuir adsorption and the Michaelis-Menten model [7]. This model fit well with the experimental data.

In addition to enzymatic hydrolysis, ethanol fermentation after enzymatic hydrolysis is necessary for ethanol production. Numerical analysis of the rate of ethanol production can provide insights into effective production methods because changes in each substance can be deduced. Additionally, Kumar et al. described a fermentation model that included substrate and product inhibition, cell numbers, and the maintenance coefficient [12]. These factors are likely to be important for establishment of an effective model of cellulose degradation using SSF.

Therefore, in this study, we aimed to establish an SSF model for cellulose degradation based on these previous models. In our model, ethanol fermentation and enzymatic hydrolysis models were fit to experimental data separately, and their model equations were then combined for calculation of experimental data, including the size of cellulose, amount of adsorbed protein, and decreases in glucose as the theoretical production values for SSF. Our numerical analysis demonstrated that these factors could be altered based on the model parameters, providing important insights into the cellulose degradation process.

2. Material and methods

2.1. Enzymatic hydrolysis

Cellulose (Sigma cell type 20; Sigma-Aldrich, Japan) were used for enzymatic saccharification. For quantification of glucose amount after enzymatic hydrolysis, glucose (Wako Pure Chemical Industries, Japan) was used as standard of high performance liquid chromatography. Enzymatic hydrolysis was carried out in a 200-mL Erlenmeyer flask. One gram of cellulose was used as a substrate for enzymatic hydrolysis, and 200 U/g substrate cellulase from *Trichoderma reesei* (Sigma-Aldrich) and 350 U/g substrate β-glucosidase (TOYOBO, Japan) were added to the flask. As enzymatic hydrolysis buffer, we used 0.01 M sodium acetate buffer which was adjusted to pH 5.0 using NaOH. Enzymatic hydrolysis was performed in an incubator with shaking. The incubation temperatures were 30 °C and 40 °C. Samples were collected at 0, 6, 12, 24, 48, and 72 h.

2.2. Ethanol fermentation

Ethanol fermentation was performed in a 200-mL Erlenmeyer flask at constant temperature (30 °C or 40 °C) and pH 5.0. Glucose (1.11 g) was used as the substrate. *Saccharomyces cerevisiae* type II (Sigma-Aldrich Japan) was precultured in YPD solution (10 g/L yeast extract, 20 g/L peptone, and 20 g/L dextrose; Difco BD Japan) at 48 h and adjusted to an OD_{600} of 1 0.0. After dilution of cell culture, 2 mL of the yeast culture was added to the flask. As the fermentation medium, peptone and yeast extract (Wako Chemicals Japan) were used at 20 and 10 g/L, respectively. Samples were collected at 0, 6, 12, 24, and 48 h.

2.3. Analysis

Glucose, cellobiose, and ethanol concentrations were analyzed by high-performance liquid chromatography (Shimadzu, Japan) operated at 60 °C with KS-802 (Shodex Japan). The eluent was deionized water, and the flow rate was 0.7 mL/min.

3. Kinetic analysis

One type of cellulase, endo-glucanase (EG), randomly cleaves internal bonds at amorphous site of cellulose, whereas cellobiohydrolase (CBH) produces cellobiose from the non-reducing terminal of cellulose. Moreover, cellobiose is hydrolyzed to glucose by β-glucosidase. Thus, we defined the reaction schemes for saccharification as cellulose → glucose and cellulose → cellobiose → glucose. Moreover, because cellobiohydrolase and EG adsorb to solid cellulose, these reactions are thought to represent liquid-solid reactions and can be based on the Langmuir adsorption model. On the other hand, glucose and cellobiose generated by hydrolysis are soluble in liquid; because the cellobiose-glucose reaction is a homogeneous reaction, this reaction model was based on the Michaelis-Menten model.

During the fermentation step, glucose is converted to ethanol. The fermentation model used in this study was described by Kumar et al. (2013) [12], and is based on the Monod equation. In SSF, saccharification and fermentation are conducted simultaneously. Therefore, it was necessary to consider the four reactions (two enzymatic hydrolysis reactions using the Langmuir model, one enzymatic reaction using the Michaelis-Menten model, and ethanol fermentation) to obtain the SSF model. Furthermore, because glucose generated by enzymatic hydrolysis is immediately converted to ethanol, glucose inhibition by β-glucosidase can be neglected.

First, because enzymes adsorbed to cellulose, we described the rates of adsorption and desorption as follows:

$$\vec{v} = K[E](N_s - N_a) \tag{1}$$

$$\overleftarrow{v} = K'N_a \tag{2}$$

where [E] is enzyme (M), and Na and Ns are the number of adsorptions and adsorption sites, respectively.

When the adsorption and desorption rates were in equilibrium, the covering rate (N_a/N_s) could be expressed as follows ($K_s = K/K'$).

$$\theta = \frac{N_a}{N_s} = \frac{K_s[E]}{1 + K_s[E]} \tag{3}$$

From Eqs. (1) and (3), the adsorption rate could be represented by Eq. (4) when the maximum adsorption rate was V_{max} ($\theta = 1$):

$$\vec{v} = \frac{dNa}{dt} = V_{max}\frac{K_s[E]}{1 + K_s[E]} \tag{4}$$

The amount of adsorbed enzyme is shown below. [EC] can be represented by Eq. (5);

$$[EC] = 4\pi r^2 K_E N_a N_C \tag{5}$$

where [EC] is the adsorbed enzyme to cellulose concentration (M), r is the radius of cellulose, K_E is a reaction constant, and N_C is the number of cellulose particles. The rate of cellulose hydrolysis can be described using the following two equations: