PRODUCTION OF ALTERNATIVE FUELS: MODELING OF CELLULOSIC BIOMASS CONVERSION TO ETHANOL

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ABSTRACT. Ethanol possesses physicochemical properties that make it an excellent alternative transportation fuel to gasoline and that can improve the atmospheric quality. Technology is being developed at SERI for ethanol production from widely available cellulosic biomass. The bioconversion of cellulose, the major biomass fraction, into ethanol is carried out in a single step, the simultaneous saccharification and fermentation (SSF) process, which enhances the kinetics and economics of biomass conversion. Although SSF is the frontrunning option for ethanol production from cellulose, a mathematical model is being developed to optimize the current technology. The quality of the substrate and enzyme and the modes of substrate-enzyme-microorganism interaction were identified as the key factors in SSF, and critical experimentation was designed for parameter determination. The model will serve as a tool for rational SSF improvement and commercialization. Optimization of the integrated bioconversion process is projected to bring the unsubsidized cost of ethanol to about $0.60/gallon, competitive with the price of gasoline from oil at $25/barrel.

1. Introduction

The United States economy is heavily dependent on imported petroleum and thus is extremely vulnerable to fluctuations in the international oil market. Alternative fuels such as ethanol, on the other hand, can be manufactured from domestic feedstocks [1] and reduce or eliminate the need for foreign oil. Cellulosic biomass, the most abundant renewable resource on earth with an annual production of approximately 1.8×10^{12} tons [2], is readily available from agricultural residues, forestry products and wastes, pulp and paper industry wastes, and municipal wastes for production of ethanol fuel. Energy crops could also be developed to produce a major feedstock for ethanol production. The high octane number, low volatility, high oxygen content, and low toxicity and photoreactivity of pure ethanol make it an excellent fuel for internal combustion engines [3].

Lignocellulosic biomass consists of cellulose (~50%), hemicellulose (~28%), and lignin and other components (~22%). Cellulose and hemicellulose are polymers of glucose and other sugars, such as xylose, respectively. The production of ethanol from cellulose involves

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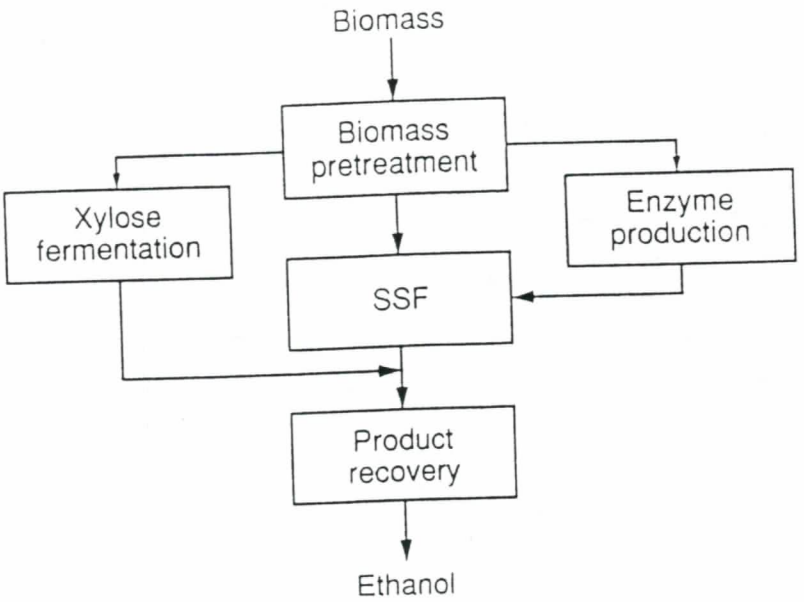

Figure 1. Schematic overview of the SERI biomass-to-ethanol conversion process.

hydrolysis of cellulose to glucose by cellulase enzymes and glucose fermentation to ethanol. Integration of these two steps into a single process called simultaneous saccharification and fermentation (SSF) enhances the kinetics of biomass conversion [4] and turns it into an economically promising process [5]. SSF leads to reduction of enzyme inhibition by cellobiose and glucose, reduces the risk of contamination due to the presence of ethanol, and results in lower capital equipment cost.

Five major steps are employed in the current biomass conversion technology developed at SERI (Figure 1): (1) chemical pretreatment of lignocellulose to render it accessible to enzymatic attack; (2) cellulase enzyme production; (3) fermentation of xylose, derived from hemicellulose during pretreatment, into ethanol; (4) SSF for conversion of cellulose to ethanol; and (5) ethanol recovery from the fermentor effluent. SSF is at the center of this process, since cellulose is the major biomass component; it has the largest contribution to the capital cost of the bioconversion process (>25%) and the final ethanol cost (>20%) [6]. It is, therefore, imperative to understand the kinetics of SSF and quantitate its dependence on variables that can be manipulated. This task can be realized in a comprehensive and rational way by developing a mathematical model that conceptualizes the performance of SSF. Unfortunately, previous mathematical models of cellulose conversion are limited to specific hydrolysis systems and conditions. This is primarily due to the fact that not all the factors affecting the kinetics of SSF, as will be presented later, have been considered.

The objective of this work is to identify and analyze the steps of the SSF process and develop a preliminary mathematical representation of its kinetics. The model, based on mathematical expressions describing the SSF kinetics, will provide valuable insight into key interactions and limitations of SSF and serve as a tool for process optimization and scale-up.

2. Substrate and Enzyme Characteristics

Cellulose is an insoluble, high molecular weight, linear polymer of D-glucose residues linked

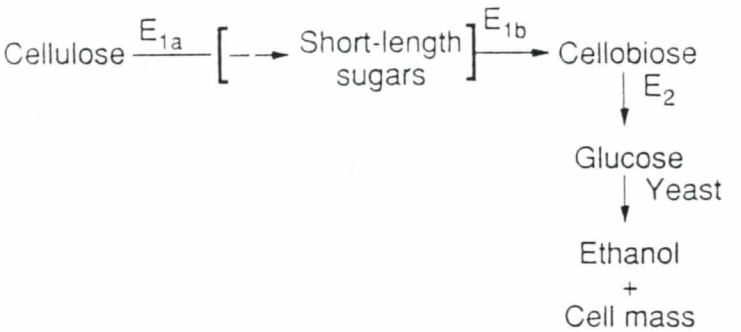

Figure 2. Simplified representation of the SSF reaction sequence (E_{1a}: endoglucanase; E_{1b}: exoglucanase; E_2: β-glucosidase).

by β-1,4-glucosidic bonds, which is usually surrounded by hemicellulose and lignin for protection from enzymatic attack. Cellulases, the enzymes that have the ability to degrade cellulose, are synthesized mostly by fungi and bacteria, both aerobically and anaerobically. Fungal cellulases consist of three major components that are believed to function synergistically [2]: endoglucanases randomly attack β-1,4-glucosidic bonds, creating shorter-length cellulose chains; exoglucanases hydrolyze these chains from the nonreducing termini, generating cellobiose residues; and β-glucosidase cleaves cellobiose to form glucose units.

3. SSF Model Description

The kinetics of SSF depend mainly on four factors: (1) quality and concentration of the cellulosic substrate, (2) quality and concentration of the cellulase and β-glucosidase enzymes, (3) mode of substrate-enzyme interaction, and (4) mode of enzyme-fermentative organism interaction. Based on these considerations, a model was developed for the conversion of cellulose into ethanol (Figure 2). Cellulase (E_1), assumed to consist of both endoglucanase and exoglucanase, adsorbs onto the solid lignocellulosic substrate and hydrolyzes cellulose (C) to shorter polysaccharides through the action of endoglucanase (E_{1a}) and finally to cellobiose (B) by exoglucanase (E_{1b}). Since the concentration of the insoluble intermediate macromolecules cannot be determined experimentally, the reaction scheme is further simplified to the direct conversion of cellulose to cellobiose. Cellobiose diffuses into the aqueous phase, where β-glucosidase (E_2) catalyzes its hydrolysis to glucose (G). Glucose serves as a carbon/energy source for yeast cells and leads to production of ethanol (E), as the major metabolic product, and cell mass (X). The model is not restricted to any particular SSF systems or conditions and refers to both anaerobic and aerobic operations.

4. SSF Model Formulation

The conversion of cellulose to ethanol involves a combination of heterogeneous and homogeneous catalyses with strong interdependence. In order to develop a comprehensive model for this complex reaction sequence, it is imperative to identify and analyze the steps that compose the cellulose-to-ethanol conversion process. They are: (1) cellulase diffusion and adsorption to cellulose; (2) hydrolysis of cellulose to cellobiose by cellulase; (3) diffusion of cellobiose into the aqueous phase; (4) hydrolysis of cellobiose to glucose by β-glucosidase; (5) uptake and catabolism of glucose by the cells; and (6) ethanol synthesis and secretion.

4.1 CELLULASE ADSORPTION AND CELLULOSE HYDROLYSIS TO CELLOBIOSE

Experimental studies [7] have indicated that enzyme diffusion is rapid relative to cellulose hydrolysis. Therefore, no mass transfer limitations are considered in this preliminary model. Cellulose hydrolysis is a heterogeneous reaction that takes place on the substrate surface. Thus, the hydrolysis rate of cellulose r_1 will depend on the concentration of cellulase adsorbed on cellulose $[E_1^*]$. Assuming that the cellulase adsorption and desorption rates are in equilibrium and taking into account the conservation of enzyme mass, r_1 becomes:

$$r_1 = k_1[E_1^*] = \frac{k_1 \alpha_t [E_1]_t}{K_e + [E_1]_t} \tag{1}$$

where $[E_1]_t$ is the total cellulase concentration, α_t is the total surface area of the biomass substrate, k_1 is the specific rate of cellulose hydrolysis, and K_e is the ratio of the specific rates of cellulase desorption and adsorption.

The internal surface area of the substrate can be measured using the solute exclusion technique [8]. However, a combination of external and internal surface area represents more accurately the total available surface area (α_t) of cellulose for enzyme adsorption. The relationship between α_t and residual substrate concentration will be determined experimentally.

In addition to cellulose, cellulosic biomass contains lignin to which both cellulase and β-glucosidase adsorb irreversibly and are rendered inactive [9]. To account for the loss of enzyme to adsorptive losses, the concentrations of active cellulase and β-glucosidase, $[E_1^*]$ and $[E_2]$, are multiplied by the corrections

$$1 - K_{1L}[L], \quad 1 - K_{2L}[L] \tag{2}$$

respectively, where K_{1L} and K_{2L} are model parameters.

In order to account for the effect of cellulose structure on the hydrolysis rate and distinguish it from the effects of available surface area and enzyme inhibition, a substrate reactivity coefficient ϕ is introduced in the model. A decrease of ϕ over time may reflect an "enrichment" of cellulose with hydrolysis-resistant regions, probably those of dense interchain bonding (crystalline cellulose). The use of the cellulose crystallinity index, determined by x-ray diffractometry [10], as a measure of ϕ will be examined.

Taking into account ϕ, cellulase adsorption to lignin, and noncompetitive inhibition of cellulase by cellobiose (B), glucose (G), and ethanol (E) [11], r_1 becomes:

$$-\frac{d[C]}{dt} = r_1 = \frac{k_1 \alpha_t \phi [E_1]_t}{(K_e + [E_1]_t)\left(1 + \frac{[B]}{K_{1B}} + \frac{[G]}{K_{1G}}\right)} \frac{K_{1E}}{K_{1E} + [E]} (1 - K_{1L}[L]) \tag{3}$$

where K_{1B}, K_{1G}, and K_{1E} are the inhibition constants of cellulase for cellobiose, glucose, and ethanol, respectively.

4.2 CELLOBIOSE HYDROLYSIS TO GLUCOSE

The hydrolysis of cellobiose to glucose is catalyzed by β-glucosidase (E_2) in the aqueous

phase. Taking into consideration substrate (B) and product (G) inhibition of β-glucosidase and adsorptive loss to lignin (L), the rate of cellobiose hydrolysis r_2 is:

$$r_2 = \frac{k_2[E_2]_o[B]}{K_m\left(1+\frac{[G]}{K_{2G}}\right)+[B]\left(1+\frac{[B]}{K_{2B}}\right)} \frac{K_{2E}}{K_{2E}+[E]}(1-K_{2L}[L]) \tag{4}$$

whereas the rate of cellobiose accumulation is:

$$\frac{d[B]}{dt} = 1.06r_1 - r_2 \tag{5}$$

where k_2 is the specific rate of cellobiose hydrolysis, K_m is the Michaelis constant of β-glucosidase for cellobiose, and K_{2B}, K_{2G}, and K_{2E} are the inhibition constants of β-glucosidase for cellobiose, glucose, and ethanol, respectively.

4.3 ANAEROBIC FERMENTATION OF GLUCOSE TO ETHANOL

Glucose, the product of cellulose hydrolysis, serves as the carbon/energy source for the fermentative organism and is metabolized anaerobically into cell mass (X), with concomitant synthesis of ethanol (E) and carbon dioxide. Other metabolic products are presumably produced in negligible amounts. A Monod kinetic expression of cell growth that includes substrate (G) inhibition is used in the model:

$$\frac{d[X]}{dt} = \mu_m \frac{[G]}{K_3+[G]+[G]^2/K_i} \frac{K_{3E}}{K_{3E}+[E]}[X] - k_d[X] \tag{6}$$

where μ_m is the maximal specific growth rate of the fermentative organism, K_3 is the Monod constant for glucose, K_i is the substrate inhibition constant, K_{3E} is the inhibition constant of cell growth caused by ethanol, and k_d is the specific rate of cell lysis.

The ethanol formation rate is considered in the model to consist of a growth-associated and a nongrowth-associated term and to also depend on the concentration of glucose (G):

$$\frac{d[E]}{dt} = \left(a\frac{d[X]}{dt} + b[X]\right)\frac{[G]}{K_4+[G]} \tag{7}$$

where a is the growth-associated ethanol formation constant, b is the nongrowth-associated specific ethanol production rate, and K_4 is a Monod constant for ethanol synthesis.

In addition to cell mass synthesis, a portion of the glucose consumed by the cells serves as a source for cell maintenance. Hence, the glucose utilization rate r_3 by the organism is:

$$r_3 = \frac{1}{Y_{XG}}\frac{d[X]}{dt} + m[X] \tag{8}$$

where Y_{XG} is an average yield coefficient of cell mass on the substrate (glucose) and m is the specific rate of substrate consumption for maintenance functions. Finally, the rate of

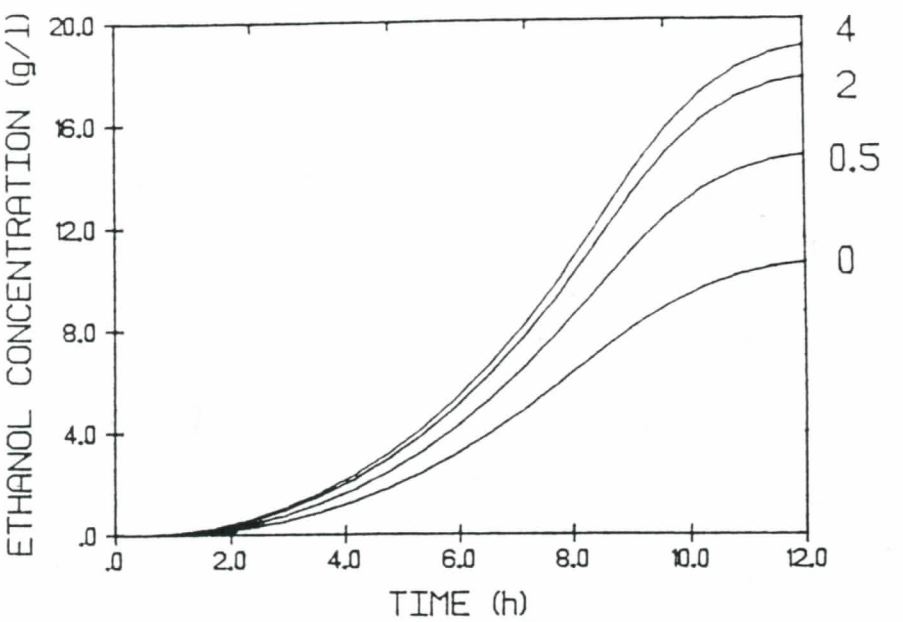

Figure 3. Model simulation of the effect of β-glucosidase concentration (in arbitrary units) on ethanol yield.

glucose accumulation will be:

$$\frac{d[G]}{dt} = 1.05 r_2 - r_3 \tag{9}$$

5. Model Calibration and Applications

The preliminary SSF model consists of five differential equations (Equations 3,5,6,7, and 9), with five dependent variables (*[C]*, *[B]*, *[G]*, *[X]*, and *[E]*) and one independent (time, *t*), assuming negligible enzyme deactivation. The outlined model can be easily modified to account for different SSF conditions and modes of operation (fed-batch, continuous).

The parameters of the model will be determined through critical experiments, each one examining the kinetics of a particular step of the SSF process isolated to the maximal feasible extent from all other steps. Nonlinear regression of the model equations to the experimental data, based on the fast-converging Levenberg-Marquardt least squares minimization procedure, will then be used to determine the parameters of the model by minimizing the weighed sum of squared residuals.

Model-based sensitivity analysis will help identify factors having a significant impact on SSF performance, as demonstrated by Figure 3, which depicts the effect of β-glucosidase concentration on ethanol yield. Although an increase of β-glucosidase concentration to two units greatly improves ethanol yield, further increase has no effect and may therefore be uneconomical. Furthermore, at the initiation of the SSF, the β-glucosidase concentration has no significant effect on ethanol yield, thus raising the possibility of a different step being rate determining at the onset of the process. These predictions of the model are in qualitative agreement with experimental results (data not shown) and underline the valuable information that a mathematical model can offer about a complex process.

6. Conclusions

A mathematical model was developed to depict the kinetics of the SSF process based on the substrate-enzyme-microorganism interactions, and critical experimentation has been designed to determine the model parameters and evaluate the predictive ability of the model. The calibrated model will be used to assess the impact that SSF variables and parameters have on ethanol productivity, identify rate limitations, and serve as a guide for process scale-up and optimization. The price of ethanol has dropped from $3.60/gallon ten years ago to $1.35/gallon now, and it is currently projected [6] that optimization of the SSF process and other unit operations of the bioconversion process, such as biomass pretreatment, cellulase production, and xylose fermentation, will further reduce the cost of ethanol to about $0.60/gallon, a price competitive with the price of gasoline on an unsubsidized basis.

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