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MODELING FOR OPTIMIZATION OF ENZYMATIC HYDROLYSIS OF CELLULOSE

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Abstract: Intrinsic kinetics of the cellulase enzyme complex as observed with hydrolysis of non-crystalline cellulose was investigated. Effect of reaction intermediates and products on intrinsic kinetics was assessed, isolating external factors such as mass transfer effects, physical properties of substrate, etc. Based on these observations a comprehensive reaction mechanism was proposed. The model predictions of cellulose, cello-oligosaccharides, cellobiose and glucose profiles, show a good agreement with experimental data. Sensitivity analysis was performed on each model parameter; this analysis provides some insights into the yield of glucose in the enzymatic hydrolysis.

Keywords: enzymatic kinetic model, non-crystalline cellulose, sensitivity analysis

1. INTRODUCTION

Kinetics and further modelling studies of hydrolysis are useful in different stages of processing of biomass to fermentable sugars. They span the entire domain of operations, namely; enzyme characterization and modification, substrate preparation, reactor design, and optimization of feeding profiles of substrate in a fed-batch operation. Mechanistic models are developed from the reaction mechanisms, mass transfer considerations and other physical parameters which affect the extent of hydrolysis. As these models address the underlying dynamics of the process, they can be extensively used in every stage.

There will be many parameters which bear direct or indirect effects on the degradation of cellulose to fermentable sugars in presence of enzyme, and can be classified as follows;

- *Enzyme Characteristics*: Adsorption of enzyme onto ligninocellulosic biomass prior to reaction; Intermediate and end-product inhibition; synergy and thermodynamic considerations of the various enzyme compounds; mass transfer limitations affecting the transport of the enzyme to substrate
- *Substrate Characteristics*: Lignin distribution; presence of other components such as hemicellulose, proteins and fats; particle size; crystallinity; degree of polymerization

Effect of physical properties and structure of substrate on enzymatic hydrolysis of biomass was presented by Perez et al., 2005. Various commercially available enzymes and their synergetic effects on hydrolysis were detailed by Howard et al., 2003. Yet, intrinsic kinetics is not completely explored. In the current work, an emphasis is given to the intrinsic kinetics and their controlling factors. The intrinsic reaction kinetics of enzymatic cellulose hydrolysis is subjected to mediation by a host of factors such as inhibitory effects from reaction intermediates and products, enzyme adsorption, etc. Furthermore, the influence of each factor is difficult to quantify in isolation, as many factors are interrelated during the hydrolytic reaction. In this work, a two step approach was adapted to investigate the intrinsic cellulase kinetics on hydrolysis of Non Crystalline Cellulose (NCC). Independent inhibition studies with reaction intermediates and products ; Comprehensive intrinsic kinetic studies of cellulase hydrolysis of NCC. To carry out this sequential study, various enzymatic hydrolysis experiments were performed on NCC.

2. MATERIALS AND METHODS

2.1 Enzyme

Cellulase enzyme, Spezyme CP (Genencor, Lot No. 301-00348-257), was obtained from NREL and has an average activity of 31.2 Filter Paper Units per mL (FPU/mL The enzyme solutions were diluted to 1, 3, and 15 FPU/mL by adding appropriate amounts of buffer solutions.

2.2 Substrate

NCC were used as substrates in multiple enzymatic hydrolysis experiments to investigate the intrinsic enzymatic kinetics. Cotton (supplied by Buckeye Tech) and Alpha cellulose which are pure forms of cellulose are used as raw materials for producing NCC. NCC was prepared in the Bio fuels/Biomass Engineering Laboratory of Auburn University by a patent pending procedure (US Provisional Patent Application #60/762,439). NCC contains 87.27% glucan, 10.51% xylan, 1.7% ash content and small amount mannan.

2.3 Enzymatic Hydrolysis

The hydrolysis of cellulose was performed in 250 mL shake flasks with a 100 mL working volume. 1 g glucan (1% w/v dry basis) was taken as the basis for each flask. 0.4 mL of tetracycline (10 mg/mL in 70% ethanol) and 0.3 mL of cyclohexamide (10 mg/mL in distilled water) were added as antibiotics to prevent any type of growth. Sodium citrate buffer (0.05 M) was used to bring the final working volume to 100 mL. The pH of 4.5 was assumed to be maintained throughout the reaction because of the buffer addition. All the components were assumed to have a density of 1g/mL in the flask. Substrate blanks and enzyme blanks were run to account for any glucose contribution from the samples, and any protein from enzymes. The flasks were heated for 1 hour at 50^{o}C before the addition of the 1 mL of cellulase enzyme, Spezyme CP (Genencor, Lot No. 301-00348-257). New Brunswick Scientific (Edison, NJ) Series 25 incubator shaker was used for agitation and

temperature control. The flasks were maintained in an incubated shaker at 50°C and 150 rpm throughout the experiments. Samples were taken at 0, 0.25, 0.5, 0.75,1, 2, 3, 4, 5, 6, 12, 24, 36, 48, 72, and 96 hours and boiled for 5 min to inactivate the enzyme, thus confirming the cessation of the reaction. Then, the samples were centrifuged, and analyzed for glucose, cellobiose, and higher cello-dextrin's using HPLC. The samples, after the carbohydrate analysis and the enzyme hydrolysis were analyzed for sugars using HPLC equipped with RI detector and Bio-Rad's Aminex HPX-87P column maintained at 85^{o}C with DI water as the mobile phase. Glucose and cellobiose were taken as the standards for measuring glucose and cellobiose concentrations, respectively. Whereas, all the other peaks which have lower retention times as compared to cellobiose and glucose were lumped together as cello-oligosaccharides and their concentration was measured against glucose standard. Remaining cellulose was calculated by subtracting glucose, cellobiose and cello-oligosaccharides concentration from initial cellulose loading.

2.4 Independent inhibition studies with reaction intermediates and products

Enzymatic hydrolysis of lignocellulosic biomass depends on competing effects such as physical properties of the substrate, enzyme synergy, mass transfer and intrinsic kinetics which can not be distinguished from each other. Accurate estimation of intrinsic kinetics requires pure form of cellulosic biomass to minimize the mass transfer resistances, and effects of physical properties of the substrate and enzyme complex. For this purpose, enzymatic hydrolysis is performed on amorphous non-crystalline cellulose. In this part of the study, inhibitory effects of soluble cello-oligosaccharides, cellobiose and glucose on enzymatic hydrolysis of NCC were quantified. These reaction intermediates and products were externally added to the substrate (NCC), initially. Later, the cellulose concentration profiles were studied after introducing the enzyme complex to the substrate solution. The series of experiments were designed in such a way that the initial concentration of one of the components (cello-oligosaccharides, cellobiose or glucose) supplemented with NCC, was varied (0%, 5% and 10% w/w) while keeping others constant. Idea behind this strategy was that the resultant variations in the hydrolysis rates may be solely attributed to the constituent whose composition was varied.

2.5 Comprehensive intrinsic kinetic model of cellulase hydrolysis of NCC

The focus of this study was not to propose altogether a new phenomenological reaction mechanism, rather to qualitatively and quantitatively analyze the underlying steps in enzymatic hydrolysis and to develop a well grounded understanding of the controlling factors of intrinsic kinetics. The analysis presented in this work highlights the dynamically changing reaction rates, inhibitory effects of reaction intermediates and products (cello-oligosaccharides, cellobiose and glucose), variability in available active enzyme. The saturating kinetics in a finite batch time was also considered. A simplified mechanism of the hydrolysis of cellulose (NCC) is given by the schematic shown in Figure 1A. Cellulose is broken down to smaller chain length cello-oligosaccharides (insoluble: degree of polymerization >7, soluble: degree of polymerization <7) by the action of endo-glucanases. Further breakdown of insoluble cello-oligomers to glucose-dimer (cellobiose) is catalyzed by exo-glucanases. β-glucosidases act on both soluble oligosaccharides and cellobiose and convert them to fermentable sugar (glucose) (Valijamae et al., 1998). The following assumptions were made to simplify the mechanism and derive the pertinent mathematical model:

- The cellulase system (E) of endo-glucanase(E_1), exo-glucanase(E_2) and β-glucosidase(E_3) is considered as having a constant composition for the given complex. These individual enzymes may be independently inhibited by the reaction intermediates and products.
- The reducing sugars inhibit the enzyme in a reversible and competitive/non-competitive manner (Holtzapple et al., 1990).
- Cellulase adsorption to the substrate surface is reversible and is governed by simple Langmuir type adsorption isotherm (Lee and Fan, 1982).
- Cellulose and insoluble cello-oligosaccharides possess similar inhibitory effects on enzymes and also their hydrolysis kinetics is assumed to be similar.
- Resistances offered by the crystallinity and composition variation with respect to the degree of hydrolysis were neglected as these studies were carried out on non-crystalline cellulose.
- As the time scale of hydrolysis is much larger than the time scale of bulk diffusion of enzyme, mass transfer resistances were considered to be negligible (Lynd et al., 2002).

Along with the simplifying assumptions that were stated earlier, it was also assumed from the intuition that the enzymes catalyzing each reaction step are inhibited by different reaction intermediates and products, as they are distinct in their action and behaviour, even though they were considered to be a single complex quantitatively. The following are the detailed inhibitory mechanisms proposed:

- Enzymes, E_1 and E_2 were subjected to non-competitive inhibition by soluble cello-oligosaccharides, cellobiose and glucose. It was observed that as the glucose concentration increases, the inhibition rates of E_1 and E_2 increase exponentially. From the numerical experiments it was found that the probability of glucose (inhibitor) binding to the enzyme is three times higher than the probability of substrate binding.
- Enzyme, E_3 was solely inhibited by glucose in competitive inhibition.

At this juncture, the mechanism may be re-drawn as shown in Figure 1B. As the properties of insoluble cello-oligosaccharides and cellulose are assumed to be the same, conversion of cellulose to cellobiose was lumped into a single step. From the aforementioned discussions, rate of each reaction can be written as follows:

$$r_1 = \frac{k_1 CE}{(K_c + C)\left(1 + \frac{B}{K_{BI}} + \frac{O}{K_{OI}} + \frac{G^3}{K_{GIn}^3}\right)(E + K_E)}$$

$$r_2 = \frac{k_2 BE}{\left(K_B\left(1 + \frac{G}{K_{GI}}\right) + B\right)(E + K_E)} \qquad \ldots(1)$$

$$r_3 = \frac{k_3 CE}{(K_c + C)\left(1 + \frac{B}{K_{BI}} + \frac{O}{K_{OI}} + \frac{G^3}{K_{GIn}^3}\right)(E + K_E)}$$

$$r_4 = \frac{k_4 OE}{\left(K_O\left(1 + \frac{G}{K_{GI}}\right) + O\right)(E + K_E)}$$

In these rate equations,

$k_i\ (i = 1,2,3,4)$ are the individual reaction rate constants,

$K_C, K_B, and\ K_O$ are cellulose saturation constant, cellobiose saturation constant and soluble cello-oligosaccharides saturation constant, respectively,

$K_{BI}, K_{OI}, and\ K_{GI}$ are inhibition constants of cellobiose, soluble cello-oligosaccharides and glucose, respectively

K_E is the desorption equilibrium constant for cellulases, from the NCC surface

$C, O, B, and\ G$ are concentrations of NCC, soluble cello-oligosaccharides, cellobiose, and glucose, respectively.

Further, the accumulation rates of cellulose, soluble cello-oligosaccharides, cellobiose, and glucose are written as,

$$\frac{dC}{dt} = -r_1 - r_3; \quad \frac{dO}{dt} = r_3 - r_4; \qquad \ldots(2)$$

$$\frac{dB}{dt} = r_1 - r_2; \quad \text{and} \quad \frac{dG}{dt} = r_2 + r_4$$

Model parameters were estimated using a nonlinear trajectory optimization.

3. RESULTS AND DISCUSSIONS

3.1 Independent Inhibition Studies

To study the individual contributions of cello-oligosaccharides, cellobiose and glucose on the inhibition of cellulase, a fixed set of enzymatic hydrolysis experiments were performed on NCC supplemented with cello-oligosaccharides, cellobiose or glucose as substrate. Figure 2A shows cellulose hydrolysis in three different cases with pure NCC; with the addition of 5% cello-oligosaccharides; with 10% cello-oligosaccharides at 1FPU enzyme loading. It is evident from the figure 2A that at higher initial oligomer concentrations, the initial reaction hydrolysis rate lasted for relatively smaller time and also resulted in reduced extent of hydrolysis after 96 hours of hydrolysis reaction. It can be inferred from these observations that oligomers strongly inhibit the hydrolysis rates. Figures 2B and 2C were plotted for different initial compositions of substrate (NCC) with cellobiose and glucose, respectively with enzyme loadings of 1 FPU/g-Glucan in each case. In both the cases, the effect of external additions was not distinguishable as the rate and extent of hydrolysis with the increase in either cellobiose or glucose additions are found to be somewhat random.

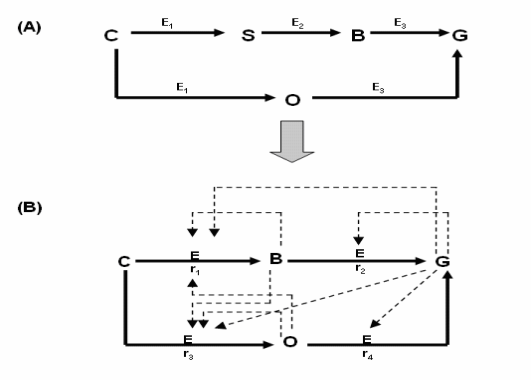

Figure 1: A) : Schematic showing the simplified mechanism of the enzymatic hydrolysis of NCC. C: Non crystalline cellulose, S: insoluble cello-oligosaccharides, O: soluble cello-oligosaccharides, B: cellobiose, G: glucose, E_1: endo-glucanases, E_2: exo-glucanases, E_3: β-glucosidases. **B)** Schematic of NCC hydrolysis mechanism after further simplification

At 5% addition of cellobiose, the higher initial rate prolonged for longer time and the extent of hydrolysis was also higher. However, further increase in external cellobiose addition to 10%, resulted in reduction in the final hydrolysis extent compared to pure NCC case. In case of 5% glucose addition, the hydrolysis extent is slightly higher than pure NCC hydrolysis, where as 10% glucose addition resulted in reduction in the extent of hydrolysis. This may have resulted due to the following reasons:

- Along with the cellulose decomposition, these additions may be triggering some intermediate steps in the cellulose hydrolysis to form glucose
- Due the accumulation of cello-oligomers, cellobiose and glucose, the effect of initial addition of either cellobiose or glucose was not distinct.
- Initial high concentrations of glucose / cellobiose may inhibit the cellulose flux towards soluble cello-oligomers and hence may reduce the inhibition on cellulases, resulting in higher extent of hydrolysis.

This study on external additions of cello-oligosaccharides, cellobiose and glucose exemplified the need to develop a comprehensive method to

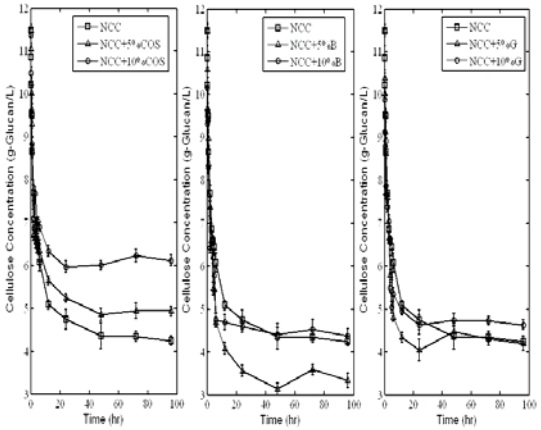

Figure 2: Enzymatic hydrolysis of NCC substrate with initial addition of **A)** Soluble Cello-oligosaccharides (COS) **B)** Cellobiose(B) and **C)** Glucose(G) in different proportions with an enzyme loading of 1 FPU/g-Glucan.

investigate the inhibitory effects of all these compounds together, as these interactions are inter-dependant in nature.

3.2 Comprehensive intrinsic kinetic studies of cellulase Hydrolysis of NCC

The reaction kinetics of enzymatic cellulose hydrolysis is subjected to mediation by a host of factors. The influence of each factor is difficult to be quantified in isolation as many factors are interrelated during the hydrolytic reaction. As discussed in the section 2.5, in synthesizing a mathematical representation of the hydrolytic reaction kinetics, a strategy was adopted to incorporate vital information with respect to the reaction mechanism, but without unnecessarily complicating the model equations for all the interwoven events in the complex heterogeneous reaction. The goal was to obtain parsimony model equations, which describe the overall kinetic behaviour of cellulose hydrolysis.

Initially, the parameters for the proposed model were identified based on the data obtained from cellulase hydrolysis of pure NCC using nonlinear constrained trajectory optimization. These model parameters are listed in Table 1. Later, the proposed methodology was validated against different sets of experimental data. From Figure 3, it is evident that all the cases, the proposed model effectively predicted the concentration profiles of cellulose, cello-oligosaccharides, cellobiose and glucose which were in close agreement with experimental data. The R^2 values in each case was provided with the figure 3. From these high R^2 values, we believe that this model can explain the entire enzymatic hydrolysis batch with high precision.

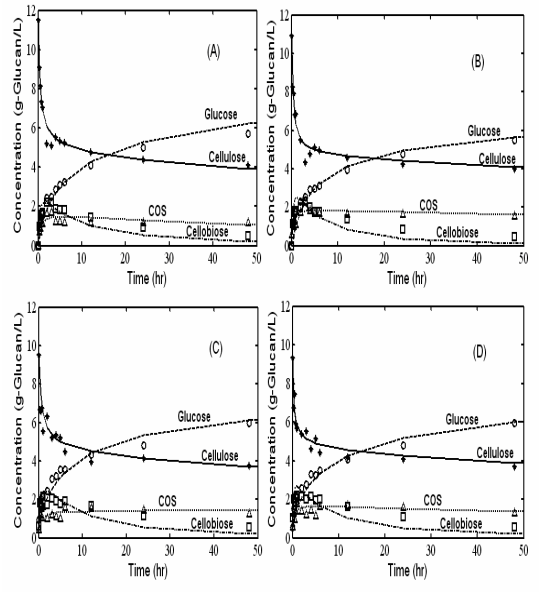

Figure 3: Experimental and predicted concentration profiles of cellulose, soluble cello-oligosaccharides, cellobiose and glucose in the enzymatic hydrolysis of **A)** pure non-crystalline cellulose (R^2 = 0.9884), **B)** non-crystalline cellulose with 5% (w/w) cello-oligosaccharides (R^2 = 0.9908), **C)** non-crystalline cellulose with 5% (w/w) Glucose (R^2 = 0.9760), **D)** non-crystalline cellulose with 5% (w/w) cellobiose (R^2 = 0.9821) with 3 FPU/g-glucan enzyme loading. Experimental data is shown by discrete points where as predictions are shown by continuous or dashed lines.

3.3 Sensitivity Analysis

Further investigation was carried out to explore the controlling factors of the enzymatic hydrolysis of cellulose by performing sensitivity analysis on the developed mathematical model. This study was performed to formulate the theoretical directives for enzyme modifications and substrate preparation to increase the extent of hydrolysis. Every kinetic parameter was varied between two limits (±100% of their nominal values) and the variation in the final extent of hydrolysis to glucose was observed. Figure 4 shows the varying final glucose concentration (g/L) in the medium, which was initially loaded with 11 g/L of NCC, with respect to the change in each kinetic parameter of the proposed model.

It can be observed that the increase in reaction rate constants k_1 and k_3 leads to the higher overall yield of glucose until a critical value, and then the extent of hydrolysis is either constant or it decreases. As k_1 and k_3 are the rates corresponding to the reactions, cellulose to cellobiose, and cellulose to soluble cello-oligosaccharides, respectively, these parameters determine the activity of the endo and exo-glucanases directly. As seen from the graphs, a three fold increase k_1 (from 38.29 to 153.16) results in approximately 30% increase in the extent of hydrolysis, where as similar change in k_3 results in an increase of 15%. Increase or decrease in k_2 and k_4 from nominal parametric values (32.92 and 14.83, respectively) results in the reduction in the extent of overall cellulose hydrolysis. If the reaction rate decreases, rate of formation of glucose also decreases. Increase in the reaction rate constants, results in either in higher amount of glucose formed, resulting in higher inhibition rates and hence reduced extent of hydrolysis. As these rate constants correspond to the reactions catalyzed by β-glucosidases, it may be concluded that further enzymatic modifications are not required to improve the catalytic activity of β-glucosidases. But as seen from the final glucose concentration *vs* K_{GI} plot, if K_{GI} is increased from the nominal value, the yield of glucose on the hydrolysis of cellulose increases in an exponential manner. This indicates that there is a scope for improvement in β-glucosidases, making them inaccessible for glucose will ensure higher extent of hydrolysis.

TABLE 1: Parameter values of proposed comprehensive kinetic model for cellulase hydrolysis of NCC

S.No.	Parameter	Description	Numerical Value
1	k_1 (g/g.min)	Rate constant	38.29625
2	k_2 (g/g.min)	Rate constant	32.92130
3	k_3 (g/g.min)	Rate constant	20.62100
4	k_4 (g/g.min)	Rate constant	14.83944
5	K_C (g/L)	Saturation constant for NCC	9.348311
6	K_B (g/L)	Saturation constant for Cellobiose	13.400910
7	K_O (g/L)	Saturation constant for COS	14.277510
8	K_{OI} (g/L)	Inhibition constant for COS	8.686783
9	K_{BI} (g/L)	Inhibition constant for Cellobiose	5.200752
10	K_{GI} (g/L)	Inhibition (competitive) constant for Glucose	0.080118
11	K_g (g/L)	Enzyme desorption constant	0.038113
12	K_{GIn} (g/L)	Inhibition (non-competitive) constant for Glucose	0.431098

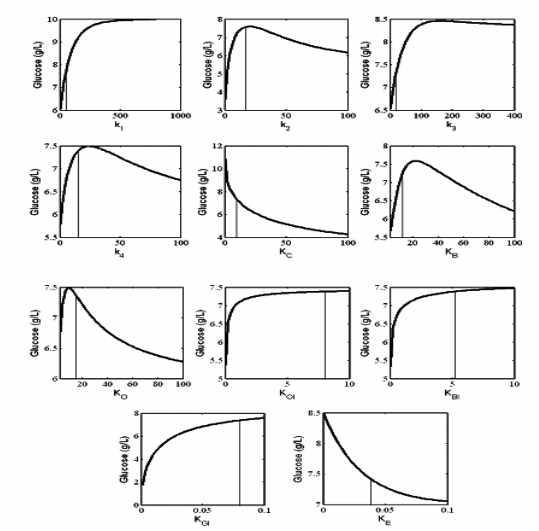

Figure 4: Effect of kinetic parameter variation on ultimate glucose yield for an NCC loading of 11 g/L.

From plot of final glucose concentration vs enzyme desorption constant K_E, it is evident that as the value of K_E decreases, final glucose concentration increases denoting higher extent of hydrolysis. Hence, if any surface modifying agents like surfactants are added to the reaction medium, they lessen the desorption constant and makes the reaction proceed further to yield higher final glucose concentration.

The individual parameter sensitivity indices over different time points of the hydrolysis reaction are estimated. A subset of these parameters, which showed noticeable patterns in their sensitivity indices over the reaction time, is plotted in Figure 5. k_3 and K_C were also presented to draw a comparison with the normal behaviour of the parameters, which is characterized by a sudden drop in the absolute value of the sensitivity index in the initial stage of hydrolysis. The sensitivity of K_{GI} dropped to 30% of its initial value over the reaction time. k_2 and K_B showed an interesting behaviour in their sensitivity indices. Surprisingly, as time of hydrolysis increased, the sensitivity indices of some parameters changed signs. At the initial stages of the reaction, increase in reaction rate constant of reaction2 increases the glucose formation, whereas at the end of the batch similar increase in k_2 results in the decrease of extent of hydrolysis. Similar behaviour is observed with K_B but in the opposite direction. These sign changes in the sensitivities were observed at 40hrs of the reaction time. From the experimental

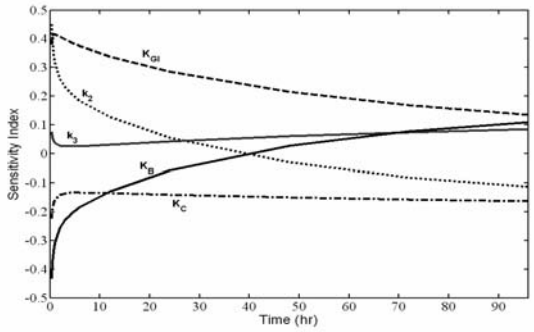

Figure 5: Variation in parametric sensitivity with batch time

observations, it was found that after this time, the concentration of cello-oligosaccharides is almost constant with no further change and cellobiose reached a minimal level. It is logical that after this time, further increase in the reaction rate constant of B to G will not be effective as already the substrate for this reaction (cellobiose) is in negligible concentrations. This observation suggests that increase of the cellulose flux towards glucose through reaction4 (O to G) in the later stages of hydrolysis, instead through reaction2 will be beneficial.

3.4 Chemical Modifications to Improve Glucose Yield

There are two ways to improve the glucose yield in enzymatic hydrolysis of cellulose; first one is chemical or physical modifications which can be done readily, and the second one is enzymatic modifications. In this work, the effect of change in enzyme complex composition and addition of surfactant was studied.

Let us consider the mole fractions of endo-glucanses, exo-glucanases and β-glucosidases are x_1, x_2, and x_3, respectively. For known composition of enzyme complex, the model equations presented earlier can be modified as;

$$r_1 = \frac{k_1 CE}{(K_c + C)\left(1 + \frac{B}{K_{BI}} + \frac{O}{K_{OI}} + \frac{G^3}{K_{GIn}^3}\right)\left(E + \frac{K_{1E}}{(x_1 + x_2)}\right)} \quad (6)$$

$$r_2 = \frac{k_2 BE}{\left(K_B\left(1 + \frac{G}{K_{GI}}\right) + B\right)\left(E + \frac{K_{2E}}{x_3}\right)}$$

$$r_3 = \frac{k_3 CE}{(K_c + C)\left(1 + \frac{B}{K_{BI}} + \frac{O}{K_{OI}} + \frac{G^3}{K_{GIn}^3}\right)\left(E + \frac{K_{3E}}{x_2}\right)}$$

$$r_4 = \frac{k_4 OE}{\left(K_O\left(1 + \frac{G}{K_{GI}}\right) + O\right)\left(E + \frac{K_{4E}}{x_3}\right)}$$

where, $K_{1E} = 0.97K_E$, $K_{2E} = 0.03K_E$, $K_{3E} = 0.8K_E$, and $K_{4E} = 0.03K_E$. The enzyme complex composition of the enzyme, final yield of glucose for a cellulose substrate loading of 11 g-glucan/L is plotted in Figure 6. The regions separated by contours shown in the Figure denote the enzyme complex compositions that give the same extent of hydrolysis. As seen from the Figure, the current enzyme complex lies in the region A; this is characterized by the region of enzyme complex mixture that yields higher hydrolysis extent. This shows that further chemical composition modifications in the enzyme complex may not result in any significant increase in the glucose yield. As discussed earlier in, reduction of the desorption constant of the enzyme-substrate complex, greatly affects the glucose yield. To validate this hypothesis experimentally, enzymatic hydrolysis was carried out in presence of surfactant Tween-20 (1 % w/w) along with sonication. Figure 7 shows the progress of enzymatic hydrolysis of NCC, sonicated NCC and sonicated NCC with small addition of Tween -20. The results of hydrolysis of sonicated NCC indicated very high initial hydrolysis rates when compared to the original NCC.

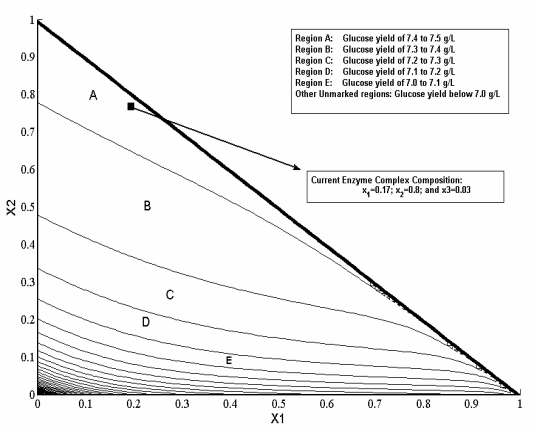

Figure 6: The ultimate glucose yield at different enzyme compositions (x1 – mole fraction of endo-glucanases; x2 – mole fraction of exo-glucanses and x3 = 1-x1-x2 is the mole fraction of β-glucosidases) for the cellulose substrate loading of 11.49g/L.

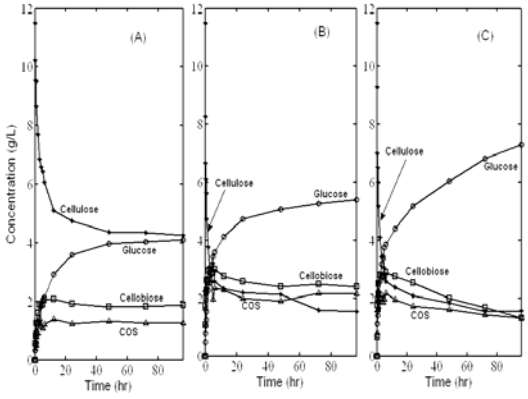

Figure 7: Effect of addition of surfactant (T-20) to the medium on enzymatic hydrolysis of NCC; **A)** Enzymatic hydrolysis of NCC, **B)** Enzymatic hydrolysis of sonicated NCC, and **C)** Enzymatic hydrolysis of sonicated NCC in presence of surfactant

The total digestibility for 96 hours was reported to be 20% higher than that of unsonicated NCC. This indicates that breaking the particle size and making a homogenous reaction mixture would favourably affect the enzyme-substrate reaction rate. The accumulation of intermediates was less when surfactant was added and most of the cellulose flux was routed to glucose even though the total hydrolysis remained the same.

4. CONCLUSIONS

This investigation coupling experimental methods with mathematical modelling and simulation analysis highlights the changing reaction dynamics of batch cellulose hydrolysis, which is influenced by substrate binding of enzyme and competitive / non-competitive product inhibition. As a result of revisiting the extensively studied subject with new analysis and experiments, some new and additional understanding of the enzymatic reaction kinetics are offered:

- Cellulose break down to smaller chain length cello-oligosaccharides (insoluble: dp>7, soluble: dp<7) by the action of endo-glucanases. Further breakdown of insoluble cello-oligomers to glucose-dimer (cellobiose) is catalyzed by exo-glucanases. β-glucosidases acts on both soluble oligosaccharides and cellobiose and converts them to fermentable sugar (glucose).
- Cellulase adsorption to the substrate surface is reversible and is governed by simple Langmuir type adsorption isotherm.
- The reducing sugars inhibit the enzyme in a reversible and competitive/non-competitive manner
- Enzymes endo-glucanases and exo-glucanases were subjected to non-competitive inhibition by soluble cello-oligosaccharides, cellobiose and glucose.
- As the glucose concentration increases, the inhibition rate of endo-glucanases and exo-glucanases increases. The probability of glucose (inhibitor) binding to the enzyme is three times higher than the probability of substrate binding.
- Enzymes, β-glucosidase were solely inhibited by glucose through a competitive inhibition mechanism.
- With NCC as substrate, accumulation of significant amounts of cellobiose and soluble cello-oligosaccharides was observed.

By sensitivity analysis, it was found that the enzyme complex is already at optimal composition for most cases, and further increase in the glucose yield by altering its composition may not be feasible. Adding surface active agents to the medium improved the enzyme-substrate adsorption which resulted in lower accumulation rates of intermediates and higher yield of glucose.

Enzyme modifications using protein engineering principles to enhance their activity by altering the properties such as; substrate binding at active site, access to the inhibitors, and allosterism are out of the scope of current work. However, the understanding of the underlying mechanism and the nature of enzyme-substrate interaction at macro-scopic level is as a starting point for such future studies.

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