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### Application of Tikhonov regularization technique to the kinetic data of the enzymatic hydrolysis of cellulose

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#### KEYWORDS

Tikhonov regularization technique, kinetic data, enzymatic hydrolysis, cellulose

#### A B S T R A C T

In this work, experimental conversion-time data for the enzymatic hydrolysis of cellulose was transformed into the concentration-rate data by the application of Tikhonov regularization technique (TRT). Converting conversion-time data into concentration-rate data through TRT transforms an otherwise ill-posed problem to one where the noise in the original data resulting in unreliable results is minimized. Enzymatic hydrolysis of cellulose is the breaking down of cellulose into smaller molecules such as glucose, cellobiose using enzyme(s). Here, three reaction kinetic models of enzymatic hydrolysis of cellulose were proposed and their kinetic parameters were determined using concentration-rate data obtained from Tikhonov regularization technique and Nelder Mead optimization technique for parameter estimation and model discrimination. Based on the fact that model 1 has no kinetic parameter with negative value and its objective functions are smaller than that of model 2, it was chosen as the best model describing the enzymatic hydrolysis of cellulose.

#### Introduction

Enzymatic hydrolysis of lignocellulosic material is an important step in the production of bioethanol. Lignocellulose exists in various forms, such as tree, grass, and wood. Bioethanol obtained from the hydrolysis of lignocellulose is accepted to be a good transportation fuel (Bommarius *et*

*al.*, 2009). Lignocellulose does not contain only cellulose; it also contains hemicelluloses and lignin (Natalija, 2007). Therefore, bio ethanol obtained from Lignocellulose is produced through four consecutive steps:

- Pre-treatment: To remove the lignin and hemicellulose.
  - Hydrolysis: To breakdown the cellulose to glucose.
  - Fermentation: Decomposition of glucose to bio ethanol.
  - Separation: For purification of the bio ethanol.
- a) Significance and objectives of the study is, in reaction kinetics investigation, determination of the rate and equilibrium constants to minimize the deviation of the computed time-concentration profile from its experimentally observed counterpart becomes more complicated for those are not simple and cannot be found analytical solution. Tikhonov regularization technique is used to convert the concentration-time data to reaction rate-concentration.
  - b) Comparison of the time-concentration against proposed time-concentration data
  - c) Proposed kinetic model
  - d) Determine the kinetic parameters of the proposed models
  - e) Scrutinize the proposed models to determine the best model

The aim of this paper is to apply Tikhonov regularization to well documented kinetic data from the previous work of Bommarius et al (1978) and to determine the kinetic parameters of three proposed kinetic models for the enzymatic hydrolysis of cellulose.

The relationship between the reaction rate  $r(t)$  and the time-concentration profile  $C(t)$  can be written as:

$$r(t) = \frac{dC(t)}{dt} \quad (1)$$

This can be rewritten as:

$$C(t) = \int_{t=0}^t r(t) + C_0 \quad (2)$$

the problem of obtaining  $r(t)$  is an ill-posed problem in the sense that if inappropriate methods are used, it will lead to inaccurate results (Engl et al., 2000). Instead of solving Equation (2) directly for  $r(t)$ , this equation, through integration by parts, can be transformed into:

$$C^C(t) = \int_{t=0}^t d[t'r(t)dt' - t'f(t')]dt' + C_0 \quad (3)$$

$$= t'r(t)]_{t=0}^t - \int_{t=0}^t t'f(t')dt' + C_0 \quad (4)$$

$$= tr(t) - \int_{t=0}^t t'f(t')dt' + C_0 \quad (5)$$

$$C^C(t) = t \left( \int_{t=0}^t f(t')dt' + r_0 \right) - \int_{t=0}^t t'f(t')dt' + C_0 \quad (6)$$

$$= \int_{t=0}^t (t-t')f(t')dt' + C_0 + tr_0 \quad (7)$$

Where  $f(t) = \frac{dr(t)}{dt}$  and  $r_0 = r(0)$  is the initial rate.

Equation (7) is the starting point of the present investigation. Inputs to this equation are the experimentally measured time-concentration data points:

$$(t_1, C_1^M), (t_2, C_2^M), (t_3, C_3^M), \dots, (t_{M_N}, C_{M_N}^M)$$

$M_N$  is the number of points in the set and is usually a relatively small number, typically around 10–50. The data points may or may not be regularly spaced out in time. From the way Equation (7) was obtained it is clear that this equation is independent of the order of the reaction and its nature.

### Discretizing the Volterra integral equation

In discretized form equation (7) becomes:

$$C_i^e = C_0 + t_i r_0 + \sum_{t_j=0}^{t_i-t_i} \alpha_{ij} (t_i - t_j) f_j \Delta t' \tag{8}$$

Where,  $t_i = 1, 2, \dots, N_D$   
 $t_j = 1, 2, \dots, N_K$

Where,  $f_1, f_2, f_3, \dots, f_{N_K}$  are the discretized  $f(t)$ . The independent variable  $0 \leq t' \leq t_{max}$  is divided into  $N_K$  uniformly spaced discretized points with step size  $\Delta t' = t_{max}/(N_K - 1)$ .

In the present investigation  $N_K$  is typically of the order of 101 to 401.  $t_{max} = t_{N_D}$  is the largest  $t_i$  in the data set.  $\alpha_{ij}$  is the coefficient arising from the numerical scheme used to approximate the integral in Eq. (8). For Simpson's 1/3 rule, used throughout the present investigation,  $\alpha_{ij} = 2/3$  for odd  $j$  (except  $\alpha_{i1} = 1/3$ ) and  $4/3$  for even  $j$ . Depending on whether the  $t_i$  of the experimental data point coincides with a discretized point, the last  $\alpha_{ij}$  associated with this point may have to be adjusted, by interpolation, to allow for fractional step size.

The deviation of  $C^C$  from  $C^M$  is given by

$$\delta_i = C_i^M - (C_0 + t_i r_0 + \sum_{t_j=0}^{t_i-t_i} \alpha_{ij} (t_i - t_j) f_j \Delta t') \tag{9}$$

$$\delta_i = C_i^M - (C_i c_0 + B_i r_0 + \sum_{t_j=0}^{t_i-t_i} \alpha_{ij} (t_i - t_j) f_j \Delta t') \tag{10}$$

Or in matrix notation,

$$\delta = C^M - C c_0 - B r_0 - A f \tag{11}$$

$C$  and  $B$  are  $N_D \times 1$  column vectors and  $A$  is a  $N_D \times N_K$  matrix of coefficient of the unknown column vector

$$f = [f_1, f_2, f_3, \dots, f_{N_K}]^T$$

While  $C$ ,  $B$  and  $A$  are given by

$$C_i = 1 \tag{12}$$

$$B_i = t_i \tag{13}$$

$$A_{ij} = \alpha_{ij} (t_i - t_j) \Delta t' \quad \text{for } t_i \geq t_j \tag{14}$$

In Equation (14)  $t_i, i = 1, 2, 3, \dots, N_D$  are the times at which the concentration is measured and  $t_j, j = 1, 2, 3, \dots, N_K$  are then uniformly discretized time  $0 \leq t' \leq t_{max}$ .  $N_K$  generally exceeds the number of data points  $N_D$ , thus  $A$  is not a square matrix and Equation (3) cannot be inverted to give a unique  $f$ ,  $C_0$  and  $r_0$ . Instead, these unknowns are selected to minimize the sum of squares of  $\delta_i$ , i.e. to minimize

$$\sum \delta_i^2 = \delta^T \delta = (C^M - C c_0 - B r_0 - A f)^T \times (C^M - C c_0 - B r_0 - A f) \tag{15}$$

### Tikhonov regularization

To obtain smooth solutions to ill-posed problems, the standard Tikhonov regularization method is most often used. Hadamard defined a linear problem to be well posed if it satisfies the following three requirements: (a) existence, (b) uniqueness, and (c) stability. A problem is said to be ill-posed if one or more of these requirements are not satisfied. A classical example of an ill-posed problem is a linear integral equation of the first kind in  $L^2(I)$  with a smooth kernel. A solution to this equation, if it exists, does not continuously depend on the right-hand side and may not be unique. When a discretization of the problem is performed, we obtain a matrix equation in  $C^m$ ,

$$Ku=f \tag{16}$$

where  $K$  is an  $m \times n$  matrix with a large condition number,  $m \geq n$ . A linear least squares solution of the system (1) is a solution to the problem.

$$\text{Min} \|Ku-f\|^2 \tag{17}$$

$$u \in C^n$$

Where, the Euclidean vector norm in  $C^m$  is used. We say that the algebraic problems (16) and (17) are discrete ill-posed problems.

The numerical methods for solving discrete ill-posed problems in function spaces and for solving discrete ill-posed problems have been presented in many papers. These methods are based on the so-called regularization methods. The main objective of regularization is to incorporate more information about the desired solution in order to stabilize the problem and find a useful and stable solution. The most common and well-known form of regularization is that of Tikhonov (Groetsch, 1984). It consists in replacing the least-squares problem (2) by that with a suitably chosen Tikhonov functional. The most basic version of this method can be presented as

$$\text{min} \{ \|Ku-f\|^2 + \alpha^2 \|u\|^2 \} \tag{18}$$

$$u \in C^n$$

Where,  $\alpha \in R$  is called the regularization

parameter. The Tikhonov regularization is a method in which the regularized solution is sought as a minimiser of a weighted combination of the residual norm and a side constraint. The regularization parameter controls the weight given to the minimization of the side constraint.

Minimizing  $\delta^T \delta$  in equation (15) will not in general result in a smooth  $f(t)$  because of the noise in the experimental data. To ensure smoothness, additional conditions have to be imposed. In the present investigation, the additional condition is the minimization of the sum of squares of the second derivative  $d^2f/dt^2$  at the internal discretization points. In terms of the column vector  $f$ , this condition takes on the form of minimizing

$$\sum_{j=2}^{N_K-1} \left( \frac{d^2 f}{dt^2} \right)_j^2 = (\beta f)^T (\beta f) = f^T \beta^T \beta f \tag{19}$$

Where,  $\beta$  is the tri-diagonal matrix of coefficients arising from the finite difference approximation of  $\frac{d^2 f}{dt^2}$

$$\beta = \begin{bmatrix} 1 & -2 & 1 & & & & \\ & 1 & -2 & 1 & & & \\ & & 1 & -2 & 1 & & \\ & & & \dots & \dots & \dots & \\ & & & & 1 & -2 & 1 & \\ & & & & & 1 & -2 & 1 \end{bmatrix} \frac{1}{(\Delta t)^2} \tag{20}$$

In Tikhonov regularization (Engl et al., 2000) instead of minimizing  $\delta^T \delta$  and  $f^T \beta^T \beta f$  separately, a linear combination of these two quantities  $R = \delta^T \delta + \lambda f^T \beta^T \beta f$  is minimized.  $\lambda$  is an adjustable



weighting/regularization factor that controls the extent to which the noise in the kinetic data is being filtered out. It balances the two requirements on  $f(t)$ :

- a. fitting the experimental data
- b. Remaining as smooth as possible.

A large  $\lambda$  will give a smooth  $f(t)$  but at the expense of the goodness of fit of the kinetic data and vice versa.

Minimizing R becomes:

$$\frac{\partial R}{\partial f_j} = 0 \quad j = 1, 2, 3, \dots, N_K \quad (20)$$

$$\frac{\partial R}{\partial C_0} = 0 \quad (21)$$

$$\frac{\partial R}{\partial r_0} = 0 \quad (22)$$

These give rise to a set of linear algebraic equations for  $\mathbf{f}$ ,  $C_0$  and  $r_0$  (assuming that both initial conditions are unknown). It can be shown (Shaw & Tigg, 1994) that the  $\mathbf{f}$ ,  $C_0$  and  $r_0$  that satisfy Equations (20) to (22) are given by

$$\mathbf{f}' = (\mathbf{A}^T \mathbf{A} + \lambda \boldsymbol{\beta}^T \boldsymbol{\beta})^{-1} \mathbf{A}^T \mathbf{C}^M \quad (23)$$

For convenience  $\mathbf{f}'$  is used to denote the column vector

$$[f_1, f_2, f_3, \dots, f_{N_K}, C_0, r_0]^T$$

incorporating  $C_0$  and  $r_0$  into  $\mathbf{f}$ .  $\mathbf{A}'$  is the composite matrix ( $\mathbf{A}$ ,  $\mathbf{C}$ ,  $\mathbf{B}$ ) derived from Equations (12) to (14) to reflect the inclusion of  $C_0$  and  $r_0$  in  $\mathbf{f}$ . Similarly  $\boldsymbol{\beta}'$  is the composite matrix ( $\boldsymbol{\beta}$ ,  $\mathbf{0}$ ,  $\mathbf{0}$ ) where  $\mathbf{0}$  is a  $(N_K - 2) \times 1$  column vector of 0 to allow for the fact that  $C_0$  and  $r_0$  play no part in the smoothness condition in Equation (19). The  $\mathbf{f}'$  given by Equation (23) can now be substituted into Equation (13) to give  $C(t)$ . It can also be substituted in the defining equation  $dr(t)/dt = f(t)$  and integrated to give the reaction rate  $r(t)$ .

As  $f(t)$  is known at a large number of closely spaced discretization points the integration for  $r(t)$  and  $c(t)$  can be carried out using any of the standard numerical integration procedures. Since integration is a smoothing process, the resulting  $r(t)$  and  $c(t)$  can be expected to be well-behaved smooth functions. This has been observed in all the examples investigated.

### Regularization parameter identification

A suitable choice of the regularization parameter  $\lambda$  has to be provided by the user in order to apply Tikhonov regularization (Yeow and Taylor, 2002). An optimal regularization parameter should fairly balance the perturbation error and the regularization error in the regularized solution.

The discrepancy principle is a-posteriori strategy for choosing  $\alpha$  as a function of an error level (the input error level must be known). The generalized cross validation method is based on a-priority *knowledge* of a structure of the input error, which means that the errors in  $f$  can be considered to be uncorrelated zero-mean random variables with a common variance, i.e., white noise.

Another practical method for choosing  $\alpha$  when data are noisy is the L-curve criterion (Hansen, 1992; Hansen and O'Leary, 1993). The idea of the L-curve criterion is to choose regularization related to the characteristic L-shaped "corner" of the graph.

The most appropriate value of  $\lambda$  depends on factors such as the noise level in the experimental data, the number of data points  $N_D$ , and discretization points  $N_K$ , and the numerical schemes used to approximate the integral in Equation (13) and the second derivative in Equation (19). It is neither a

property of the reaction under investigation nor a (Yeow and Taylor, 2002) constant determined by the concentration measurement technique or instrument employed.

If  $\lambda$  is set too small the determinant of the matrix  $A^T A + \lambda B^T B$  in Equation (23) may become close to zero and the inverse of the matrix becomes ill conditioned. This is a manifestation of the ill-posed nature of the problem of obtaining reaction rates from time-concentration data.

### Applied mathematical methods and available software package

Methods that were previously thought to be too effort consuming are now commercially available in a number of software packages such as Mathematica, MAPLE, or MATLAB.

### Simpson rule

Standard numerical integration procedures include the Simpson's rule and the trapezoidal rule.

Simpson's rule is

$$A \approx S/3 [(F + L) + 4E + 2R]$$

Where S is the width of each strips.

### Model discrimination and parameter estimation

The laws governing the behaviour of a system, and can then derive equations describing the relationship among the observed quantities (Bard, 1974). Such an equation is termed a model, and the process of determining the model's parameter values is parameter estimation. The model is expressed as a mathematical program i.e one in which the objectives and constraints are

given as mathematical functions and frictional relationships. When the model equations are non-linear, the determination process may also be described as non-linear estimation. The determination of which the derived rate best fits the observed rates will give an insight into the actual mechanism of the reaction and the chosen equation can then be used in future predictions.

### Optimization technique

The general objective in optimization is to choose a set of values of variables (or parameters) subject to the various constraints that produce the desired optimum response for the chosen function (Edgar et al., 2001). The objective function in this case is the sum of squares of residuals between experimental and predicted rates of reaction.

$$S = \sum_{i=1}^n (r_i^{cal} - r_i^{obs})^2 \quad (24)$$

The smaller the value of S, the better the model fits the data and the more reliable the value of the kinetic parameters thus obtained. The method used in the present investigation is called Flexible tolerance method (Paviani and Himmelblau, 1969) which incorporates portions of the polyhedron method with the additional advantage of not restricting intermediate iteration to feasible region. The method of Nelder-Mead is the method that alters the shape of the simplex to suit local topology. The tolerance criterion is reduced within the region of an optimum till it reached a present small value.

### The flexible polyhedron search

The Nelder-Mead's modified simplex method minimizes a function of independent variables using (n+1) vertices of a flexible polyhedron in  $E_n$ . Each vertex is defined by

a vector  $x$ . the vertex in  $E_n$ , which yields the highest value of  $f(x)$  by better point until minimum  $f(x)$  is found.

The details of the algorithm are as follows:

Let  $xi(k) = [xi^{(k)} \dots \dots \dots, xij^{(k)}]$ ,  $I=1, \dots, n+1$ , be the vertex(point) in  $E_n$  on the  $k^{th}$  stage of search.  $K=0, 1, \dots$ , and let the value of the objective function at  $xi(k)$  be  $f(xi^{(k)})$ .  $X_{n+2}$ . In addition, we need to label  $x$  vectors in the polyhedron that gives the maximum and minimum values of  $f(x)$ .

Define

$$f(xh^{(k)}) = \max\{f(xh^{(k)}) \dots f(xn+I^{(k)})\}$$

With corresponding,  $xi^{(k)} = xh^{(k)}$

In addition,  $f(xh^{(k)}) = \min\{f(xi^{(k)}) \dots f(xn+I^{(k)})\}$

With the corresponding  $xi^{(k)} + xh^{(k)}$  since the polyhedron in  $E_n$  is made up of  $(n+1)$  vertices,  $X_n, \dots, X_{n+1}$ , let  $X_{n+2}$  be the centroid of all vertices excluding  $X_n$ . The coordinates of the centroid are given by:

$$X_{n+2,j}^{(k)} = \frac{1}{n} [\sum_{j=1}^n X_{ij}^{(k)} - X_{hj}^{(k)}]$$

Where, index  $j$  designates each co-ordinate direction. The initial polyhedron usually is selected to be a regular simplex.

**Methodology**

**Processing the time-conversion data into reaction rate- concentration data**

Bommarius et al. (1978) investigated the kinetics of the enzymatic hydrolysis of cellulose and obtained the experimental data in form of conversion of cellulose against time. The data was for the reaction occurring

at enzyme concentration; 1.5U, 15U and 30U.

The stage wise analysis of the conversion of the data from conversion-time data to reaction rate-concentration data, taking the initial conversion of glucose to be zero for 1.5U enzyme concentration:

$$t_{max} = 95, N_D = 9, N_K = 1000$$

Step one: Divide the independent variable, time(t), where  $0 \leq t' \leq t_{max}$  is divided uniformly spaced discretization points  $N_K$  with step size

$$\Delta t' = \frac{t_{max}}{N_K - 1} \quad t_{max} = 95, N_K = 1000$$

Step two: Generate  $\alpha_{ij}$  from simpson's 1/3 rule, where  $\alpha_{ij} = 2/3$  for odd  $j$  (except  $\alpha_{i1} = 1/3$ ) and 4/3 for every even  $j$

Step three: Compute  $(t_i - t_j)$  in equation (14)

Step four: Compute  $\Delta t'$  in equation (14)

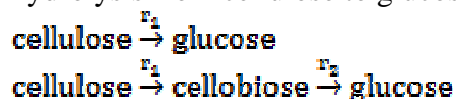
Step five: Compute  $A_{ij}$  using equation (14)

Step six: Compute matrix **A**, where **A** is a 9 x 1000 matrix of coefficients of unknown column vector

Step seven: Compute matrix **C**, where **C** is a 9 x 1 matrix of ones



cellulose for the subsequent conversion to glucose. Finally, the most implemented one added to this second type the direct hydrolysis from cellulose to glucose.



### Factors affecting enzymatic

Factors affecting enzymatic hydrolysis of cellulose can be classified into two groups.:

- Enzyme related factors
- Substrate related factors

### Enzyme related factors

Enzymes concentration, adsorption, synergising, end-product inhibition, mechanical deactivation, thermal inactivation and irresistible binding to lignin

### Physical proportion of substrate affecting hydrolysis

Crystalline of cellulose, degree of polymer reaction, accessible surface area, structural organisation and presence of associated materials such as hemicellulose and lignin.

### Mechanistic models development

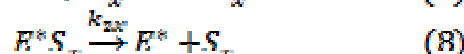
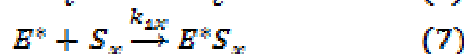
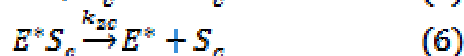
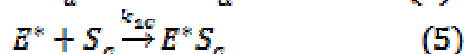
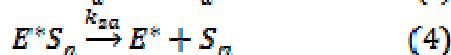
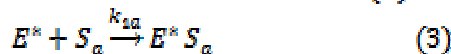
#### Mechanism 1

The first kinetic model to be used was proposed by Ryu et al. (1982). The kinetic model for the enzymatic hydrolysis of cellulose was derived based on the following assumptions;

1. The cellulosic material  $S_0$  is composed of amorphous matter  $S_a$ , crystalline matter  $S_c$ , and nonhydrolyzable parts  $S_x$  and their rates of enzymatic degradation are different.

2. The Cellulases system can be represented quantitatively by single enzyme E.
3. The Cellulases enzyme is first adsorbed  $E^*$  on the surface of cellulose, followed by enzyme-substrate formation  $E^*S$  and the hydrolysis to release both product and enzyme.
4. The products (glucose and cellobiose) inhibit the cellulase enzyme competitively.

The reaction scheme based on the above assumptions can be written as follows;



Where P is product (Glucose and cellulose) The adsorption of the enzyme, equation (1) and (2) can be described by Langmuir-type adsorption isotherm.

$$\frac{C_{E^*}}{C_{E^*_{max}}} = \frac{K_E C_E}{1 + K_E C_E} \quad (13)$$

Where  $C_{E^*_{max}}$  is concentration of the maximum adsorbed enzyme and  $K_E$  is the adsorption constant.

At low enzyme concentration;

$$K_c C_E \ll 1$$

And equation (13) becomes

$$C_{E^*} = K_c C_E C_{E_{max}}^* \quad (14)$$

Where  $K_d = K_c C_{E_{max}}^*$

The rate determining steps are equation (11) and (12). Therefore, the rate of product formation from equation (11) and (12) is, if we assume total enzyme content is constant.

$$\frac{dC_P}{dt} = k_{2a} C_{E^*} S_a + k_{2c} C_{E^*} S_c \quad (15)$$

If we assume that the total enzyme content is constant

$$C_{E0} = C_E + C_{E^*} + C_{E^*} S_a + C_{E^*} S_c + C_{E^*} S_x + C_{EP} \quad (16)$$

Using steady-state approximation, the change in intermediate concentrations is given as;

$$\frac{dC_{E^*} S_a}{dt} = k_{1a} C_E C_{S_a} - (k_{2a} + k_{3a}) C_{E^*} S_a \cong 0$$

$$k_{1a} C_E C_{S_a} = (k_{2a} + k_{3a}) C_{E^*} S_a$$

$$C_{E^*} S_a = \frac{k_{1a} C_E C_{S_a}}{(k_{2a} + k_{3a})}$$

$$C_{E^*} S_a = \frac{C_E}{K_a} C_{S_a}$$

$$C_{E^*} = \frac{K_a C_E C_{S_a}}{C_{S_a}}$$

Where  $K_a = \frac{(k_{2a} + k_{3a})}{k_{1a}}$

$$\frac{dC_{E^*} S_c}{dt} = k_{1c} C_E C_c - (k_{2c} + k_{3c}) C_{E^*} S_c \cong 0$$

$$k_{1c} C_E C_c = (k_{2c} + k_{3c}) C_{E^*} S_c$$

$$C_{E^*} S_c = \frac{k_{1c} C_E C_c}{(k_{2c} + k_{3c})}$$

$$C_{E^*} S_c = \frac{C_E}{K_c} C_c \quad (19)$$

Where  $K_c = \frac{(k_{2c} + k_{3c})}{k_{1c}}$

$$\frac{dC_{E^*} S_x}{dt} = k_{1x} C_E C_{S_x} - (k_{2x} + k_{3x}) C_{E^*} S_x \cong 0$$

$$k_{1x} C_E C_{S_x} = (k_{2x} + k_{3x}) C_{E^*} S_x$$

$$C_{E^*} S_x = \frac{k_{1x} C_E C_{S_x}}{(k_{2x} + k_{3x})}$$

$$C_{E^*} S_x = \frac{C_E}{K_x} C_{S_x} \quad (20)$$

Where  $K_x = \frac{(k_{2x} + k_{3x})}{k_{1x}}$

$$\frac{dC_{EP}}{dt} = k_{1p} C_E C_P - k_{2p} C_{EP} \cong 0$$

$$k_{1p} C_E C_P = C_{EP}$$

$$C_E = \frac{k_{2p} C_{EP}}{k_{1p} C_P}$$

$$C_E = \frac{C_{EP}}{K_p} \quad (21)$$

Where

$$K_p = \frac{k_{2p}}{k_{1p}} \quad (18)$$

Dividing equation (17) by (18), we obtain

$$\frac{C_{E^*} S_a}{C_{E^*} S_c} = \frac{K_c C_c}{K_a C_{S_a}} \quad (22)$$

From equation (15), we obtain

$$\frac{dC_P}{dt} = \left( k_{2a} + k_{2c} \frac{C_E C_{S_c}}{C_E C_{S_a}} \right) C_{E^*} S_a \quad (23)$$

Substituting equation (21) into (22), we obtain



$$\frac{dC_s}{dt} = \left( k_{2a} + k_{2c} \frac{K_a C_{S_c}}{K_c C_{S_a}} \right) C_{E^* S_a} \quad (24)$$

From equation (14), we obtain

$$C_E = \frac{C_{E^*}}{K_d} \quad (25)$$

Substituting equation (17) into (14), we obtain

$$C_E = \frac{K_a C_{E^* S_a}}{K_d C_{S_a}} \quad (26)$$

Substituting equation (26) into (20), we obtain

$$C_{E^* S_c} = \frac{C_{S_c} K_a C_{E^* S_a}}{K_x C_{S_a}}$$

From equation (21), we obtain

$$C_{E^* S_c} = \frac{K_a C_{S_c}}{K_c C_{S_a}} C_{E^* S_a} \quad (27)$$

Substituting equation (26) into (21), we obtain

$$C_{EP} = \frac{K_a C_p C_{E^* S_a}}{K_p C_{S_a} K_d}$$

Substituting equation (24) to (27) into (16) yields

$$C_{E0} = \frac{K_a}{C_{S_a}} \left( 1 + \frac{1}{K_p} + \frac{C_{S_a}}{K_a} + \frac{C_{S_c}}{K_c} + \frac{C_{S_x}}{K_x} + \frac{C_p}{K_p K_p} \right) C_{E^* S_a}$$

$$C_{E^* S_a} = \frac{C_{E0}}{\frac{K_a}{C_{S_a}} \left( 1 + \frac{1}{K_p} + \frac{C_{S_a}}{K_a} + \frac{C_{S_c}}{K_c} + \frac{C_{S_x}}{K_x} + \frac{C_p}{K_p K_p} \right)} \quad (29)$$

Substituting equation (29) into (24) yields

$$\frac{dC_p}{dt} = \frac{\left( k_{2a} + k_{2c} \frac{K_a C_{S_c}}{K_c C_{S_a}} \right) C_{E0}}{\frac{K_a}{C_{S_a}} \left( 1 + \frac{1}{K_p} + \frac{C_{S_a}}{K_a} + \frac{C_{S_c}}{K_c} + \frac{C_{S_x}}{K_x} + \frac{C_p}{K_p K_p} \right)}$$

$$\frac{dC_p}{dt} = \frac{\left( k_{2a} \frac{C_{S_a}}{K_a} + k_{2c} \frac{C_{S_c}}{K_c} \right) C_{E0}}{1 + \frac{1}{K_p} + \frac{C_{S_a}}{K_a} + \frac{C_{S_c}}{K_c} + \frac{C_{S_x}}{K_x} + \frac{C_p}{K_p K_p}} \quad (30)$$

If we define

$$\varphi = \frac{C_{S_c}}{C_{S_c} + C_{S_a}}$$

And

$$\gamma = \frac{C_{S_x}}{C_{S_c} + C_{S_a} + C_{S_x}}$$

Substituting equation (31) and (32) into (30) and rearranging yield

$$\frac{dC_p}{dt} = \frac{[k_{2a}(1-\varphi) + k_{2c}\varphi\frac{K_a}{K_c}] C_{E0} (1-\gamma) C_p}{K_a \left( 1 + \frac{1}{K_p} + \frac{C_p}{K_p K_p} \right) + [(1-\varphi)(1-\gamma) + \varphi\frac{K_a}{K_c} + \gamma\frac{K_a}{K_x}]}$$

From literature, the value of  $\varphi$  for non-pretreated avicel is 0.8,  $\gamma$  is 0.04 and average degree of polymerization is 325

The second kinetic model to be used was proposed by Shen and Agblevor (2008a). The kinetic model for the enzymatic hydrolysis of cellulose was derived based on the following assumptions;

- (28) The endo- $\beta$ -1,4-glucanase, exo- $\beta$ -1,4-cellobiohydrolase and glycosidase enzymes were assumed to form a single combined effect on the hydrolysis of insoluble substrate to breakdown substrate to produce reducing sugar
2. The surface and structure of insoluble substrate was considered homogeneous (i.e. there is no distinction between crystalline and amorphous region). This assumption is also in agreement with requirement of the Langmuir adsorption model.
3. Ineffective production of enzyme-substrate complexes.
4. The enzyme deactivation was assumed to be second order reaction and was consider the main factor influencing the hydrolytic rate.
5. There is only one reaction that occurred: direct transformation of cellulose to glucose

The reaction scheme based on the above assumptions can be written as follows;



Ineffective enzyme adsorption;



From the assumption (4)



$$\frac{dC_{ES_{in}}}{dt} = k_{de} C_E^2 \quad (37)$$

Mass balance

$$C_s = C_{S_0} - C_{ES} - C_{ES_{in}} - C_P \quad (38)$$

Where

$C_{S_0}$  = initial concentration of substrate

$C_P$  = concentration of product

Assuming  $C_{ES_{in}} \ll C_{ES}$ , equation (35) becomes

$$C_s = C_{S_0} - C_{ES} - C_P$$

From (33), (34) and (36), we obtain

$$\frac{dC_{ES}}{dt} = k_{ad} C_E C_s - (k_d + k_{cat}) C_{ES} \quad (40)$$

Using steady-state approximation, equation (34) becomes

$$k_{ad} C_E C_s - (k_d + k_{cat}) C_{ES} \cong 0$$

$$k_{ad} C_E C_s = (k_d + k_{cat}) C_{ES}$$

$$C_{ES} = \frac{k_{ad}}{(k_d + k_{cat})} C_E C_s$$

$$C_{ES} = \frac{C_E C_s}{K_M}$$

$$C_{E0} = C_E + C_{ES}$$

Substituting equation (38) into (36), we obtain

$$C_s + \frac{C_E C_s}{K_M} = C_{S_0} - C_P$$

$$C_s \left(1 + \frac{C_E}{K_M}\right) = C_{S_0} - C_P$$

$$C_s = \frac{C_{S_0} - C_P}{\left(1 + \frac{C_E}{K_M}\right)} \quad (43)$$

Substituting equation (40) into (38), we obtain

$$C_{ES} = \frac{C_E}{K_M} \left(\frac{C_{S_0} - C_P}{1 + \frac{C_E}{K_M}}\right)$$

$$C_{ES} = \frac{C_E (C_{S_0} - C_P)}{K_M + C_E} \quad (44)$$

From equation (36)

$$\frac{dC_P}{dt} = k_{cat} C_{ES} \quad (45)$$

Substituting equation (44) into (45), we obtain

$$\frac{dC_P}{dt} = k_{cat} \frac{C_E}{K_M} \left(\frac{C_{S_0} - C_P}{1 + \frac{C_E}{K_M}}\right) \quad (46)$$

$$\frac{dC_{ES_{in}}}{dt} = -\frac{dC_E}{dt}$$

$$\frac{dC_E}{dt} = -k_{de} C_E^2 \quad (47)$$

Integrating equation (47) using boundary conditions;

$C_E = C_{E0}$  at  $t = 0$  and  $C_E = C_E$  at  $t = t$ , we obtain

$$C_E = \frac{C_{E0}}{1 + k_{de} C_{E0} t} \quad (48)$$

Substituting equation (48) into (46), we obtain

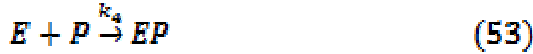
$$\frac{dC_P}{dt} = \frac{k_{cat} (C_{S_0} - C_P) C_{E0}}{K_M (1 + k_{de} C_{E0} t) + C_{E0}} \quad (49)$$

(41) The third kinetic model to be used was derived from Michaelis-Menten approach (40) product (glucose) inhibition. The kinetic model for the enzymatic hydrolysis of cellulose was derived based on the following assumptions;

1. The endo-β-1,4-glucanase, exo-β-1,4-cellobiohydrolase and glycosidase enzymes were assumed

to form a single combined effect on the hydrolysis of insoluble substrate to breakdown substrate to produce reducing sugar

- The cellulose and cellulase system is assume to be homogeneous



$$\frac{dC_P}{dt} = k_3 C_{ES}$$

From equation (50) and (51), we obtain

$$k_1 C_E C_S = k_2 C_{ES}$$

$$\frac{C_E C_S}{C_{ES}} = \frac{k_2}{k_1}$$

$$\text{let } K_s = \frac{k_2}{k_1}$$

$$C_E = \frac{K_s}{C_S} C_{ES} \quad (56)$$

From equation (53) and (54), we obtain

$$k_4 C_E C_P = k_5 C_{EP}$$

$$C_{EP} = \frac{k_4 C_E C_P}{k_5}$$

$$\text{let } K_I = \frac{k_5}{k_4}$$

$$C_{EP} = \frac{C_E C_P}{K_I} \quad (57)$$

Substituting equation (56) into (57), we obtain

$$C_{EP} = \frac{C_P K_s}{K_I C_S} C_{ES} \quad (58)$$

Enzyme balance

$$C_{E0} = C_E + C_{ES} + C_{EP} \quad (59)$$

Substituting equation (56) to (58) into (59), we obtain

$$C_{E0} = C_{ES} + \frac{K_s}{C_S} C_{ES} + \frac{C_P K_s}{K_I C_S} C_{ES}$$

$$C_{E0} = C_{ES} \left( 1 + \frac{K_s}{C_S} + \frac{C_P K_s}{K_I C_S} \right)$$

$$C_{ES} = \frac{C_{E0}}{\left( 1 + \frac{K_s}{C_S} + \frac{C_P K_s}{K_I C_S} \right)} \quad (60)$$

Substituting equation (55) into (60), we obtain

$$\frac{dC_P}{dt} = k_3 \frac{C_{E0}}{\left( 1 + \frac{K_s}{C_S} + \frac{C_P K_s}{K_I C_S} \right)}$$

$$(55) \quad \frac{dC_P}{dt} = k_3 \frac{C_{E0} C_S}{\left( 1 + K_s + \frac{C_P K_s}{K_I} \right)} \quad (61)$$

These are three kinetic models to be used are;

$$\frac{dC_s}{dt} = \frac{[K_{2s}(1-\phi) + K_{2s}\phi \frac{K_s}{K_s}] C_{E0} (1-\gamma) C_s}{K_s \left( 1 + \frac{1}{K_s} + \frac{C_s}{K_p K_s} \right) + [(1-\phi)(1-\phi) + \phi \frac{K_s}{K_s} + \gamma \frac{K_s}{K_s}]}$$

$$\frac{dC_P}{dt} = \frac{k_{cat} (C_{S0} - C_P) C_{E0}}{K_M (1 + k_{ds} C_{E0} t) + C_{E0}}$$

$$\frac{dC_P}{dt} = k_3 \frac{C_{E0} C_S}{\left( 1 + K_s + \frac{C_P K_s}{K_I} \right)}$$

### Parameter estimation

Estimation of the kinetic parameters of the six proposed rate models will be accomplished by least square fitting of the rate equations into the concentration reaction rate curve. The Nelder Mead simplex method (Polyhedron search method) is used to achieve this using MATLAB software package.

### Results and Discussion

Figure 1-6 show that Tikhonov regularization technique has successfully converted the concentration-time data to rate-time data. Looking at the kinetic

parameters obtained for three models, it is only the second model that satisfied this condition. Which is the model proposed by Shen and Agblevor (2008a, b). Therefore, model 2 is the best model describing enzymatic hydrolysis of cellulose.

The conclusion of the study is Tikhonov regularization provides a reliable way of converting the time-concentration data of reaction kinetics into concentration-reaction rate data. The procedure is independent of reaction rate model and is applicable to a wide variety of reactions as noise amplification is kept under control, the resulting concentration-reaction rate curve allows the rate constants in any rate model used to describe the reaction to be determined with relative ease and reliability.

Using the results obtained from Tikhonov regularization technique, model 2 was determined to be the best model describing enzymatic hydrolysis of cellulose

$$\frac{dC_p}{dt} = \frac{k_{cat}(C_{S_0} - C_p)C_{E0}}{K_M(1 + k_{ds}C_{E0}t) + C_{E0}}$$

The following recommendations are given based on the challenges faced during the course of the project

- More reaction mechanisms should be obtained to determine their kinetic parameters for comparison
- Further researches should be carried out on Tikhonov Regularization technique to be able to applied it to other types of reactor rather than batch reactor

Objective function and parameter estimation for model 1

Enzyme Concentration	1.5U	15U	30U
$k_{3a}$	0.174	0.380	0.089
$k_{3c}$	0.051	0.042	0.113
$K_a$	0.005	0.01	0.077
$K_c$	0.061	0.051	0.172
$K_d$	0.157	0.215	0.137
$k_p$	0.281	0.035	0.031
Objective function	3.10586E-4	6.27935E-4	5.7264E-3

Objective function and parameter estimation for model 2

Enzyme Concentration	1.5U	15U	30U
$k_{cat}$	0.958	0.960	0.980
$K_M$	0.459	0.442	0.395
$k_{ds}$	0.384	0.372	0.255
Objective function	9.85942E-3	3.0248E-3	5.12647E-3

Objective function and parameter estimation for model 3

Enzyme Concentration	1.5U	15U	30U
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$k_{cat}$	1.184	0.082	0.038
$K_M$	0.051	-0.018	-0.037
$K_I$	0.06	0.034	0.022
Objective function	2.76554E-4	4.01324E-3	2.047543E-3

**Table.1** Measured conversion and time for enzyme concentration of 1.5U

Time(h)	Conversion
2	0.08
8	0.14
10	0.16
12	0.34
20	0.51
50	0.54
60	0.54
70	0.60
95	0.68

**Table.2** Measured conversion and time for enzyme concentration of 15U

Time(h)	Conversion
2	0.19
4	0.25
6	0.35
8	0.40
12	0.46
20	0.59
29	0.62
50	0.72
60	0.76
70	0.78
82	0.82
90	0.84

**Table.3** Measured conversion and time for enzyme concentration of 30U

Time (h)	Conversion
40	0.88
50	0.94
60	0.94
70	0.96
8	0.46
10	0.52
20	0.78
29	0.82

**Table.4** For enzyme concentration 1.5U

rate	concentration
0.0001	0.0159
0.0126	0.0647
0.0073	0.0810
0.0095	0.0972
0.0161	0.1623
-0.0059	0.4062
0.0047	0.4876
0.0075	0.5689
-0.0152	0.7722

**Table.5** For enzyme concentration 15U

Rate	Concentration
0.0229	0.0454
0.0483	0.0672
0.0257	0.0890
0.0325	0.1108
-0.0000	0.1544
0.0263	0.2416
-0.0023	0.3397
0.0011	0.5686
0.0078	0.6776
0.0118	0.7866
0.0095	0.9174

**Table.6** For enzyme concentration 30U

Rate	Concentration
-0.0127	0.0690
0.0150	0.1523
0.0152	0.3188
-0.0152	0.4020
0.0110	0.8183
-0.0064	1.1929
-0.0239	1.6508
-0.0308	2.0671
-0.0028	2.4833



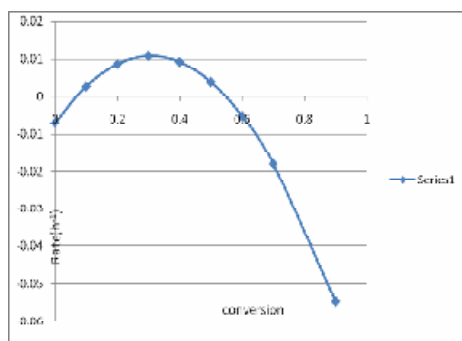
**Table.7** initial reaction rate and conversion

Enzyme concentration	Initial rate	Initial concentration
1.5U	0.0081	0.0642
15U	0.0109	0.1411
30U	0.3201	0.0129

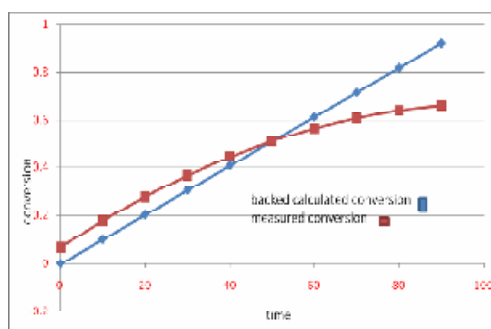
**Figure.1** Graph of

rate-conversion for enzyme concentration of 1.5U

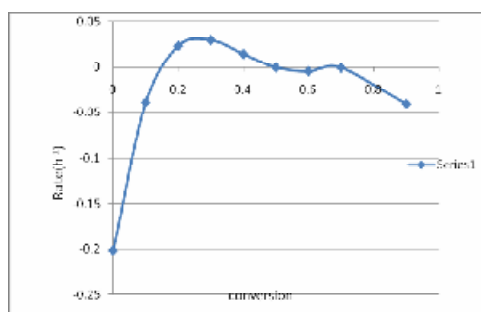
regularized reaction



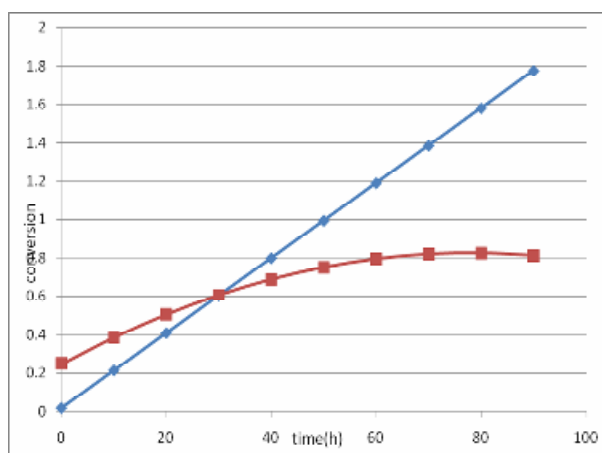
**Figure.2** Graph of back calculated and measured conversion against time enzyme concentration of 1.5U



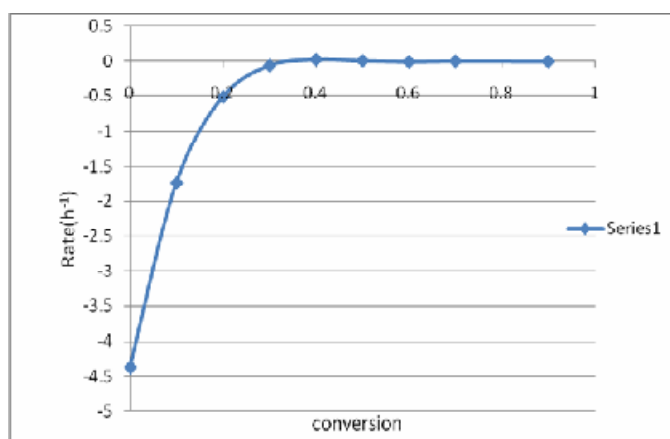
**Figure.3** Graph of regularized reaction rate-conversion for enzyme concentration of 15U



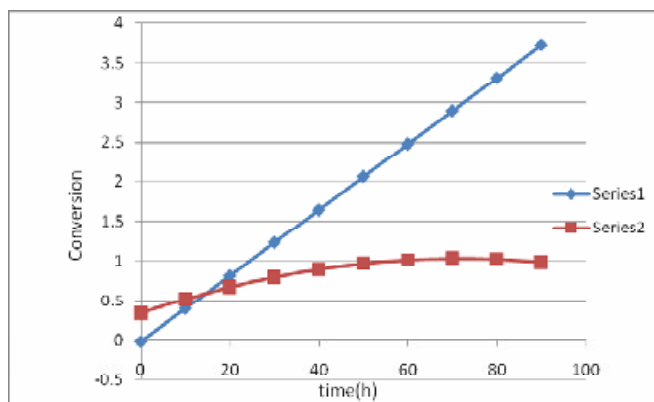
**Figure.4** Graph of back calculated and measured conversion against time enzyme concentration of 15U



**Figure.5** Graph of regularized reaction rate-conversion for enzyme concentration of 30U



**Figure.6** Graph of back calculated and measured conversion against time enzyme concentration of 30U



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