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REVIEW

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第 I 報 : Trichoderma Viride 에서 抽出된 酵素에 依한 셀루로오즈의 分解

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Enzyme Engineering
Part I : Kinetics of Cellulose Hydrolysis by Trichoderma Viride Cellulase

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This paper is intended to give the reader a review on an area of study called "enzyme engineering" by describing several sub-topics and to explain the present status of development. The present part deals with one of the sub-topics.

Introduction

Cellulose is an abundant renewable natural resource. Approximately 0.1% of the solar energy that is incident on earth is fixed by green plants yielding about $15\sim20\times10^{10}$ tons of organic plant substance of which one half is cellulose.[7,8]

The demand on food is steadily increasing due to the fast growth of world population. Since much of the plant is inedible, food shortages are foreseen.

The development of any process that promises its economic utilization offers a possible contribution to the present acute worldwide food and energy shortages. The hydrolysis of cellulose to sugars by enzymes derived from Trichoderma viride appears to have considerable merits.

The objective of this paper is to elucidate the details of the process kinetics of which to date relatively little is known. The formation of an adequate kinetic rate equation should provide information in evaluating the economic feasibility of a large scale plant as well as an optimization of the process.

A kinetic model based on theoretical hypotheses and experimental observations is proposed considering the structural nature of cellulose and the mode of action of cellulase components.

This model describes the enzymic binding on the cellulose surface to form an enzyme-substrate complex, the decomposition of the complex into products, the inhibition of the adsorbed by products and substrate, and the inactivation enzyme. It is conceivable that the adsorption of enzyme plays an important role due to the hetreogeneous nature of the system. Most of the initially uptaken enzyme remains on the cellulose surface and is gradually released to the bulk solution due to the depletion of the cellulose concen-

tration as reaction progresses. Once released, little seems to be readsorbed. This suggests that the enzymic binding cannot be described by any common types of adsorption isotherms and that there is an optimal initial enzyme-cellulose concentration ratio, and thus the effect of bulk phase enzyme concentration is not significant once the initial concentration exceeds the value determined by this ratio. The inhibitory step, which becomes apparent when the products concentration builds up, is depicted as an unusual type rather than two common kinds of inhibition frequently discussed in the literature. With proper agitation of the slurry reaction mixture to maintain good suspension of cellulose particles, various mass transfer resistances appear to be negligible indicating a kinetic controlled system.

The transient solution for the nonlinear interactive system of equations, of which an analytical solution is impossible, was obtained by a digital computer simulation technique. The use of IBM S/360 CSMP and the MIMIC program furnished by the USE Program Library Interchange greatly simplifies the procedures to obtain the numerical solution as well as for parameter determinations.

Experiments have been conducted to obtain the necessary data to test the validity of the model. Experimental observations seem to confirm the postulated individual steps and the agreement between the data and the predicted results is good. An approximate analytical solution with certain limiting conditions is also found and compared with the numerical solution. It is shown that the transient kinetics is favored over the steady state solution, although the steady state approach sometimes is useful in gaining a quick estimate of expected reaction rates.

Cellulose/Cellulase System

(a) Mass transfer limitations

The cellulose/cellulase biochemical system is very complex becaue cellulose is an insoluble polymer which contains a range of substrates varying from amorphous and reactive to crystalline and highly resistant parts and cellulase is a mixture of several enzyme components. It forms a mixture of solid cellulose particles suspended in liquid enzyme solution. The enzyme is catalytic. Unlike many heterogeneous catalytic systems, the enzyme (catalyst) molecules migrate to the cellulose (reactant) and the product released after digestion.

Due to the heterogeneity, it is conceivable that various mass transfer resistances may play significantt roles in overall reaction rate. The bulk phase and film mass transfer rates depend on the size of cellulose particles, the cellulose concentration and the degree of agitation or the Reynolds' number of the mixture being stirred. Experiments have been carried out to study the mass transfer limitations. When the agitation speed exceeded 100 r. p. m. in a batch reactor using pure cellulose (SFBW 200) as a substrate with concentration levels of 2~10 wt. %, it was observed that the mass transfer resistances were negligible. This is an indication that the bulk and film resistances can be made negligible with proper experimental conditions. Thus, with considerable size reduction of cellulose and an adequate mixing so that the cellulos particles are maintained in good suspension in the slurry mixture, it is safe to assume that there is negligible enzyme concentration gradient in the solution. This considerably simplifies the mathematical handling. However, further investigation may be proper since there is energy demand to meet the forementioned experimental conditions. No experiments were conducted regarding the pore diffusion of enzyme; however, it is assumed that the pore diffusion resistance is likewise insignificant due to the macromolecular nature of enzyme molecules.

(b) Cellulase Modes of Action

The adsoption of cellulase in contact with the cellulose particles provides the only mass transfer resistance and it is important to establish a relation which describes the adsorption characteristics. Since the cellulose and the cellulase consist of more than one component, the modes of action of cellulase on different portions of cellulose are of fundamental significance in the elucidation of cellulase adsorption and the understanding of overall kinetic process.

At present there are two distinct theoretical postulations, attributed largely to Reese and Mandels[1] and Wood et al[2] to explain the modes of action (*cf.* *Fig.* 1)

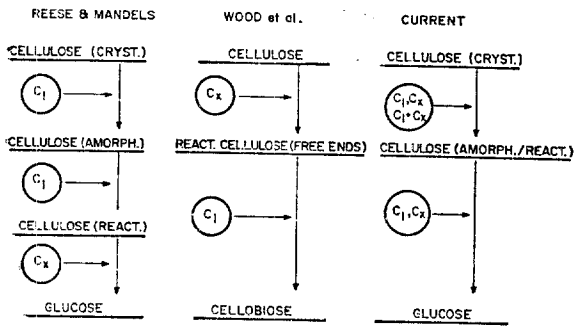

Fig. 1. Cellulase modes of action.

Postulate 1:

C_1 is an enzyme that reduces bonds between cellulase chains by opening up the crystalline structure to convert the crystalline cellulose to amorphous and/or reactive cellulose. C_x(endo-and exo-glucanases) hydrolyzes the more susceptible amorphous and/or reactive cellulose by removing glucose units endwise from the nonreducing ends (exo-) and by primarily random fission of longer chain length (endo-).

Postulate 2:

C_x acts on the crystalline cellulose to generate free ends which are more susceptible to enzymic attack and C_1 is an enzyme which hydrolyzes the reactive ends of the cellulose produced by C_x action.

The postulated theories are based on the specific experimental observations and offer similar qualitative explanations for the separate and distinctive actions of the cellulase components. In either case, it is an essential requirement that both components are needed in order to achieve saccharification of cellulose material to a significant extent. The catalytic actions by these components are synergistic. The rate of degradation of the crystalline cellulose is shown to be very slow in comparison to the hydrolysis rate of the amorphous and/or reactive cellulose. It is thus, difficult to determine a meaningful concentration ratio of C_1 to C_x or vice versa. The postulations are not affirmative and leave room for further intensive investigation.

Current study assumes somewhat different enzymic action. The solution enzyme is considered as a single component enzyme and distinctive catalytic actions appear only when enzyme is adsorbed on different portions of cellulose matrix. The crystalline bond breaking enzyme is postulated as having C_1+C_x complex form and C_1 or C_x separately acts on the hydrolysis step to produce the reducing sugars. This scheme not only maintains basic similarity to above-mentioned theories, but also eliminates difficulties in determining the concentrations of C_1 and C_x separately. The initial conditions for the cellulase can readily be defined in terms of total enzyme concentration in the rate equations.

(c) Cellulase Adsorption

Cellulase is strongly adsorbed by cellulose. The amount of cellulase adsorbed depends on the available sorption site which is a function of cellulose particle size and concentration of cellulose at fixed experimental conditions (50°C, *pH* 4.8). The initial adsorption is fast and the adsorption is continued at a slower rate for a short time period. The rapid initial uptake is due to high cellulose-cellulase concentration ratio. More than 90% of initial adsorption took place by 10% cellulose (SFBW 200) at prescribed experimental conditions in a batch reactor. Before an appreciable production of glucose (initial 3~4 hrs) the adsorption continuously takes place until the cellulase concentration reaches a state of "pseudosaturation". Once the cellulose is pseudosaturated with cellulase, negligible amount of uptake is observed, thus bulk solution cellulase concentration remaining almost at a constant level. The cellulase adsorbed forms cellulose-cellulase complex and digestion starts.

As digestion continues to produce glucose on a conversion level of 40%, the cellulase concentration in the solution increases indicating the release of cellulase from cellulose. Typical digestion curves are shown in *Fig.* 2.

After an hour of reaction, 15% volume of sample was taken and supernate seprated from the centrifuged sample. Added with fresh buffer soution into precipitate and let the reaction continue in a shaker-incubator. As shown in *Fig.* 2. glucose production rate (curve B) is nearly equal as in continued reaction in the original batch reactor (curve A). A slight lagging of curve B compared with curve A is believed

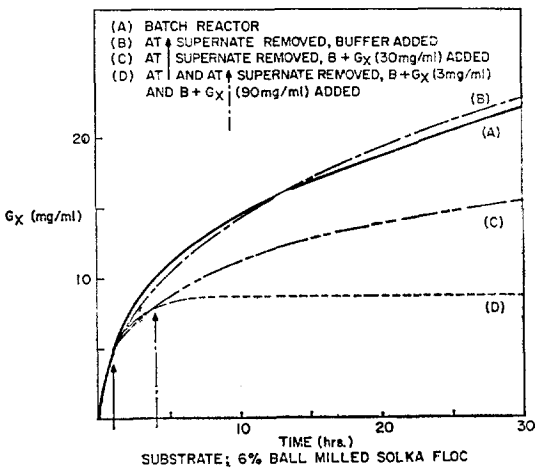

Fig. 2. Cellulose degradation.

to be due to continued adsorption for a short initial time. At about 8 hrs of reaction curve B overtakes curve A indicating a possible effect of product inhibition. These data were reproducible leading to a conclusion that most of the adsorbed enzyme is held by cellulose and is primarily responsible for the cellulose degradation. The enzyme is released when digestion is continued for a prolonged time and the cellulose is depleted.

(d) Product Inhibition

The hydorlysis takes place fast for the initial time period (1~8 hrs) and levels off considerably for a prolonged reaction time (*Cf. Fig.* 2). This is believed to be due to the inhibitory action by product or changes of cellulose susceptibility or both. In modeling standpoins the effect of susceptibility change can be depicted in the reaction step for the degradation of the crystalline cellulose.

The retardness of the hydrolysis rate which becomes apparent when the product concentration builds up is mainly attributable to the inhibition by the reaction product. The most common type of product inhibition, i.e., a reaction between the solution enzyme and the product is not likely to affect the productivity since this system is heterogenous and the enzyme adsorbed on the cellulose matrix for the short initial time period is primarily responsible for the reaction. The most probable inhibitory step is due to side reactions between the enzyme-substrate complexes and the product. These reactions usually are reversible processes whose equilibria maintain the resulting reaction velocities to a certain constant level at specified initial conditions.

These experimental observations along with theoretical considerations lead one to establish the modeling policy (Ill. 1) and a proposed kinetic scheme (*Fig.* 3, Ill. 2, Ill. 3) This kinetic model thus represents one of the most probable reaction path for this system.

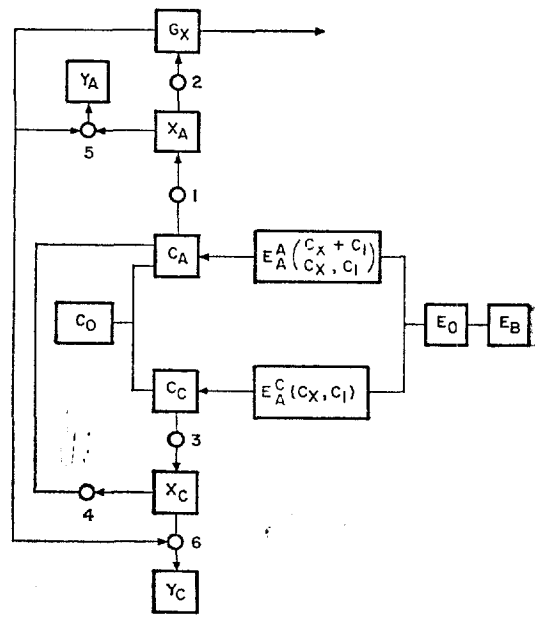

Fig. 3. Reaction pathways.

Initial Conditions

(a) The cellulose concentration

Before an actual simulation is performed, it is required to specify initial conditions to integrate the system differential equations. To assign initial values to each cellulose component and the cellulase is one of the difficulties encountered since there exist numerous factors by which the cellulose structure can be altered. The cellulose is pretreated for the size reduction to fine particles before it enters the digestion

vessel. Experimental observation shows significant differences in the reactivites of cellulose which are pretreated by various mechanical means under different physical conditions[3]

Even though it appears rather crude the current method to determine the composition (the ratio of crystalline to noncrystalline) is by hydrolyzing the specific substrate sample by p. w. *) cellulase culture for an extended long time period. P. w. cellulase contains an enzyme component capable of digesting noncrystalline part of cellulose only. Approximately 12% of the cellulose used in this study (SFBW 200, 200 mesh) can be hydrolyzed by p. w. cellulase in 48 hrs and thereafter almost negligible digestion takes place for prolonged time. This indicates only 12% of total SFBW 200 cellulose is of readily reactive form leaving 88% crystalline, resistant part.

At present there is not any decisive way to estimate the compositions of various forms of cellulose thus forcing the use of forementioned experimental determinations.

It is enhanced, however, that further investigation to be carried out in regard to the development of any deterministic relations between the cellulose compositions and various physical and mechanical factors involved in processes of cellulose pretreatment.

Added significance for the extended study in this regard can be attributed to the fact that there are energy and cost demands in these processes to increase the cellulose accessibility and reactivity.

(b) The Enzyme Concentration (Activity or Strength)

The interesting and important feature of adsorbed enzymes is their mixed function catalytic action. The bulk solution enzyme is homogeneous, while the catalytic action of enzyme adsorbed on the substrate matrix is heterogeneous.

In cellulose/cellulase system the catalytic action of adsorbed enzyme is of particular significance, for the enzyme adsorbed during a short initial period of reaction appears to be primarily responsible in its catalytic action. The requirement of information on the adsorbed enzyme activity is essential in the kinetic study of this system.

Currently utilized information on cellulase activity and concentration provides:

1) Total Protein Content (Pr)
2) Filter Paper Activity (FPA)
3) IUB Unit

Total protein content is a physical entity that can be measured in definite quantity. It is algebraically additive in the amount present in bulk solution and on substrate matrix. Not all the proteins possess the catalytic action of enzyme and it is difficult to make any presumption that;

a) the concentration ration of enzyme-protein(EPr) and nonenzyme-protein(NPr) might be proportional to total protein concentration

b) regardless whether a) is true or not the ratio EPr/NPr in adsorbed state may have a defined correlation with total adsorbed protein. Neither total protein content nor total protein adsorbed thus appears to measure proper enzyme action.

The FPA determined by a standard assay procedure in the laboratory**) is in general acceptable and is being used as the "effective" catalytic action of cellulase.

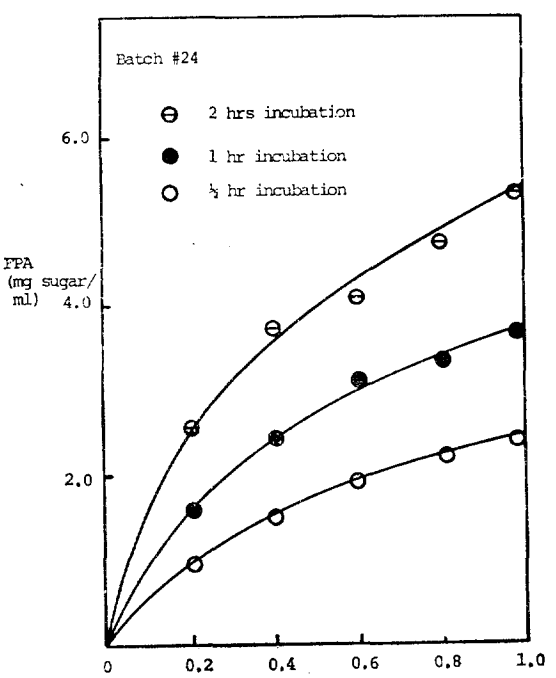

Fig. 4. FPA *VS.* dilution (d)

* Pestalotiopsis westerdijkii
** Food Science Lab.
US Army Natick Labs., Natick, Mass. 01760, U. S. A.

FPA is expressed in the amount of sugar produced from 50mg of standard substrate (Whatman #1 filter paper) in 1/2 ml of enzyme preparation and 1 ml of buffer (Na citrate) mixture after an hour of "reaction" under certain optimal conditions (50°C, *pH* 4.8).

A typical FPA *vs.* dilution (or total protein content) curves are shown in *Fig.* 4.

The enzyme preparation from QM9414 mutant of T. V. fungus was diluted from 1/10 to full strength (therefore protein concentrations were also diluted). The FPA obtained measures the catalytic action of enzyme adsorbed on the filter paper. As shown the activities of enzyme are not linearly proportional with the dilution factors.

A simple hyperbolic relation (Eq. 1) between the FPA and Pr was tested and found to fit the observed FPA *vs.* dilution data within the accuracy of possible experimental error.

In all cases the Lineweaver-Burke type plots[5] (*Fig.* 5*a and* 5*b*) show reasonably good linearities between the variables plotted. The B_1 values were very close (approximately 5.0) in four different enzyme preparations when 50mg of filter paper was used, and they differ but slightly when 100mg and 25mg of filter paper was used. This indicates the dependency of B_1 on the amount of substrate, hence its adsorption capacity. B_2 values, however, varied with the amount of substrate as well as with the different enzyme preparations (different batches) thus showing the dependency on the adsorption capacity and the ratio of NPr/EPr (See *Tables* 1 *and* 2).

Figures 5*a* and 5*b* were prepared from the data obtained from Dr. M. Mandels

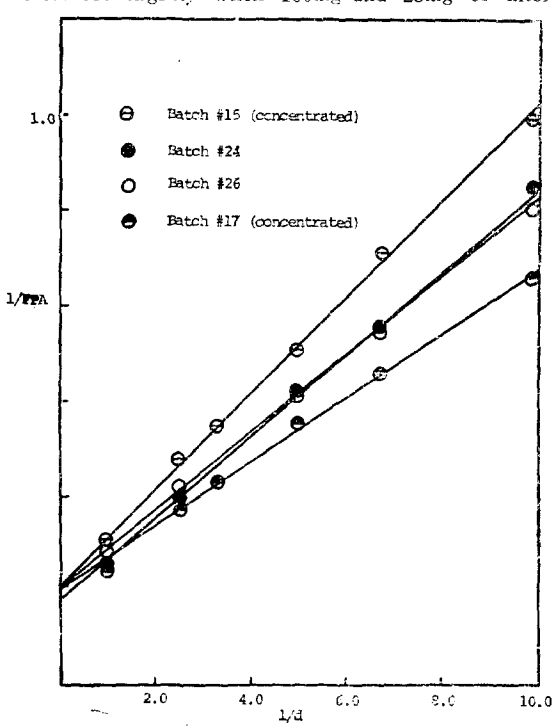

Fig. 5a. Lineweaver-burke type plot for FPA and d.

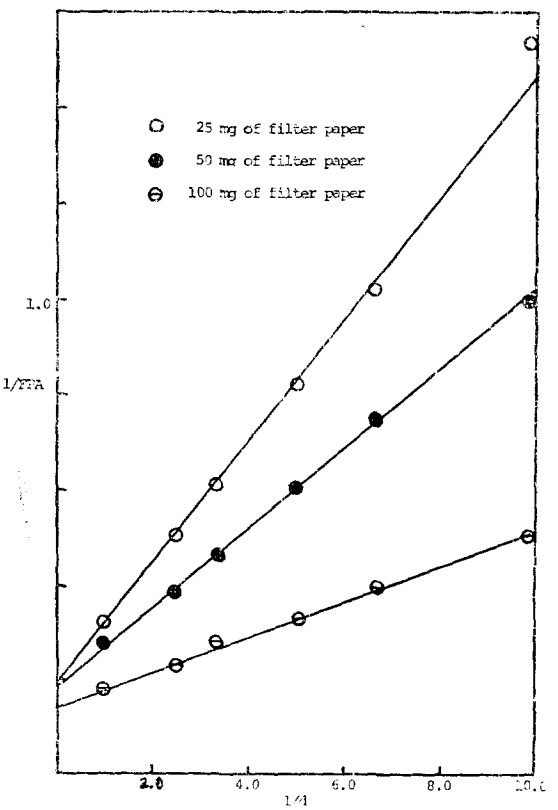

Fig. 5b. Lineweaver-burke type plot for FPA and d.

Table 1. Effect of the amount of filter paper on B_1 and B_2 in Eq. A

Filter Paper (mg)	B_1	B_2
25	5.0	0.89
50	5.3	0.63
100	7.1	0.44

Cellulase Batch #24 (QM 9414, SW 40)
Pr=1.4mg/*ml* at full strength
FPA=3.68 mg sugar/*ml* at full strength

Table 2. B_1 and B_2 for different culture batches of cellulase

Batch #	FPA (mg sugar/ml)**	Pr(mg/ml)	B_1	B_2
24	3.68	1.4	5.3	0.63
26	3.67	1.5	4.9	0.61
15*	3.39	1.5	4.9	0.76
17*(NP)	4.60	2.72	5.0	0.92

*concentrated
**at full strength
QM 9414=second generation of TV fungus mutant QM 9123, SW 40=pure cellulose, NP=news paper used as growth medium
The FPA and Pr data were obtained fromDr. M. Mandels of this lab.

In the hydrolysis system, if the initial adsorption of the total protein is determined, the corresponding FPA can be estimated by the equation

$$\text{FPA}=\frac{B_1 P_r}{B_2+P_r} \quad (1)$$

Using this relation, the FPA or the enzyme concentration can be estimated once the total protein content of the cellulase being used is known.

For the initial cellulase concentration adsorbed, Eq. (1) is modified to give

$$(\text{FPA})_{ad}=\frac{B_1(\alpha Pr)}{B_2+(\alpha Pr)} \quad (2)$$

where $\alpha=\dfrac{\text{total protein adsorbed}}{\text{total protein}}$

Simulation

(a) The CSMP (III. 4) and MIMIC

Continuous system simulation languages are extremely useful tools in modeling continuous systems as well as in finding optimal parameters in the system differential equations.

In developing mathematical models of chemical reaction systems, it is well recognized that the system differential equations are large and nonlinear. One method of attacking the general problem to obtain the solutions of these rate equations as well as to determine parameters, is to program the model equations on an analogue computer and fit the generated curves to the experimental data. This could be achieved by simply changing the settings of potentiometer. The difficulties in using analogue machine are:

(1) it becomes impossible to keep track of the response to one of many parameters as the number of dependent variables and/or parameters increases.

(2) as the ranges of variations of depndent varibles and/or parameters ane widely spread (in complex kinetic systems this often is true), the scaling of variables into reasonable voltage levels becomes intractable.

The use of analogue computer, thus, sometimes makes it difficult to handle complicated problems in spite of easy accessibilty due to its parallel nature.

On the other hand, the serial nature of the digital computer along with its lack of hardware integrator requires a skilled programming to solve these problems. The coding of integration routines to handle large and complicated system equations can also be extremely tedious and time consuming.

The simulation languages used in this investigation are digital programs which blend the best of both analogue and digital computers; the parallel nature of the analogue with the large dynamic range of digital. With these languages the model can simply be written down either in the form of block diagrams or in the differential equations. All the variational equations are written in a structural statement form. The complexities of the integrations are carried out in the translation of the structural statements to Fortran. They are also very flexible to provide various use-oriented input, output control statements as well as to accept Fortran statements and subprograms. The ability to to accept and Fortran statements and user-written Fortran programs allows the user to readily implement the use of these languages in parameter determinations.

(b) The GELG

The Gauss-Newton iterative technique is being used to determine optimal parameters in the system. The use of I.B.M. S.S.P. GELG is of great help in minimizing sequence of the least square steps and in checking convergence criterion.

(c) Parameter Determinations

The kinetic rate equations form a system of differential equations, Eqs. (3) and (4):

$$C_j=f_j(t, C_1, C_2 \ldots C_n;\ P_1, P_2 \ldots P_m)\ \ (j=1, 2, \ldots J) \quad (3)$$

$C_j(o)=C_{jo}$ $(j=1, 2, ...J)$ (4)

Where the C_j's are the dependent variables (concentrations) P_r's are the rate constants and system parameters, and the f_j's represent the desired functional relations of C and t. Equation (4) gives the known initial conditions. The rate equations are, in general, nonlinear in C_j' s but more often linear with respect to related parameters (rate constants). Often they are highly interacting to one another thus, overall nonlinearity increases. When time appears explicitly in f_j's the system is nonautonomous.

The existence and uniqueness of the solutions to Eqs. (3) and (4) are guaranteed if f_j's possess continuously uniformly bounded partial derivatives with respect to C_j's in the region of interest[6]. This condition is usually met with the rate equations that describe any physical systems.

If $C^i{}_j$ and C_{ji} are denoted as the predicted and the experimental values measured at times t_i, respectively, one criterion that can be used for the estimation of the best vaules of Pr's is to minimize the sum of the squares of the weighted deviations.

In the following equations the summation convention for the repeated indices is used.

The expression of the function, Γ^2, to be minimized is

$$\Gamma^2=[Q_{ji}(C_{ji}-C^i{}_j)]^2 \qquad (5)$$

Where Q_{ji} is the weighting factor associated with each deviation. Most frequently used weighting factors are:

(1) $Q_{ji}=1$ equal weighting for each deviation

(2) $Q_{ji}=1/C_{ji}$ relative deviation

(3) $Q_{ji}=\left[\sum_{i=1}^{R}\left(C_{ji}-\left(\frac{1}{R}\right)\sum_{i=1}^{R}C_{ji}\right)\right]^{-1/2}$ weighting to the variance of C_{ji}

The solution $C_j(t;p)$ can be expanded in Taylor series about the initial guesses of the parameter values,

$$C_j(t;p^*)=C_j(t;p^o)+C_{j,\,p_k}(t;t^o)\ \bar{P}_k +C_{j,\,p_k}\ (t;p_o)\bar{P}^2{}_k+ \cdots\cdots (6)$$
$$(k=1, 2, ..., m)$$
$$(j=1, 2, ..., J_o)$$

where J_o=number of the variables C_j for which the experimental data are available. Fq. (6) can be simplified by the following considerations;

(1) through the initial rate study it is possible to obtain close estimates of the constants, k_1, k_1, k_2

(2) since the parameters are expressed relative to k_1, the forward reaction rate constant in the fast reaction step, the values are less than one and the rough estimates within the accuracy of the order of magnitudes can be made.

The Equation (6) can now be truncated to give

$$C_j(t;\bar{p}^*)=C_j(t;\bar{p}^o)+C_{j,\,p_k}(t;\bar{p}^o)\bar{p}_k \qquad (7)$$
$$C^i{}_i(t_i;\bar{p}^*)=C^i{}_j(t_i;\bar{p}^o)+C^i{}_{j,\,p_k}(t_i;\bar{p}^o)\bar{p}_k$$

where P^*=optimal parameter vector

and $\bar{p}_k=p^*{}_k-p^o{}_k$

The equations (5) and (7) are combined to obtain

$$\Delta^2=[Q_{ji}(C_{ji}-C^i{}_j(p^o)-C^i{}_{j,\,p_k}(p^o)\bar{p}_k)]^2 \qquad (8)$$

This is a quadratic functionof p'_ks with only one minimum in N-dimensional space. By setting all the first partial derivatives of Γ^2 with respect to p_k' equal to zero, a system of algebraic equations is obtained

$$\Gamma^2,\ \bar{p}_k=0\ \ (k=1, 2, ..., m) \qquad (9)$$

which, given equal weighting, lead to

$$[C_{ji}-C^i{}_j(\bar{p}^0)]\ \ C^i{}_{j,\,p_l}(\bar{p}^o)=C^i{}_{j,\,p_k}(\bar{p}^o)C^i{}_{j,\,p_l}(\bar{p}^o)\bar{p}_k \qquad (10)$$

The integration was performed by 4 the fourth order Runge-Kutta routine in the dynamic section of the CSMP (5) and the Gauss-Newton method was programmed to solve Eq (10) for $p_k's$ in the terminal segment. Fifteen iterations were required to obtain the parameter values within the deviations of 5 to 7%

Initial Rate, Steady State Kinetics

The concept of steady state in chemical kinetices has been a useful notion itself and was justified as a physical reality by reliable experimental techniques. Whereas the notion has also been purely conceptual without good reasoning, and used for the convenience of mathematical simplification. In complicated kinetic systems, it is virtually impossible to obtain the analytical solution for any species of interest without simplification. Steady state assumption on certain intermediates sometimes greatly simplifies the mathematical procedure by reducing the number of differential equations thus leading one to a closed form of analytical solution. Yet, not to mention of mathemati-

cally nonsensical assumptions or of the incompatibility in initial conditions, the solution obtained by the assumption of steady state has its limited application. The question is then whether the criteria for time after which the steady-state hypothesis is tolerable is satisfactory.

With modern computer technology there is no doubt that more reliable solution can be obtained by using computers without the risk of inaccuracy. Approximate solution under the steady state. assumption can be useful to provide a quick estimate of the system variables and/or parameters.

In *Fig.* 7 a comparison is made between exact and approximate soluitons, the initial rates (INI) and the steady state kinetics (SS).

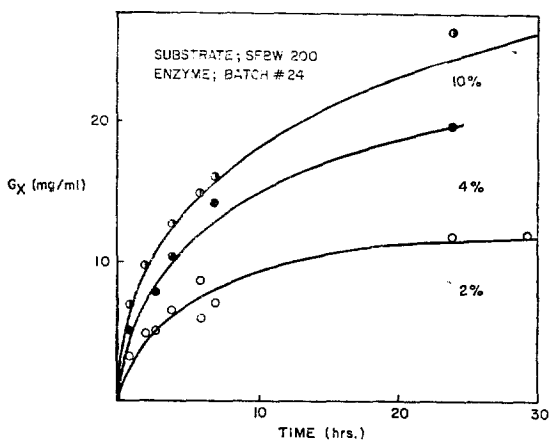

Fig. 6. Glucose production. prediction by model and expermantal results.

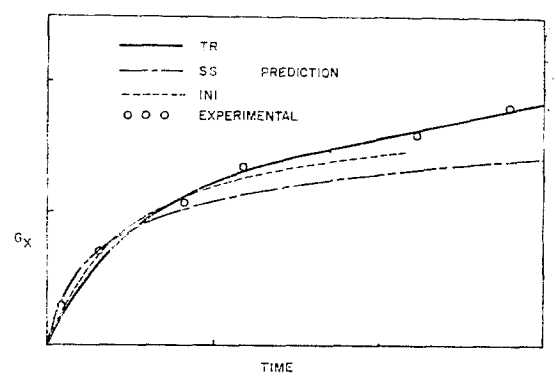

Fig. 7. Comparion of transient kinetics with steady state and initial rate.

A comparison between predictions by the proposed model and the experimental data is shown in *Fig.* 6 with a good agreement. For a chemically reacting system alternative kinetic schemes may sometimes serve to estimate approximate rates. It should be pointed out however, that a slight difference in rate estimation may often cause a considerable production cost change in an optimization sequence for a large scale plant operation.

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Modeling Policy

(1) Bi-Composition of Cellulose
(2) Cellulsse Modes of Action
(3) Negligible Mass Transfer Resistances
(4) Cellulase Adsorption, Complex Formation
(5) Decomposition of the Complex
(6) Product Inhibition
(7) Cellulase Deactivation

Hypotheses, Assumptions

(1) Cellulose Fibre is of Long Cylindrical Form
(2) The Amount Cellulase Adsorbed on Each Portion of Cellulose is Proportional To its concentration
(3) Exponential Decay of the Adsorbed cellulase

I11. 1
Overall Kinetic Scheme

(A) Complex Formation, Decomposition

$$C_A+E^A{}_A \underset{k-1}{\overset{k_1}{\rightleftarrows}} \times A \xrightarrow{k_2} G_X+E^A{}_A$$

$$C_C+E^A{}_A \underset{k-3}{\overset{k_3}{\rightleftarrows}} X_c \xrightarrow{k_4} C_A+E^C{}_A$$

(B) Inhibition

$$X_A+G_X \underset{k-5}{\overset{k_5}{\rightleftarrows}} Y_A$$

$$X_C+G_X \underset{k-9}{\overset{k_6}{\rightleftarrows}} Y_C$$

(C) Enzyme Deactivation

$$E^A{}_A \xrightarrow{k_7} E^d{}_A$$

$$E^C{}_A \xrightarrow{k_7} E^d{}_A$$

I11. 2
Rate Equations

$$\dot{C}_A=-PA+\alpha X_C$$
$$\dot{C}_C=-PC$$
$$G_X=\gamma X_A-QA-QC$$
$$\dot{X}_A=\gamma X_A+PA-QA$$
$$\dot{X}_C=\alpha X_C+PC-QC$$
$$Y_A=QA$$
$$Y_C=QC$$

Where

$$PA=\frac{E_{AO}}{C_O{}^{1/2}}\ \frac{C^2{}_A}{C^{1/2}}\exp(-\xi_r)-X_A/P_{K1}$$

$$PC=\beta\left[\frac{E_{AO}}{C_O{}^{1/2}}\ \frac{C^2{}_c}{C^{1/2}}\exp(-\xi)-X_c/P_{k3}\right]$$

$$QA=\delta(X_A G^m{}_x-Y_A/P_{k5})$$

$$QC=\eta(X_C\, G^m{}_x-Y_c/P_{k5})$$

$$\cdot\equiv\frac{d}{d\tau}\quad \tau=k_1 t\quad t=\text{time}$$

$$\alpha=\frac{k_4}{k_1}\quad \beta=\frac{k_3}{d_1}\quad \gamma=\frac{k_2}{k_1}\quad \delta=\frac{k_5}{k_1}$$

$$\eta=\frac{k_6}{k_1}\quad \xi=\frac{k_7}{k_1}$$

I11-3
Csmp

Initial
Param P^O.....Initial parameter values
Incon C_{jo}.....Initial conditions
Afgen C_{ji}.....Experimentel data
:
Dynamic

$$\dot{C}_j=F_j$$
$$C_j=\int F_j$$

:
Te rminal

$$\nabla^2{}_{,pk}=0$$

Fortran Subprograms (IBM SSP)
T imer
Print C_j, P^0

I11. 4. Continuous system modeling program