Economic Implications of Purification of Glucose Isomerase prior to Immobilization

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We examined the impact on total process cost of purifying enzymes prior to immobilization for use in industrial processes. Glucose isomerase extracted from Bacillus cells was fractionated with acetic and ammonium sulfate yielding preparations of three different specific activities. These were immobilized on porous alkylamine glass beads. Catalyst activity and stability, monitored in a plug-flow reactor, all increased with specific activity of the soluble isomerase immobilized. To examine economic consequences of enzyme purification, we compared reactors producing 10^6 lb of fructose per year. Total process cost fell dramatically with enzyme purity. Our analysis suggests economic gain may accompany purification prior to enzyme immobilization.

Introduction

Glucose isomerase (β-xylene ketol-isomerase, EC 5.3.1.5) is used commercially in free and immobilized form as catalyst in the domestic production of hundreds of millions of pounds annually of high fructose corn syrup (Schneider, 1972). Although crystalline glucose isomerase has been obtained from three sources [α Streptomyces species (Takasaki et al., 1989), Lactobacillus brevis (Yamanaka, 1963; 1968); Bacillus coagulans (Dann, 1970)], its partial purification prior to immobilization for commercial use has not, to our knowledge, been considered. This is unfortunate, since the cost of support and immobilization chemicals significantly affects the economics of commercial processes that use relatively inexpensive enzymes (Swanson et al., 1976). Commercially useful enzymes that have been purified prior to immobilization include glucoamylase (α-1,4-glucan hydrolyase, EC 3.2.1.3) and amylase (EC 3.5.1.1). These were immobilized on alkylamine glass (Westall and Havewals, 1973) and on a controlled pore ceramic (Westall and Detar, 1974), respectively, although the economic impact on overall process cost was not considered.

Our purpose was twofold: (1) to develop procedures for purification of glucose isomerase sufficiently simple and inexpensive for use in industry, and (2) to examine the economic advantages that might accrue from the use of partially purified glucose isomerase to prepare immobilized catalyst.

Materials

Noncellular cells of a Bacillus species treated to facilitate extraction of glucose isomerase were a generous gift of the Novo Enzyne Corporation, Mamaroneck, N.Y. Porous alkylamine glass (ENCOR, 55 mm, 40/80 mesh) was from Cornings, Biomaterials, Plattsburgh from PolyScience, Inc., and boron serum albumin (Type F, essentially fatty acid free) was from Sigma Chemical Co. The glucose Analyzer used was generously loaned by Beckman Instruments Inc., Fullerton, Calif.

Methods

Protein. To determine soluble protein in fractions that included ammonium sulfate precipitates, we used a biuret reagent containing NaH₂O₂ that is insensitive to interference by N₄H₅. Biuret reagent (0.54 M NH₄OH·0.64 M CuSO₄·5.4 M carbonate-free NaOH), 2.0 mL, and 1.0 mL of sample (0.5-5 mg of protein) were mixed and maintained at room temperature for 10 min. The intensity of the violet color complex formed was then measured at 540 nm against a reagent blank. Protein concentration was calculated from a standard curve generated using solutions made from dry bovine serum albumin. Protein is thus reported as mass of dry protein.

Glucose Isomerase Activity. While the reaction of industrial interest is glucose → fructose, analytical considerations led us to assay the reverse reaction: fructose → glucose. These data were recalculated for the reaction of industrial interest before use in reactor modeling. Our choice was dictated by the availability of an analytical instrument that determines glucose in the presence of a large excess of fructose and permitted us to perform multiple assays at 60-70 intervals.

The enzymatic activity of soluble glucose isomerase preparations was calculated by measuring the initial rate of conversion of fructose to glucose. A 1.0-mL portion of appropriately diluted isomerase was mixed with 1.0 mL of 200 mM D-fructose in 5.0 mM MgCl₂, 0.5 mM CoCl₂, 35 mM sodium succinate, pH 6.8 at 25°C, and incubated at 60°C. At intervals from 10 to 30 min, 10-μL portions were assayed for glucose. Under these conditions, less than 2% of the fructose is converted to glucose, and isomerase activity is linear with respect both to incubation time and quantity of isomerase present. One unit (EU) of glucose isomerase activity is that quantity of enzyme that catalyzes the formation of 1 μmol of glucose/min at 80°C. Specific activity is expressed as EU per milligram of protein.

Imobilized glucose isomerase activity was monitored in a plug flow reactor consisting of a 5.7 x 100 mm Pasteur pipet containing a 47 mm high catalyst bed. Thoroughly degassed feedstock (1.0 M D-fructose in 10 mM MgSO₄·10 mM NaCl·0.4 mM Na₂SO₄·50 mM sodium maleate, pH 7.0 at 25°C) was passed through a 1-nm coil of polyethylene tubing immersed in a 60°C water bath and then to the reactor, which was also immersed in the water bath. Maleate buffer is non-inhibitory, does not complex with Co⁴⁺, and does not precipitate with Mg²⁺ (Lloyd et al., 1972). Flow, metered by a peristaltic pump, was at a superficial velocity of 2-4 cm/min, the velocity required to eliminate mass transfer resistance in the fluid around the catalyst. The reaction-diffusion modulus based on observed reaction rate (Satterfield, 1970) was a
decade or more below the critical value for all runs, indicating that diffusion in the pores was not a problem in any of the work. The steady state concentration of glucose in the effluent stream was determined and used to calculate reactor productivity, which is expressed as EU per milliliter of reactor volume. This may be converted to the basis of dry carrier by the apparent density of porous glass, 0.46 g/mL.

**Purification of Glucose Isomerase.** To facilitate application of purification techniques to industry, we considered and used only those procedures which are rapid, technically undemanding, and inexpensive. To avoid costly cooling equipment, all manipulations were conducted at or near ambient temperature (30°C). In the procedures described below, centrifugation was carried out at 12,000 g for 10 min. For industrial use, the centrifuge might be replaced by filters.

**Extraction.** Enzyme was extracted from Bacillus cells by shaking them with 10 volumes of water. Maximal extraction of isomerase activity occurred after 5 h. The slurry was centrifuged and the supernatant liquid was decanted and saved. The damp cells were resuspended in water, centrifuged again, and the two supernatant liquids were combined to produce the Crude Extract.

**Acetone Fractionation.** Room temperature acetone, 40 mL, was added dropwise, with stirring, to 50 mL of Crude Extract. The white precipitate was collected by centrifugation and dissolved in phosphate/iron solution of composition 5 mM MgCl₂-0.5 mM CoCl₂-100 mM sodium phosphate, pH 7.0 at 25°C. The resulting cloudy solution was clarified by centrifugation, the precipitate was discarded, and the supernatant liquid was retained as the Acetone Fraction. The quantity of acetone required was 49 mg per EU of activity recovered.

**Ammonium Sulfate–Acetone Fractionation.** Ammonium sulfate solution (saturated at 30°C), 75 mL, was added dropwise, with stirring, to 50 mL of Crude Extract. After 1 h, the precipitate was collected by centrifugation and was discarded. To the supernatant liquid, 65 mL of room temperature acetone was added dropwise, with stirring. The resulting precipitate formed a thin layer between the immiscible ammonium sulfate and acetone phases upon centrifugation. This layer was collected, dissolved in 16 mL of phosphate/iron solution (described above), clarified by centrifugation, and retained as the Salt–Acetone Fraction. The quantities of agents used were 74 mg of ammonium sulfate and 85 mg of acetone per EU of activity recovered. In an industrial process, saturated ammonium sulfate solution could be replaced by solid material.

Properties of these three enzyme preparations are summarized in Table I.

**Immiscibility of Glucose Isomerase.** The three fractions of glucose isomerase obtained as above were immiscible on porous alkydane glass as described by Weetall and Vann (1976). All isomerase preparations were prepared, immobilized, and evaluated simultaneously. The Crude Extract, Acetone, and Salt–Acetone fractions were diluted with phosphate/iron solution prior to immobilization to give enzyme preparations having a protein concentration of 10.5 mg of protein/mL and activities of 10.2, 21.5, and 35.8 EU/mL, respectively.

Alkaline glass, 800 mg, was added to 12.8 mL of 2.5% (v/v) glutaraldehyde in 100 mM sodium phosphate, pH 7.0, at room temperature and placed under vacuum for 1 h. The glass was then washed with water, added to 10 mL of glucose isomerase (10.5 mg of protein/mL) in phosphate/iron solution and maintained at 4°C for 8 h. A portion, 1.2 mL, of the glass was placed in a tubular reactor and washed with 50 mL of feed. The reactor was then connected to the preheater coil and the entire apparatus was immersed in a 60°C water bath. The catalyst, monitored for activity as described above, initially exhibited about 60% of the added activity for all three immobilized preparations, a yield we have encountered with many supports and with other enzymes that are not denatured during immobilization.

**Experimental Results and Discussion**

**Enzyme Stability.** As anticipated, the initial activity of the immobilized isomerase increased with the specific activity of the soluble isomerase used for immobilization (Table I). An unexpected benefit was the increased stability we observed when isomerase preparations of higher specific activity were immobilized. This result appears to reflect the enhanced stability of the higher specific activity preparations of soluble glucose isomerase (Table I, Figure 1).

**Economic Implications.** The preliminary economic analysis of the effect of enzyme purity on cost presented below has the basic (1) The reactor is a single module producing one million pounds of fructose per operating year (330 days) in the form of syrup containing 42% fructose and 50% glucose (dry basis), a specification common in industry. (2) The feed is 50% aqueous glucose (specific gravity 1.23) containing no fructose. (3) Flow rate is reduced as catalyst ages to maintain constant product composition at all times. A more complex approach is to maintain approximately constant flow rate by employing several parallel modules with staggered regeneration times (Pitcher and Weetall, 1976). The difference in costs between the two models is small, and hence, the simpler one is used. (4) Any decrease of the capacity of the carrier for immobilized enzyme with time is compensated for by adding a small quantity of fresh carrier after each regeneration step. While selection of an optimum number of cycles after which carrier is added permits a more refined estimate of the design parameters, the effect on total cost is negligible (Swanson et al., 1976).

Cost factors considered include enzyme, purification and immobilization reagents, carrier, and the cost of the tubular reactor. Other cost factors such as overhead and labor are not included. Labor can be important in purifications which ap-

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Table I. Properties of Glucose Isoamylase Preparations before and after Immobilization. (The Data Are for Isoamylase Preparations Prepared, Immobilized, and Analyzed as Described in the Test.)

<table>
<thead>
<tr>
<th>Soluble</th>
<th>Immobilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific activity, EU/mg of protein</td>
<td>t1/2 at 60°C, h</td>
</tr>
<tr>
<td>Crude extract</td>
<td>0.94 (1.0)</td>
</tr>
<tr>
<td>Acetone fraction</td>
<td>2.1</td>
</tr>
<tr>
<td>Salt–Acetone fraction</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Note: Measured in 250 mM D-fructose at pH 6.8 (see "Methods").
proach purity, as pointed out by Colton and Whitesides (1974), but it is a secondary factor in the purification considered here, consisting as it does of no more than two precipitations.

The total cost of production, $C$ (dollars $\times$ year$^{-1}$), is given by

$$C = \left( C_1 \frac{\delta}{\theta} + C_2 \right) V \quad (1)$$

where $\delta$ = cycle time (h), $V$ = reactor volume (L), $C_1$ = cost parameter independent of cycle time (dollars $\times$ h $\times$ year$^{-1}$), and $C_2$ = cost parameter independent of cycle time (dollars $\times$ L$^{-1}$ $\times$ year$^{-1}$). $C_1$ and $C_2$ are defined by

$$C_1 = |C_{EB}V_0 + C_{IT} + C_{EB} (1 - L)|/h \quad (2)$$

$$C_2 = 2C_{PE}D_p \quad (3)$$

where $a$ = a conversion factor of $10^{-11}$ (m$^2$ $\times$ L$^{-1}$), $A$ = empty cross-sectional area of the reactor (m$^2$), $C_{P}$ = cost of the reactor shell (dollars $\times$ m$^{-2}$), $C_{C}$ = cost of immobilization reagents (dollars $\times$ m$^{-2}$), $C_{C}$ = cost of carrier (dollars $\times$ L$^{-1}$), $D_p$ = fractional rate of depreciation (year$^{-1}$), $h$ = total annual time on stream (h $\times$ year$^{-1}$), $I$ = immobilization reagent used for each regeneration ($g$ $\times$ L$^{-1}$ of reactor volume), $L$ = fraction of carrier capacity remaining after each regeneration (dimensionless), $V_0$ = initial immobilized enzyme activity (EU $\times$ L$^{-1}$ of reactor), and where the cost of immobilized enzyme, $C_{E}$ (dollars $\times$ EU$^{-1}$), is given by

$$C_E = C_{EB}Y_e \quad (4)$$

where $C_{EB}$ = cost of purification reagents (dollars $\times$ EU$^{-1}$ of purified, soluble enzyme), $Y_e$ = fraction of added activity recovered in immobilized form (dimensionless), and $Y_p$ = fraction of activity recovered after purification (dimensionless). Values assumed are shown in Table II.

The reaction velocity, $v$ (g $\times$ min$^{-1}$ $\times$ L$^{-1}$), for the conversion of glucose to fructose may be written as (Lee et al., 1976)

$$v = V'G^* / (K^* + G^*) \quad (5)$$

where $V'$ = a velocity term (g $\times$ min$^{-1}$ $\times$ L$^{-1}$), $G$ = concentration of glucose ($g$ $\times$ L$^{-1}$), and $K'$ = an apparent rate constant ($g$ $\times$ L$^{-1}$). $V'$, $G^*$, and $K'$ are given by eq 6-8.

$$V' = \frac{K_p + 1}{K_p - K} \frac{K_p}{K_p - K} \frac{V_{max}}{G^*} = G^* = G - G^* \quad (6)$$

$$K' = [(K_p + G^*)G + K_pK] \quad (7)$$

$$K_p = K^* \quad (8)$$

and where the equilibrium constant, $K_p$, (dimensionless), is given by

$$K_p = \frac{E_b}{G^*} \quad (9)$$

where $F$, $G$ = concentrations of fructose and glucose, respectively ($g$ $\times$ L$^{-1}$), $V_{max}$ = limiting reaction velocity when $G$ is very high and $F = 0$ ($g$ $\times$ min$^{-1}$ $\times$ L$^{-1}$), and $K_p$, $K_P$ = Michaelis constants for fructose and glucose, respectively ($g$ $\times$ L$^{-1}$). The subscript $p$ and $s$ denote initial and equilibrium conditions, respectively. Diffusion has been assumed to be of no importance in writing these equations.

The reaction equation for a plug-flow reactor is

$$-\frac{dG}{dt} = v \frac{dV}{q} \quad (10)$$

where $q$ = flow rate (L $\times$ min$^{-1}$) and $V$ = packed bed volume (L).

Substituting in eq 5 for $v$ and integrating gives

$$\frac{V'}{q} = G^* - G^* \ln (1 - x) \quad (11)$$

where $x$, the fractional conversion, with glucose as substrate, is given by eq 12

$$x = \frac{G_1 - G}{G_1 - G_s} = \frac{G^* - G^*}{G^*} \quad (12)$$

For fructose as a substrate, $G_1$, and $G_s$ would be replaced by $F$, $F_1$, and $F_s$, respectively.

We assume in the integration that $V'$ is constant, i.e., that enzymatic activity is constant during the residence time or time it takes a units' quantity of fluid to pass through the reactor.
Table III. Total Production Cost for Immobilization of Glucose Isomerase of Differing Specific Activities. Calculations Assume a Carrier Cost of $20/L.

<table>
<thead>
<tr>
<th>Isomerase preparation</th>
<th>Cost of isomerase, a $/10^6 EU</th>
<th>Total production cost, b $/lb fructose</th>
<th>Fractional cost, c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract (SA = 0.94)</td>
<td>5</td>
<td>0.58</td>
<td>0.03</td>
</tr>
<tr>
<td>Acetone fraction (SA = 2.1)</td>
<td>78.4</td>
<td>0.46</td>
<td>0.47</td>
</tr>
<tr>
<td>Salt-Acetone Fraction (SA = 3.8)</td>
<td>173</td>
<td>0.19</td>
<td>0.79</td>
</tr>
</tbody>
</table>

a Cost of isomerase includes cost of soluble crude enzyme, purification reagents, and enzyme losses incurred in the purification procedure. 
b Based on the sum of enzyme, immobilization reagents, carrier and reactor shell costs. 
c SA = Specific activity (EU X mg^-1 of protein).

Though this is only an approximation, especially for less stable enzymes, a more rigorous approach would show the relative economic advantage to be even greater than that to be indicated by the following analysis.

From the data of Sproull (1974), who reported Kf = 1.14, we can calculate the minimum V/V=V0 for the level of glucose conversion considered here. We have, accordingly, ignored the term G1t* in what follows. This gives the working equation

\[ V = K' \ln (1 - x) \frac{Q}{V} \]  

(13)

Equation 13 is the same as that obtained using a different derivation where it is assumed that Kf = K0, which is approximately true (Sproull, 1974). To apply eq 13 to the experimental data at hand, for the reverse reaction fructose -> glucose, these data are substituted into eq 13 to obtain a combined constant K'/V' which is then corrected for the difference in maximum velocity between forward and backward reactions by multiplying the computed value of K'/V' by the factor 1.72 (Sirootti et al., 1975).

The volume of a reactor required to produce a given quantity of fructose depends on the initial activity and stability of the immobilized enzyme. The rate of deactivation of immobilized enzyme is taken to be first order with time, as it is for many immobilized enzyme preparations and was taken for the preparations considered here, as shown in Figure 1. The equation is

\[ V = V'_r \exp \left( \frac{-x}{t} \right) \]  

(14)

where \( V'_r \) is a constant representing immobilized enzyme activity at time zero (g X min^-1 X L^-1), \( t \) is time on stream (h), and \( x \) is a stability time constant (h) calculated from eq 14.

In our reactor we constrain conversion as constant. Hence, neglecting any diffusional effects, the flow rate, \( q \), must decrease in proportion to enzymic activity as given in eq 15

\[ q = q'_1 \exp \left( \frac{-x}{t} \right) \]  

(15)

where \( q'_1 \) = flow rate at time zero (L X min^-1).

The total flow, \( Q_t(L) \), over the time of one cycle, \( t \), is

\[ Q_t = \int_0^t q \, dt \]  

(16)

where \( q \) is defined by eq 15. Integration of (16) and multiplication by h/\( b \) (the number of cycles per year), results in an expression for total annual throughput, \( Q_{tot}(L) \).

\[ Q_{tot} = q_1 \, h \left( 1 - \exp \left( \frac{-x}{t} \right) \right) \frac{1}{\theta/r} \]  

(17)

Combination of eq 17 and 13 gives

\[ V = \frac{K'}{V'_r} \ln \left( 1 - x \right) \frac{Q_{tot}}{h} \frac{1}{1 - \exp \left( \frac{-x}{t} \right)} \]  

(18)

Combining eq 18 with eq 1, differentiating the result with respect to \( \theta \) and setting the derivative equal to zero gives an expression from which optimum cycle time, \( \theta_{opt} \), may be determined

\[ \frac{C_1}{C_2} \frac{1}{\theta_{opt}} \exp \left( \frac{\theta_{opt}}{t} \right) - \frac{\theta_{opt}}{t} - 1 = b \]  

(19)

Substitution of eq 19 into (1) and incorporation of a factor \( h \) gives

\[ C = h \, K' \frac{V}{V'_r} \ln \left( 1 - x \right) \frac{Q_{tot}}{h} \frac{C_2}{C_3} \exp \left( \frac{\theta_{opt}}{t} \right) \]  

(20)

where \( b \) is a conversion factor of 0.0167 (h X min^-1). Equation 20 gives the minimum yearly cost corresponding to optimum cycle time.

Discussion and Conclusions

Table III summarizes the production costs, exclusive of feed costs, for three preparations of immobilized glucose isomerase of differing specific activity computed from eq 20 using data given in Tables I and II and the text of this paper. The economic optimum is affected by the cost of purification, the improvement in the stability and loading of enzyme on the carrier with increased specific activity, and the cost of carrier and immobilization reagents. The fractional contribution of enzyme to total costs rises dramatically (from 0.03 to about 0.08) with increasing specific activity of the soluble isomerase preparations immobilized. This increase is, however, more than counteracted by the accompanying decline in the fractional contribution of carrier cost (from 0.28 to 0.06) and in the fractional contribution of immobilization reagents (0.69 to 0.15). Reactor shell cost, in all cases, makes an insignificant contribution. Although carrier and immobilization costs have a significant effect on production cost for immobilized enzyme prepared from crude extract, these costs are relatively small at the highest specific activity. Production cost becomes less sensitive to carrier cost with increasing specific activity (Figure 21). Despite the increase in the contribution made by the purification reagents to production cost with increasing specific activity of isomerase, overall production cost decreases (Table 312).
Simulation of High-Temperature Water–Gas Shift Reactors

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A rate equation for water–gas shift reaction over a high-temperature shift catalyst (Fe$_2$O$_3$–Cr$_2$O$_3$) has been developed. This rate equation accounts for the effects of temperature, pressure, age of the catalyst, and H$_2$S content of the reacting gases on the catalyst behavior. The effect of diffusion of reactants and products inside the catalyst pores has also been considered. Subsequently, this rate equation has been used in a mathematical model developed for design and simulation calculation of high-temperature water–gas shift reactors. Agreement between experimental data and the calculated results is generally very good.

This paper describes the development of a rate equation for the water–gas shift reaction and a mathematical model based on it for design and simulation calculations of water–gas shift reactors using a chromia-promoted iron oxide catalyst (72% Fe$_2$O$_3$–8% Cr$_2$O$_3$). The reaction describing the process is

\[ \text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{H}_2 \]  

(1)

Some workers propose a first-order (Atwood et al., 1906; Laupichler, 1938; Mars, 1961) or a second-order (Moe, 1962; Padovani and Lotteri, 1977) rate equation to explain the conversion due to this reaction, but many others (Atros-
chenshot, 1958, 1962; Barkley et al., 1952; Bohbro, 1964; Bor-
tolmii, 1958; Goodridge and Quazi, 1967; Hulbert and Sini-
vason, 1961; Kirzilloff, 1959; Kodama et al., 1952, 1955; Kul-
kova and Temkin, 1949; Pivovat, 1967; Puri et al., 1973) propose
different rate equations. Each of the latter rate equations is
equivalently derived from those proposed by others.

It has experimentally been observed that catalysts of different
manufacturers give different rate equations even under
identical conditions (Goodridge and Quazi, 1967). The specific
surface area of the catalyst is reduced considerably during use
due to sintering (Hoogschagen and Zwietering, 1953) and the
surface composition of the catalyst changes depending on the
composition of the reacting gases which are a mixture of oxi-
dizing and reducing gases. All these may help in qualitatively
explaining the difference in rate equations obtained by dif-
ferent workers, but it is unable to help us in getting a general
rate equation which is not available in the literature. So in this
work our approach is to start with a rate equation which
explains the conversion under some constants. Each of these
constraints is subsequently eliminated, one at a time, by in-
roducing suitable correction terms. These are mostly em-
pirical correlations obtained from experimental results of
different workers on the same catalyst.

Development of the Rate Equation

The rate equation most frequently referred to in the litera-
ture is first order or pseudo first order in carbon monoxide
concentration or partial pressure (Atwood et al., 1950; Lau-
pichler, 1958, Mars, 1961) and all attempts to correlate the rate
data by power function models give reaction rates which are
almost consistently first order with respect to partial pressures
of carbon monoxide (Attschensker et al., 1962; Bohlino, 1964;
Goodridge and Quazi, 1967). So a first order rate equation of
the form given by eq 2 has been selected.

\[ r = k(P_{CO})^{n} \]  

where \( r \) = rate of reaction, \( k \) = rate constant, \( P_{CO} \) = partial
pressure of CO, and \( P_{CO} \) = partial pressure of CO in equi-
librium conditions. It is noted that this rate equation has been
used under low pressures (of the order of 1 atm), high steam
to carbon monoxide ratio and below a temperature of 500°C.
In commercial reactors the latter two conditions are met but
the operation pressure is generally high (15 to 30 atm). So we
use the above rate equation at a constant pressure of 1 atm, and
account for the variation in pressure separately. At 1 atm
pressure, eq 2 can be written as

\[ r = k \times P_{CO} \]  

where \( k \) = rate constant, \( P_{CO} \) = mole fraction of CO, \( x_{CO} \) = mole
fraction of CO in equilibrium conditions = \( \frac{x_{CO}}{x_{CO}} \times \frac{x_{H2} \times \frac{x_{H2}}{x_{CO}} \times K_{eq}} \), and \( K_{eq} \) = equilibrium con-
stant = exp(9998.227 - 10.515 \times 4.746 \times 10^{-7} - 0.453 \times 10^{-7} - 0.201 \times \ln T/R); T in K.

The rate constant, \( k \), is generally expressed in the
form of the Arrhenius equation as

\[ k = ae^{-E/RgT} \]  

where \( a \) is a preexponential factor, \( E \) is the activation energy,
\( Rg \) is the universal gas constant, and \( T \) is absolute temperature.

The value of activation energy, \( E \), to be used in this
equation is reported from 13000 to 32000 cal/g-mol (Chandra et
al., 1972; Goodridge and Quazi, 1967; Nakamish and Tomaru,
1953), but mostly it lies in the range 21.4 to 27.3 kcal/g-mol
(Ruthevinn, 1920). For the present catalyst (of P and D, FCI)
we calculate the value of \( E \) as follows.

A pilot plant study using pellets of this catalyst and feed gas
containing high CO content (40.8%, dry basis) is reported in

the literature (Chandra et al., 1973). In this, the calculated
value of energy of activation for the catalyst without taking into
account any mass or heat transfer resistance between the
bulk gas and catalyst pores; i.e., the apparent energy of acti-
vation for the catalyst pellets, is

\[ E' = 13.88 \text{ kcal/g-mol} \]  

For commercial shift reactor operating conditions, heat
transfer and the external mass transfer resistances may be
considered insignificant, but the intra-pellet mass transfer
resistance is very high (Moe, 1962). In such a case, the true
energy of activation, \( E \), can be taken as twice the apparent
energy of activation, \( E' \) (Smith). Therefore

\[ E = 2E' = 27.76 \text{ kcal/g-mol} \]  

In the above evaluation any temperature difference between
the catalyst pellets and the bulk gas or between different
points in a catalyst pellet has been taken to be insignificant.
The close agreement in values of \( E \) thus obtained and those
reported by different workers (Ruthevinn, 1920) who used small
(<1 mm size) catalyst particles, lower pressures, and lower CO
concentrations in their studies, suggests that it is so. However,
if some temperature difference exists, its effect is absorbed
in the rate equation and hence need not be considered sepa-
rate.

The preexponential factor, \( a \), is not a constant. It depends
on temperature because the specific surface area varies with
 temperature (Hoogschagen and Zwietering, 1953). In this
work, it has been evaluated at a temperature of 350°C by

\[ a = 2.32 \times 10^{13} \text{ cm}^{2}/\text{h g of catalyst} \]  

It has been used throughout this work.

Error incurred in the calculation of a catalyst pellet activity
due to taking a constant value of \( a \) is corrected for temperature
as discussed in the section dealing with catalyst age. From eqs
3, 4, 6, and 7 we have the intrinsic rate of reaction for the
catalyst

\[ r = 2.32 \times 10^{13} (x_{CO} - x_{CO}^{*}) \times \exp(-27760.0/RgT) \]  

The rate of reaction for the pellet is obtained by incorpo-
rating the effects of diffusional resistances, age of the catalyst,
pressure, and \( H_{2}S \) concentration in the reacting gases. These
factors are discussed in the above order in the following
paragraphs.

(a) Diffusional Resistances. As mentioned earlier, only
intra-pellet diffusional resistance is to be considered. This can
be done by using a suitable effectiveness factor. For calculating
effectiveness factor, catalyst pellets have been considered to
be isothermal (Carberry, 1961) and Wheeler's method (1961)
has been used. This gives the effectiveness factor

\[ Ef = \frac{h}{n} \]  

where

\[ h = L \left( \sqrt{2} \frac{D_{p}}{D_{s}} \right) \]  

\[ L = \frac{\sqrt{2} V_{p}}{S_{e}} \]  

\[ V_{p} = \text{volume of the catalyst/pellet}, S_{e} = \text{external surface}
\]  

\[ D_{s} = \frac{1}{D_{p} + 1/\rho_{p}} \]