Kinetics of Fermentation by Enzymes: A Mathematical Approach

Semiu Adebayo Kareem, Haruna Mavakumba Kefas[+], Ternenge Joseph Chior[++] and Ganiyu Kayode Latinwo[+++]

Department of Chemical Engineering, Federal University of Technology Yola, Adamawa State, Nigeria
E-mail: <sakareem_1@futy.edu.ng>

Abstract

To explain the kinetics of enzyme-substrate reactions, Michaelis and Menten (1913) came up with a mechanism, which uses an equilibrium assumption. Briggs and Haldane (1925), on the other hand, employed a steady-state assumption in place of the equilibrium assumption and came up with their own mechanism. In this work, the method outlined by Boudart (1968) for surface reaction was applied to mechanism of enzyme-substrate reactions. The Cramer's rule was applied to solve the sets of algebraic equations obtained from the method. The results obtained are similar to those of Michaelis and Menten as well as those of Briggs and Haldane. This work shows the power of applied Mathematics to explain natural phenomena and attestation to the fact that enzyme-catayzed reactions are another form of surface reaction.

Keywords: *fermentation, rate-determining step, steady state approximation and quasi-equilibrium.*

Introduction

Fermentations are reactions wherein a raw organic feed is converted into product by the action of microbes or by the action of enzymes. Enzyme fermentations can be represented by enzyme E:

Organic feed, A $\xrightarrow{Enzyme}$ product chemical, R;

Microbial fermentations can be represented by microbe C;

Organic feed, A $\xrightarrow{Microbe}$ product, R + more cells, C.

The key distinction between these two types of fermentation is that in enzyme fermentation the catalytic agent, the enzyme, does not reproduce itself, but acts as an ordinary chemical, while in microbial fermentation the catalytic agent, the cell or microbe reproduces itself. Within the cells it is the enzyme which catalyses the reaction, just as in enzyme fermentation; however, in reproducing itself the cell manufactures its own enzyme (Levenspiel 1999).

Kinetic equations, which describe the activity of an enzyme or a microorganism on a particular substrate, are crucial in understanding many phenomena in biotechnological processes. Quantitative experimental data is required for the design and optimization of biological transformation processes. A variety of mathematical models have been proposed to describe the dynamics of metabolism of compounds exposed to pure cultures of microorganisms or microbial populations of natural environment (Minton 2001). Characterization of the enzyme or microbe-substrate interactions involves estimation of several parameters in the kinetic models from experimental data. In order to

+ Department of Chemical Engineering, Federal University of Technology, Yola, Adamawa State, Nigeria.
E-mail: <hmkefas@yahoo.com>.
++ Department of Chemical Engineering, Federal University of Technology, Yola, Adamawa State, Nigeria.
E-mail: ternenge123@yahoo.com
+++ Department of Chemical Engineering, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria.
E-mail: <kayodelatinwo@yahoo.com>.

describe the true behavior of the system, it is important to obtain accurate estimates of the kinetic parameters in these models (Olsen 2006).

Both derivative and integrated forms of equations derived for enzyme catalyzed reactions have been used to estimate kinetic parameters of microbiological processes. Estimates of kinetic parameters K and K_M have been calculated by fitting data to either integrated (Goudar and Delvin 2001) or derivative (Acuña-Argüelles *et al*. 2003) forms of Michaelis-Menten and Monod equations. Different approaches have been proposed for estimating the kinetic parameters, but progress curve analysis is the most popular because substrate depletion or product formation data from a single experiment are enough for parameter estimation (Duggleby and Wood 1989).

The basic hypothesis of biodegradation kinetics is that substrates are consumed via catalyzed reactions carried out only by the organisms with the requisite enzymes. Therefore, rates of substrate degradation are generally proportional to the catalyst concentration (concentration of organisms able to degrade the substrate) and dependent on substrate concentration characteristic of saturation kinetics (e.g. Michaelis-Menten and Monod kinetics). Saturation kinetics suggests that at low substrate concentrations (relative to the half-saturation constant), rates are approximately proportional to substrate concentration (first order in substrate concentration), while at high substrate concentrations, rates are independent of substrate concentration (zero order in substrate concentration). In the case of substrates that contribute to the growth of the organisms, rates of substrate degradation are linked to rates of growth (i.e. the concentration of the biomass increases with substrate depletion). The mathematical analysis of such growth-linked systems is more complex than those situations where growth can be ignored (Schnell and Maini 2000). There are a number of situations where it may not be possible to quantify the concentration of substrate-degrading organisms in a heterogeneous microbial community. However, the rate of substrate depletion can be measured. There are also situations in which the organism concentration remains essentially constant even as the substrate is degraded (i.e. no growth situation). Given these various features of biodegradation kinetics, different models including first-order, zero-order, logistic, Monod (with and without growth) and logarithmic models can be used to describe biodegradation (Srere 1967).

Biodegradation kinetics is used to predict concentrations of chemical substances remaining at a given time during ex situ and in situ bioremediation processes. In most cases, information is based on loss of parent molecule targeted in the process (Segel and Slemrod 1989). The key interest is frequently the decrease in toxicity concentration. Nevertheless, toxicity measurements require bioassays, which are always very difficult and tedious. Therefore, efficacy of biodegradation is based on chemical measurements, e.g. disappearance of parent molecule, appearance of mineralization products or disappearance of other compounds used stoichiometrically during biodegradation of a compound, for instance, electron acceptors (Cox *et al*. 2000). In the derivation of kinetic equations for homogeneous and heterogeneous catalytic reactions, it is necessary to use some approximation in order to reduce the derived equation to manageable forms. Chemical reactions occur through some reactive intermediates. These intermediates can be conceived as energized reactants with the requisite energy to pass into the final state and form a product. These reactive intermediates could be atoms or free radicals. In heterogeneous catalysis, these intermediates adsorb species with mobility to search for energetically favourable sites for subsequent conversion and desorption. In order to derive the rate of a chemical reaction, the various elementary reactions are tabulated and appropriate differential equations are written for individual reactions. The concentrations of the intermediates are then eliminated and the resulting equations are solved to obtain the rate of reaction (Susu 1997).

The steady state approximation allows a simple procedure where the intermediates, whose concentrations are low, are assumed to

have a constant concentration during the course of the reaction. By this approximation, simple expressions for the concentration of intermediates are derived and hence the overall reaction rate determined. The concept of the rate-determining step is a formalized way of arriving at the simpler forms of the rate expression. The concept is simply stated thus: 'In a sequence of elementary steps, one step is postulated to be rate determining and all the other steps in the sequence are assumed to be in a quasi-equilibrium'. The consequence of the concept is that only that step is kinetically significant as it is the only step that appears in the rate expression. The rate constants of the other steps appear as the ratios that are equal to the equilibrium constants of those steps (Susu 1997). In this work, both the steady state approximation and the rate-determining step approaches shall be applied to the Boudart approach to investigating the kinetics of enzymatic fermentation.

Methodology

The methods of steady state approximation and rate-determining step as outlined by Boudart (Susu 1997) for heterogeneous catalysis was applied to enzymic fermentation reactions as shown below.

Steady- State Approximation

A simple enzyme-catalyzed reaction can be illustrated as shown below:

$\underline{E} + S \rightleftharpoons \underline{ES}$, (1)

$\underline{ES} \rightleftharpoons P + \underline{E}$. (2)

Here E denotes the enzyme that is a catalyst and thus provides the active site(s) on which the reaction will take place while S is the substrate and its concentration, C. All the surface species are underlined. The forward rate of the i^{th} step is denoted by k_i while that of the reverse step is k_{-i}.

Using the method outlined by Boudart (Susu 1997):

$a_1 = k_1[A]$, (3)

$a_{-1} = k_{-1}[A]$, (4)

where the 'a's are the pseudo-first-order rate constants.

The net rate of each step, r_i, is set according to the steady state approximation:

$r_1 = r_2 = r$. (5)

Thus, the two algebraic equations necessary for the solution of E and ES are

$a_1E - a_{-1}ES = r$, (6)

$-a_{-2}E + a_2ES = r$, (7)

and

$$\frac{a_1E}{r} - \frac{a_{-1}ES}{r} = 1, \quad (8)$$

$$\frac{-a_{-2}E}{r} + \frac{a_2ES}{r} = 1. \quad (9)$$

The unknowns, $\frac{E}{r}$ and $\frac{ES}{r}$, can be solved by applying the Cramer's rule to the following sets of rearranged equations:

$a_1E_1 - a_{-1}E_2 = 1$, (10)

$-a_{-2}E_1 + a_2E_2 = 1$, (11)

where:

$$E_1 = \frac{E}{r} \text{ and } E_2 = \frac{ES}{r}.$$

The solution is given by

$$E_i = \frac{N_i}{D}, \text{ i = 1, 2,} \quad (12)$$

where N_i and D are determinants:

$$D = \begin{vmatrix} a_1 & -a_{-1} \\ -a_{-2} & a_2 \end{vmatrix},$$

$$N_1 = \begin{vmatrix} 1 & -a_{-1} \\ 1 & a_2 \end{vmatrix},$$

$$N_2 = \begin{vmatrix} a_1 & 1 \\ -a_{-2} & 1 \end{vmatrix}.$$

Thus.

$D = a_1a_2 - a_{-1}a_{-2}$, (13)

$N_1 = a_2 + a_{-1}$, (14)

$N_2 = a_1 + a_{-2}$. (15)

Therefore,

$$\sum_{i=1}^{2} N_i = a_2 + a_{-1} + a_1 + a_{-2}. \quad (16)$$

If L is the total concentration of active sites on the enzyme surface, then:

$L = E + ES$, (17)

Thus,

$$\frac{L}{r} = \frac{E}{r} + \frac{ES}{r},$$

$$\frac{L}{r} = \frac{N_1}{D} + \frac{N_2}{D},$$

$$\frac{L}{r} = \frac{\sum_i N_i}{D},$$

$$r = L\frac{D}{\sum_i N_i}. \quad (18)$$

The expression for the rate of reaction is

$$D = k_1k_2C - k_{-1}k_{-2}P, \quad (19)$$

$$\sum_{i=1}^{2} N_i = k_2 + k_{-1} + k_1C + k_{-2}P. \quad (20)$$

Thus,

$$r = \frac{k_1k_2C - k_{-1}k_{-2}P}{k_2 + k_{-1} + k_1C + k_{-2}P}. \quad (21)$$

The Rate-Determining Step Method

Applying the concept of the rate-determining step to the chemical reaction illustrated in equations 1 and 2 above, if step 1 determines the rate of the overall reaction i.e. the rate–determining step, then *ES* in the second step is in quasi-equilibrium with *E* and *P* and vice versa. Then:

$$a_2ES = a_{-2}E, \quad (22)$$

so that (conservation of active site)

$$L = ES + E. \quad (23)$$

Hence,

$$ES = \frac{L}{1 + \frac{a_2}{a_{-2}}}, \quad (24)$$

and

$$E = \frac{L}{1 + \frac{a_{-2}}{a_2}}, \quad (25)$$

$$r_1 = a_1E - a_{-1}ES, \quad (26)$$

$$r_1 = L\left(\frac{a_1a_2}{a_2 + a_{-2}} - \frac{a_{-1}a_{-2}}{a_2 + a_{-2}}\right). \quad (27)$$

Setting *L* to unity, then:

$$r_1 = \frac{k_2k_1C - k_{-1}k_{-2}P}{k_2 + k_{-2}P}. \quad (28)$$

If the second step determines the rate of the overall reaction, then:

$$a_1E = a_{-1}ES, \quad (29)$$

so that,

$$E = \frac{L}{1 + \frac{a_1}{a_{-1}}}, \quad (30)$$

and

$$ES = \frac{L}{1 + \frac{a_{-1}}{a_1}}, \quad (31)$$

$$r_2 = a_2ES + a_{-2}E, \quad (32)$$

$$r_2 = L\left(\frac{a_1a_2}{a_1 + a_{-1}} - \frac{a_{-1}a_{-2}}{a_1 + a_{-1}}\right). \quad (33)$$

Setting *L* to unity, then:

$$r_2 = \frac{k_2k_1C - k_{-1}k_{-2}P}{k_1C + k_{-1}}. \quad (34)$$

These methods assume a single substrate whose transformation is catalyzed by a single enzyme. Furthermore, the substrate is readily available to the enzyme with no hindrances associated to mass transfer factors. Also, the enzyme is specific for the substrate.

Results and Discussion

The result obtained from the steady-state approximation method is as shown in Eq. (21). In the methods of the rate-determining step, the results are two; the one obtained when the formation of the substrate-enzyme complex as depicted in Eq. (1) limits the reaction, the obtained result is Eq. (28). On the other hand, the expression in Eq. (34) is obtained when Eq. (2), which is the decomposition of the substrate-enzyme complex to form the product, limits the enzymic fermentation reaction. The three equations further showed that enzyme-catalyzed reactions are a type of heterogeneous catalytic reactions. Generally, from these three results it can be seen that the rate of reaction decreases as the product is formed and the reactant concentration is decreasing, this account for the negative sign that always accompany the rate of substrate consumption, (-d*C*/d*t*), in rate expressions.

The study of kinetics of biodegradation and other bio-reactions is essentially to evaluate the persistence of organic pollutants and to assess their exposure to the environment. Thus, in many instances, the information on the kinetics comes only from the evaluation of the loss of parent molecules (Schnell and Turner 2004). This is so because the reactions are slow and takes a longer time for the products to form to such an appreciable level that analytical tools can measure their concentrations. In view of this, the concentrations of the product(s) are normally ignored. For this work, if the product

concentration is ignored a new set of expressions for Eqs. (21), (28) and (34) are obtained. Equation (21) becomes

$$r = \frac{k_1 k_2 C}{k_2 + k_{-1} + k_1 C},$$

which on further manipulation becomes

$$r = \frac{k_2 C}{\frac{k_2 + k_{-1}}{k_1} + C},$$

an expression similar to the Briggs-Haldane equation for the rate expression of enzyme-catalyzed reactions. Briggs and Haldane (1925) also used the steady-state assumption in their work. The difference in the two results is that the Briggs-Haldane equation did not account for the product formed in the reaction as contained in Eq. (21) of this work.

For the rate-limiting steps approach, Eq. (28) becomes $r_1 = k_1 C$,

when the concentration of the product is assumed negligible while Eq. (34) is reduced to

$$r_2 = \frac{k_2 k_1 C}{k_1 C + k_{-1}},$$

and subsequently to

$$r_2 = \frac{k_2 C}{C + \frac{k_{-1}}{k_1}},$$

when the first and second step limits the reaction rate, respectively. It has been observed that the result obtained for the second step limiting the reaction rate correspond to that obtained by Michaelis and Menten (1913). They made this assumption in their work, that the formation of the enzyme-substrate complex is very rapid, however, like the work of Briggs and Haldane (1925), their work did not also account for the product formed in the reaction as contained in Eq. (34) of this work. In view of these observations, one may infer that the method of Boudart (1968) is a more comprehensive way of arriving at rate expressions of heterogeneous catalytic reactions. Furthermore, enzyme-catalyzed reactions are an extension of heterogeneous catalytic reactions. The unique result in this work is the consequent result of Eq. (28) that portrays kinetics of enzyme-catalyzed reactions as first order when the formation of the enzyme-substrate limits the reaction. The consequence of this is the rapid decomposition of the enzyme-substrate complex to form the product. Many researchers prefer working with the first-order rate because of the ease associated with its usage. On the other hand the Briggs-Haldane and Michaelis-Menten equations are very tedious and require special skills of mathematics to handle.

The Briggs-Haldane and Michaelis-Menten equations are the same and represent the kinetic expression for enzyme-catalyzed reactions. At high substrate concentration the rate of its consumption is independent of the concentration implying that the reaction at that stage follow the zero-order kinetics. However, at low substrate concentration, the rate of reaction is first-order with respect to the concentration. On the other hand, Kareem (2010) observed that *Desulfobacterium anilini* and *Desulfobacterium indolicum* consumption of sulfur in thiophene contained kerosene followed the zero-order kinetics pattern for a period of 72 hours, although the initial concentration of the thiophene was 9 mg/l. This means that the order need not be zero-order kinetics preceding first-order kinetics always, each pattern could prevail entirely. The mechanism of the first-order has been shown here while that of the zero-order kinetics is not clearly understood yet.

Nomenclatures

k_i	rate constants
K	equilibrium constants
r_i	rate of reaction
A	substrate in an enzyme-catalyzed reaction
E	concentration of free enzyme
E_0	concentration of total enzyme
ES, X	concentration of enzyme-substrate complex
C	cells of microbes
K_M	Michaelis-Menten constant
A	pseudo-first-order rate constants
D, N	Determinants of matrices
L	total concentration of active sites on an enzyme
P, R	products obtained from an enzyme-catalyzed reaction

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