Simple Models of Enzyme Kinetics with and without Inhibitors

Jeffrey D. Varner*

School of Chemical and Biomolecular Engineering*
Cornell University, Ithaca NY 14853

Introduction

We developed material balances for nutrients, products and cells for batch, fed-batch and continuous stirred tank bioreactors where we treated the cells as black boxes that consumed nutrients and produced products. Given our black box model of cells, we also considered the *best* way to operate bioreactors, for example, to maximize product titer or productivity by varying process parameters, such as volumetric flow rate. However, since we treated the cells as black boxes, we have not considered the problem of changing the *intracellular* operation of cells. To do that we need to understand how cells operate, and particularly how they process nutrients such as sugars into products. Cells operate huge connected intracellular biochemical *networks* collectively called metabolism using a special class of proteins called *enzymes*. Enzymes are proteins that catalyze the chemical reactions occurring inside simple and complex cells. Enzymes are responsible for processing starting materials such as sugars into products of interest, waste products and eventually more cells. Enzymes are also responsible for information processing, and biophysical functions such as transporting molecules across membranes. Enzymes can be classified in one of six classes, based on the chemical reactions they catalyze (Fig. 1). Just like other types of catalysts, enzyme do *not* change the overall energetics of a chemical reaction, rather they lower the activation barrier for the reaction to occur (Fig. 2)

Idealized models of enzyme kinetics. In general, modeling the kinetics (rate) of enzyme catalyzed reactions, for example the rate of starch degradation by α-amylase, is a difficult problem. However, we can gain insight into this difficult problem, and the general arguments of how to formulate the problem, by studying a simple idealized example. Let's assume we have a well mixed test tube containing an enzyme E (a protein that catalyzes chemical reactions) which converts substrate S (the starting compound) into product P:

$$S \xrightarrow{E} P \tag{1}$$

by an idealized *lock and key* mechanism (Fig. 3). The enzyme E converts the substrate S into the

Class	General Reaction	Example
Oxidoreductase	$A^- + B \rightarrow A + B^-$	Cytochrome oxidase
Transferase	A-X + B $\rightleftharpoons$ A + B-X	Acetate kinase
Hydrolase	A-B + $H_2O \rightleftharpoons$ A-H + B-OH	Lipase
Lyase	A-B $\rightleftharpoons$ A + B	Isocitrate lyase
Isomerase	A $\rightleftharpoons$ A'	Triophosephate isomerase
Ligase	A + B + ATP $\rightleftharpoons$ A-B + ADP + P_i	DNA ligase

Fig. 1: Enzymes are proteins that carry out specific chemical transformations inside a cell. There are six general classes of enzymes: Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases and Ligases.

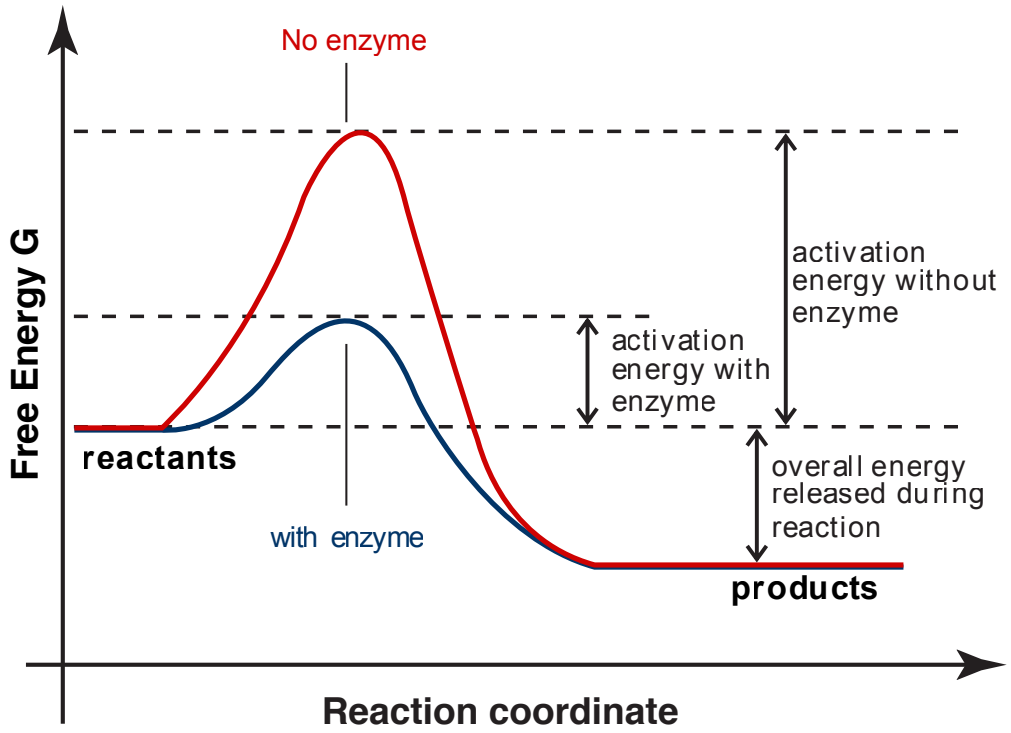

Fig. 2: Enzymes catalyze chemical reactions by lowering the activation energy required for the reaction to proceed. However, enzymes do not alter the overall free energy change of the reaction.

product P according to the three elementary reactions:

$$E + S \rightleftharpoons E:S \tag{2}$$

$$E:S \longrightarrow E + P \tag{3}$$

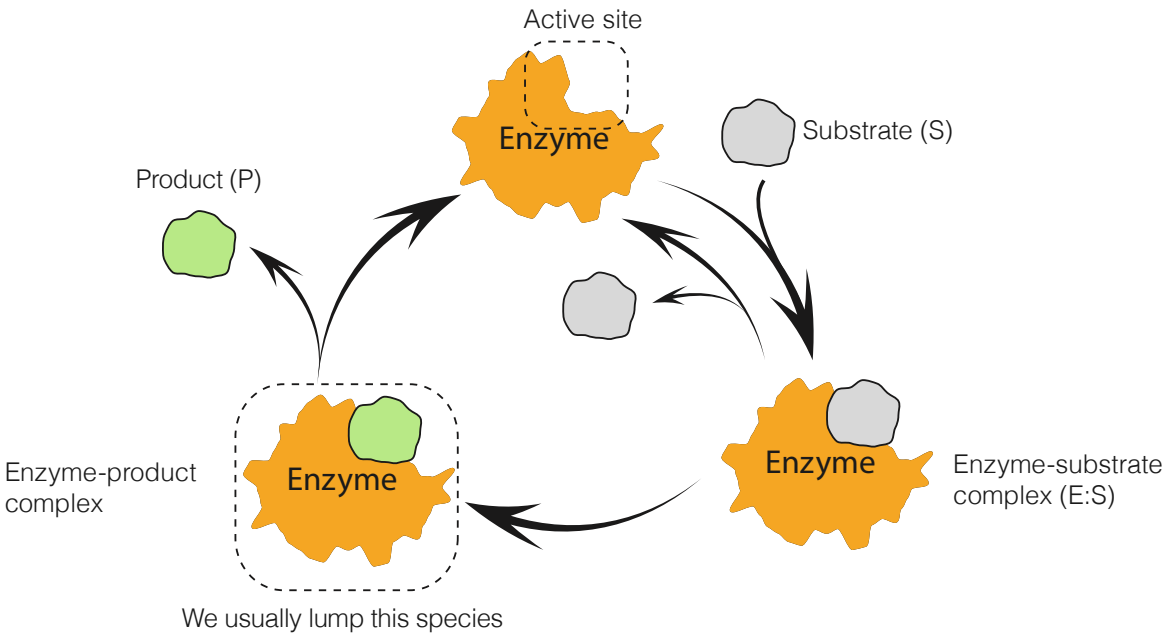

Fig. 3: Schematic of the idealized *lock and key* mechanism of the enzyme catalyzed conversion of substrate S to product P by an enzyme E.

The kinetics of each elementary step can be written using *mass-action* kinetics, i.e.,

$$r_1 = k_1 [E] [S] \tag{4}$$

$$r_2 = k_2 [E:S] \tag{5}$$

$$r_3 = k_3 [E:S] \tag{6}$$

where $[\cdot]$ denotes a species concentration, and k_j denotes the rate constant governing the jth elementary reaction. The rate r_1 describes the *association* rate between the enzyme and substrate, r_2 describes the rate of *dissociation* of the enzyme substrate complex and r_3 denotes the rate of *chemical conversion* of the bound substrate into product (where we assume the dissociation of the product from the enzyme is fast). Lastly, enzyme concentration must obey the relationship:

$$[E_T] = [E] + [E:S] \tag{7}$$

where $[E_T]$ denotes the total enzyme concentration in the tube, $[E]$ denotes the *free* enzyme concentration (not bound to substrate) while $[E:S]$ denotes the enzyme substrate complex.

In order to estimate the *overall* rate of enzymatic conversion (v) of S to P, we need to stipulate a single rate limiting step out of the set of elementary reactions describing the conversion. Let's assume that the rate of chemical conversion (r_3) is the *slowest* step, i.e., the substrate bounces on/off the enzyme quickly with only a fraction of these binding events resulting in a successful

chemical transformation. Thus, the overall rate of S to P is then given by:

$$v = k_3 \left[E : S\right] \tag{8}$$

Let's also assume that we already know (or can estimate) the rate constants k_1, k_2 and k_3. When this is true, the only unknown in Eqn. (8) is $[E : S]$. However, we can relate $[E : S]$ to variables we know (E_T and at least initially S) through the enzyme balance, and a second assumption called the *pseudo-steady-state assumption* for the reaction intermediate $[E : S]$:

$$\frac{d\left[E : S\right]}{dt} = k_1 \left[E\right]\left[S\right] - k_2 \left[E : S\right] - k_3 \left[E : S\right] \simeq 0 \tag{9}$$

Rearranging Eqn. (9) and solving for $[E : S]$ gives the relationship:

$$\left[E : S\right] \simeq \frac{k_1}{k_2 + k_3} \left[E\right]\left[S\right] \tag{10}$$

where the ratio of rate constants is defined as the Michaelis Menten saturation coefficient or K_M:

$$\frac{1}{K_M} \equiv \frac{k_1}{k_2 + k_3} \tag{11}$$

Substituting Eqn. (10) into the overall rate yields:

$$v = k_3 \frac{\left[E\right]\left[S\right]}{K_M} \tag{12}$$

However, in v we still do not know $[E]$, the free enzyme concentration. To get $[E]$ we have to use the total enzyme balance. Substituting Eqn. (10) into the enzyme balance Eqn. (7) and solving for $[E]$ yields:

$$\left[E\right] = \frac{\left[E_T\right] K_M}{K_M + \left[S\right]} \tag{13}$$

Lastly, we can substitute Eqn. (13) into Eqn. (12) to arrive at the final expression for v:

$$v = V_{max} \frac{\left[S\right]}{K_M + \left[S\right]} \tag{14}$$

where $V_{max} \equiv k_3 \left[E_T\right]$. Michaelis Menten kinetics are a type of saturation kinetics where the change in the reaction rate as a function of substrate concentration saturates (slows down) as we increase substrate beyond a critical limiting value (Fig. 4). Similar to Monod growth kinetics, when $S \gg K_M$ the rate becomes close to V_{max}. Conversely, when $S \ll K_M$ the rate appears to be linear with respect to substrate concentration. Lastly, it is easy to show that when $K_M \simeq S$ the reaction rate equals $v \simeq 1/2 V_{max}$.

How can we estimate V_{max} and K_M from data? There is no general first-principles methodology to estimate V_{max} and K_M for an arbitrary enzyme catalyzed reaction. Thus, we must estimate these parameters from experimental measurements. For the simple reactions we have derived we

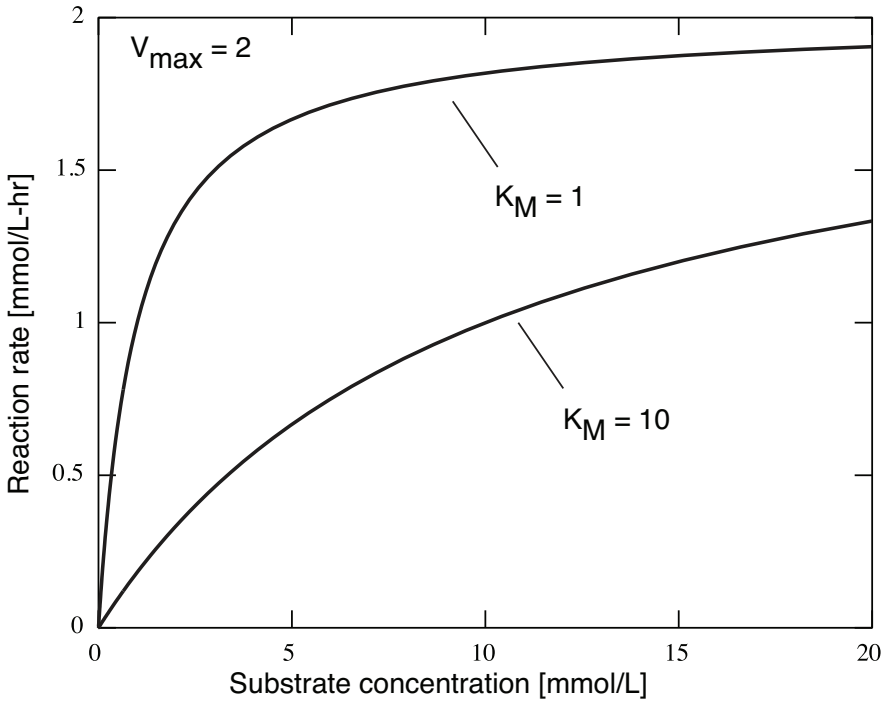

Fig. 4: Reaction rate versus substrate concentration for a Michaelis Menten rate form.

can make a *Lineweaver-Burk* plot (LBP) (1). LBPs are a double reciprocal plot that allows us to estimate *both* the K_M and the V_{max} of an enzyme substrate pair. Suppose we could measure the overall rate of reaction for a given substrate level. If we invert Eqn. (14) and collect terms we arrive at:

$$\frac{1}{v} = \frac{K_M}{V_{max}}\frac{1}{S} + \frac{1}{V_{max}} \tag{15}$$

Eqn. (15) is a *linear* equation; if we let $1/v$ equal the dependent variable (y-axis), and $1/S$ equal the independent variable (x-axis), then $1/V_{max}$ is the y-intercept and K_M/V_{max} equals the slope (Fig 5).

Aside: What is the physical meaning of the K_M? We have already seen that the K_M is the substrate concentration where reaction rate equals half the maximum rate. However, K_M also has a relationship with *affinity* of an enzyme for its substrate. The MichaelisMenten constant K_M is *inversely* proportional to the *affinity* of the enzyme for its substrate. To show this true, let's revisit our rate limiting step hypothesis. Our development was based on the chemistry step being *slow* relative the binding. When this is true, $k_2 \gg k_3$ which implies:

$$K_M \simeq \frac{k_2}{k_1} \tag{16}$$

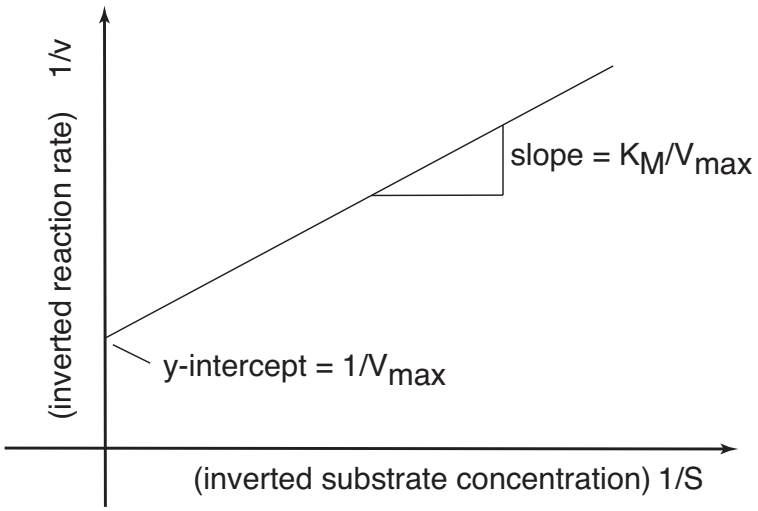

Fig. 5: Example Lineweaver-Burk plot for a single substrate reaction in the absence of inhibitors.

The ratio k_2/k_1 is inversely related to the affinity K_A:

$$K_M \simeq \frac{k_2}{k_1} = \frac{1}{K_A} \tag{17}$$

Thus, if an enzyme binds a substrate strongly, it has a *small* K_M. Conversely, if the substrate is *not* tightly held by the enzyme, then the K_M is large. Different K_M values impact the reaction rate (Fig. 4); as K_M increases (the affinity decreases) more substrate is required to achieve the same reaction rate. In other words, more binding events between substrate and enzyme are required before the enzyme holds the substrate long enough for the chemistry to occur.

Simple models of enzyme kinetics in the presence of inhibitors. One of the standard laboratory (and potentially even therapeutic) tools to manipulate enzyme activity (the ability to carry out chemical function) are inhibitors. Typically, inhibitors are small molecules that interfere with the ability of an enzyme to function in some way. *Competitive inhibitors* bind the enzyme active site (location on the enzyme where the chemical conversion of substrate to product occurs) and block the ability of the substrate to bind. Conversely, *uncompetitive* and *noncompetitive* inhibitors allow substrate binding, but modulate product formation by potentially binding regulatory sites on the enzyme (allosteric sites) or by binding both free and bound enzymes.

Competitive inhibitors. Let's assume we have a well mixed test tube containing an enzyme E (a protein that catalyzes chemical reactions), a competitive inhibitor I and a substrate S (the starting compound). The enzyme E converts the substrate S into the product P according to the normal elementary reactions:

$$E + S \quad \rightleftharpoons \quad E:S \tag{18}$$

$$E:S \quad \longrightarrow \quad E + P \tag{19}$$

$$E + I \quad \rightleftharpoons \quad E:I \tag{20}$$

in addition to an elementary reaction describing the reversible association of the inhibitor with the enzyme, Eqn. (20). Again, the kinetics of each elementary step can be written using *mass-action* kinetics:

$$r_1 = k_1 [E] [S] \tag{21}$$
$$r_2 = k_2 [E:S] \tag{22}$$
$$r_3 = k_3 [E:S] \tag{23}$$
$$r_4 = k_4 [E] [I] \tag{24}$$
$$r_5 = k_5 [E:I] \tag{25}$$

where $[\cdot]$ denotes a species concentration, and k_j denotes the rate constant governing the jth elementary reaction. The rate r_1 describes the *association* rate between the enzyme and substrate, r_2 describes the rate of *dissociation* of the enzyme substrate complex and r_3 denotes the rate of *chemical conversion* of the bound substrate into product (where we assume the dissociation of the product from the enzyme is fast). The last two rates (r_4 and r_5) describe the rate of association and dissociation of the enzyme-inhibitor complex. Lastly, enzyme concentration must obey the relationship:

$$[E_T] = [E] + [E:S] + [E:I] \tag{26}$$

where $[E_T]$ denotes the total enzyme concentration in the tube, $[E]$ denotes the *free* enzyme concentration (not bound to substrate), $[E:S]$ denotes the enzyme substrate complex and $[E:I]$ denotes the enzyme inhibitor complex. In order to estimate the *overall* rate of enzymatic conversion (v) of S to P, we postulate a single rate limiting step out of the set of elementary reactions describing the conversion. Let's assume that the rate of chemical conversion (r_3) is the *slowest* step, i.e., the substrate bounces on/off the enzyme quickly with only a fraction of these binding events resulting in a successful chemical transformation. Thus, the overall rate of conversion is then given by:

$$v = k_3 [E:S] \tag{27}$$

Let's also assume that we already know (or can estimate) the rate constants k_j. When this is true, the only unknown in Eqn. (27) is $[E:S]$. However, we can relate $[E:S]$ to variables we know (E_T and at least initially S) through the enzyme balance, and the *pseudo-steady-state assumption* for the reaction intermediates $[E:S]$ and $[E:I]$:

$$\frac{d[E:S]}{dt} = k_1 [E] [S] - k_2 [E:S] - k_3 [E:S] \simeq 0 \tag{28}$$
$$\frac{d[E:I]}{dt} = k_4 [E] [I] - k_5 [E:I] \simeq 0 \tag{29}$$

Rearranging Eqn(s). (28) and (29) and solving for $[E:S]$ and $[E:I]$ gives the relationships:

$$[E:S] \simeq \frac{k_1}{k_2+k_3}[E][S] \tag{30}$$

$$[E:I] \simeq \frac{k_4}{k_5}[E][I] \tag{31}$$

As was true without inhibitor, the ratio of rate constants in Eqn (30) is the Michaelis Menten saturation coefficient or K_M:

$$\frac{1}{K_M} \equiv \frac{k_1}{k_2+k_3} \tag{32}$$

while the ratio of inhibitor constants:

$$K_I \equiv \frac{k_5}{k_4} \tag{33}$$

is defined as the equilibrium inhibition constant (units of concentration). For *tight* inhibitors $K_I \ll 1$, conversely for *loose* inhibitors $K_I \gg 1$. Previously, we substituted our expression for $[E:S]$ into the enzyme balance and then solved for free enzyme. In the presence of an inhibitor we do the same thing, except we substitute both $[E:S]$ and $[E:I]$ into the enzyme balance to arrive at:

$$[E_T] = [E] + \frac{[E][S]}{K_M} + \frac{[E][I]}{K_I} \tag{34}$$

Solving Eqn. (34) for $[E]$ yields:

$$[E] = \frac{[E_T]}{1+\frac{[S]}{K_M}+\frac{[I]}{K_I}} \tag{35}$$

Now that we have an expression for free enzyme, we can eliminate this unknown from our overall rate expression:

$$v = k_3\frac{[E][S]}{K_M} \tag{36}$$

and group terms to arrive at:

$$v = \frac{V_{max}[S]}{\hat{K}_M+[S]} \tag{37}$$

where $V_{max} \equiv k_3[E_T]$ and:

$$\hat{K}_M \equiv K_M\left(1+\frac{[I]}{K_I}\right) \tag{38}$$

Competitive inhibitors *seemingly* influence the *affinity* of the enzyme for its substrate (Fig. 6).

Uncompetitive and Noncompetitive inhibitors. The derivation for the overall enzyme catalyzed reaction rate in the presence of uncompetitive or noncompetitive inhibitors follows similar arguments to the competitive case, with the exception that uncompetitive or noncompetitive inhibitors can have multiple biological mechanisms for inhibition. Uncompetitive inhibitors bind the enzyme substrate complex, while noncompetitive inhibitors can bind both free enzyme and bound enzyme. In these cases, the overall rate of conversion of substrate S to product P is modulated by the inhibitor (but not necessarily blocked). Lets consider a uncompetitive inhibitor (inhibitor binds the

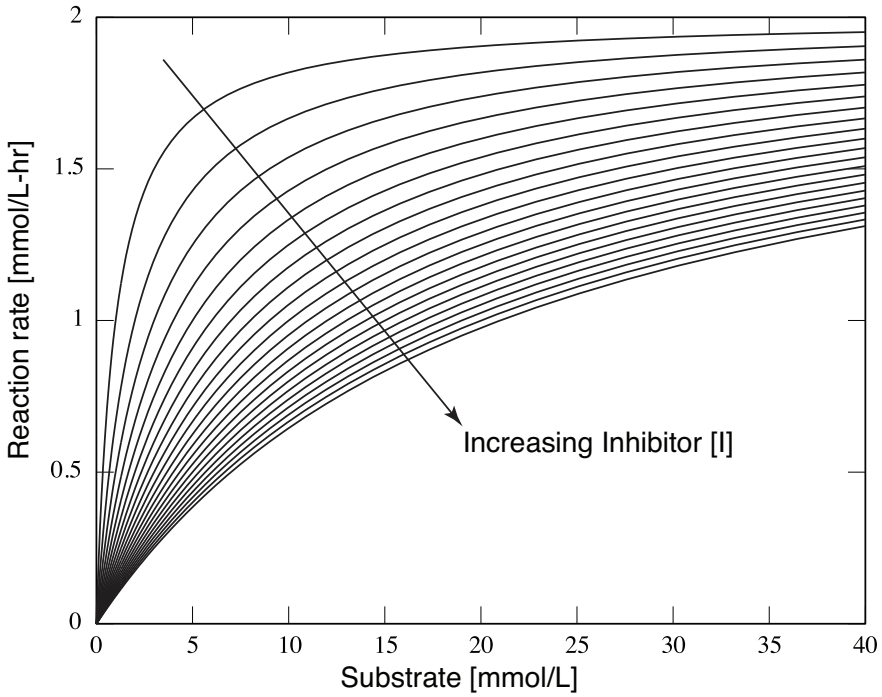

Fig. 6: Reaction rate versus substrate concentration for a Michaelis Menten rate form in the presence of a competitive inhibitor.

enzyme-substrate complex). The rate of conversion of substrate S to product P by enzyme E in the presence of an uncompetitive inhibitor I is given by:

$$v = \frac{\hat{V}_{max}\,[S]}{K_M + [S]} \tag{39}$$

where $\hat{V}_{max}$ is given by:

$$\hat{V}_{max} \equiv \frac{k_3\,[E_T]}{1 + \frac{[I]}{K_I}} \tag{40}$$

Uncompetitive inhibitors *seemingly* influence the *maximum rate* that an enzyme can convert its substrate to product (Fig. 7). On the other hand, the rate of conversion of S to P in the presence of a noncompetitive inhibitor is given by:

$$v = \frac{\hat{V}_{max}\,[S]}{\hat{K}_M + [S]} \tag{41}$$

where both the V_{max} and K_M are influenced.

References

1. Lineweaver H, Burk D (1934) The determination of enzyme dissociation constants. JACS 56: 658 - 666.

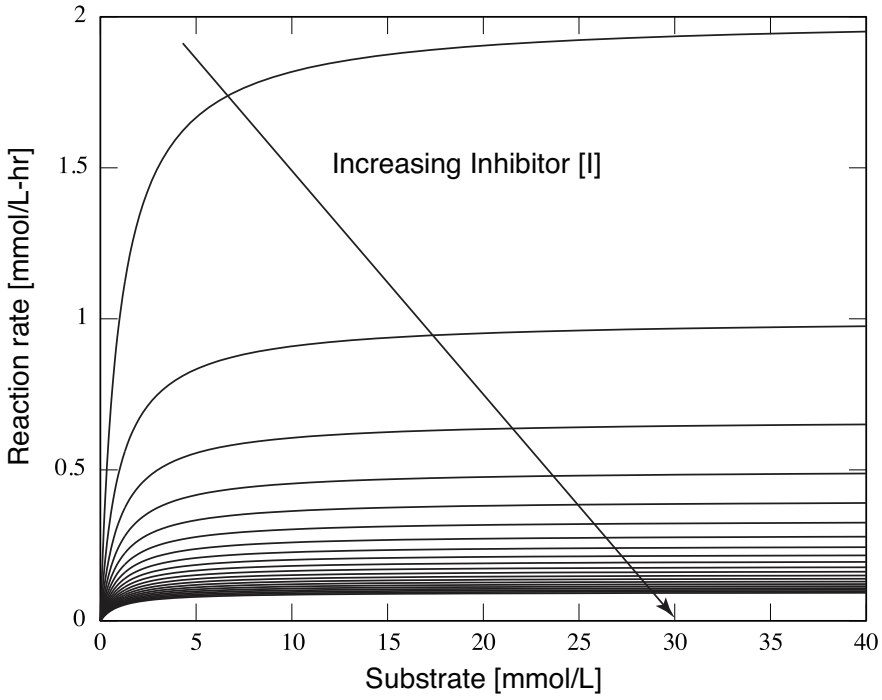

Fig. 7: Reaction rate versus substrate concentration for a Michaelis Menten rate form in the presence of a non-competitive inhibitor.