

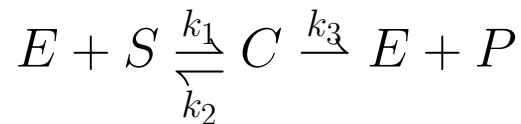
## **Analysis of Enzyme Kinetics in Invertebrates**

- Derivation of the Michaelis-Menten Relation
- Experimental Use of the Michaelis-Menten Equation
- Enzyme Kinetics Experiment

## Experimental Use of M-M Equation

### I. Review

#### A. Chemical equation



where  $E$ =enzyme,  $S$ =substrate,  $C$ =complex, and  $P$ =product.

#### B. Mathematical Equations

$$\frac{dE}{dt} = -k_1SE + k_2C + k_3C$$

$$\frac{dS}{dt} = -k_1SE + k_2C$$

$$\frac{dC}{dt} = k_1SE - (k_2 + k_3)C$$

$$\frac{dP}{dt} = k_3C$$

#### C. Leads to the Michaelis-Menten equation

$$\frac{dP}{dt} = \frac{V_{\max}S}{K_m + S}$$

where  $V_{\max}$  is the maximum rate of product formation and  $K_m$  is the 1/2-saturation coefficient.

### II. Experimental Uses Effects of experimental treatments on enzyme kinetics (e.g., temperature, drugs, etc).

Compare  $K_m$  or  $V_{\max}$  at different levels. Therefore, need estimates of the parameters under different conditions.

### III. Two Kinds of Regression Problems

A. Models (equations) that are **Linear in the Parameters** (LITP). E.g.,

$$y = mx + b$$

All parameters ( $m = \text{slope}$  and  $b = \text{intercept}$ ) appear as a constant (e.g.,  $b$ ) or as a simple multiplication of a constant (e.g., straight line above).

Another example:

$$y = a_0 + a_1x + a_2x^2$$

The variable  $y$  is not linear over values of  $x$ , but it is linear if  $a_0$ ,  $a_1$ , and  $a_2$  are considered variables and  $x$  is considered constant.

$$y = a_0 + a_1x_1 + a_2x_2$$

Also LTIP, but has 2 independent variables (problem is to find slope and intercept of a plane, not a line).

B. Not LITP

Operations involving the parameters that cause it to not be LTIP: raise to power, use in denominator, use inside a function (e.g.,  $\sin(bx)$ ,  $e^{bx}$ ). For example,

$$y = ax^b$$

$a$  is okay, but the problem is  $b$ ; it is not simple addition or multiplication.

$$y = \frac{ax}{b+x}$$

$b$  is in the denominator.

IV. LTIP models can be solved using linear regression

A. Basic idea: (above) observed  $y$  equals straight line plus or minus some **error** ( $\epsilon_i$ ).

$$y_i = a_0 + a_1x_i + \epsilon_i$$

where  $a_0$  and  $a_1$  must be estimated from data.

Another example:

$$y_i = a_0 + a_1x_i + a_2x_i^2 + \epsilon_i$$

B. Problem: What function to use for  $\epsilon$ ?

Let the error be the sum of squared deviations of the model from the data:

$$\epsilon = \sum_{i=1}^N [y_i - (a_0 + a_1 x_i)]^2$$

where  $N$  = number of data points.

C. Linear Regression is an Optimization (Minimization) Problem

Choose  $a_0^*$  and  $a_1^*$  such that

$$\epsilon = \min (\sum [y_i - (a_0^* + a_1^* x_i)]^2)$$

D. Regression Terminology (next page)

V. What if the model is not in the form  $y = mx + b$ ?

A. Transformation

How to transform this?

$$y = ax^b$$



Regression takes place in this space:

B. Lineweaver-Burke Transform of the M-M equation

Inverse transformation: invert both sides

$$\frac{1}{y} = \frac{K_m}{V_{\max}} \frac{1}{S} + \frac{1}{V_{\max}}$$

where  $S$  is the substrate concentration,  $y$  is the rate of product formation. (Algebra left for students.)

Regression takes place in this space:

C. SAS (Statistical Analysis System) Program and Output

(Next page)

D. Eadie-Hofstee Transformation

$$\frac{y}{S} = \frac{V_{\max}}{K_m} - \frac{y}{K_m}$$

(algebra left for students). SAS program and output on next pages.

Regression in this space:

E. Hanes

$$\frac{S}{y} = \frac{S}{V_{\max}} + \frac{K_M}{V_{\max}}$$

(algebra left for students). SAS program and output on next pages.

Regression in this space:

## VI. Problems with the Inverse Transformation

Can inflate the  $R^2$  of the model by creating groups of data. E.g., suppose we have these evenly spaced data:

$x$	1	2	3	4	5
$y$	1	2	3	4	5

Transformed:

$1/x$	1	0.5	0.333	0.25	0.2
$1/y$	1	0.5	0.333	0.25	0.2

Notice “clumping” of data; data are no longer uni-

formly distributed. This produces really just 2 “points” near 1.0 and small values near 0.25. Fitting a straight line to 2 points always gives a good  $R^2$ .

For this and other reasons, transformation is to be avoided if at all possible. Modern statistical packages make it easy to do regression without transforming data.

## VII. Estimating parameters when the model is not LITP

$$y = ax^b$$

Error function is the same (usually)

$$\epsilon = \sum_{i=1}^N [y_i - (ax_i^b)]^2$$

where  $N$  = number of data points.

## VIII. Can not solve for $a$ and $b$ directly.

Assume we don't want to do a log transform.

A. The problem:

- B. Use computer iteration to move from point in parameter space that has high error to a point with low error.
- C. Start at some point and choose new point to reduce  $\epsilon$ .

- D. Stop computer iteration until sufficiently close to minimum.
- E. DANGER: Can stop at local minimum

F. How to choose new point? Levenberg-Marquardt

method.

SAS program and output

## IX. Advantages and Disadvantages

### A. Linear Regression

Easy, quick, exact; but only straight lines and may require transformations that may distort the data.

### B. Nonlinear Regression

Any function, any number of parameters, no transformations; but complex to program, may need multiple starting points