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# LINEAR TRANSFORMATIONS OF THE MICHAELIS-MENTEN KINETIC EQUATION IN NATURAL MICROBIAL COMMUNITIES RESEARCH

Timo Tamminen[1)]

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The Michaelis-Menten kinetic formula has been widely utilized in ecological studies for describing the active transport of substrates into microbial cells. Since the original formula is in hyperbolic form, several linear transformations have been presented to generate kinetic parameters in laboratory studies of microbial cultures. In aquatic ecological research with natural populations or communities, the so-called Lineweaver-Burk transformation has almost solely been used. In this paper, two other linear transformations for the study of natural microbial communities are presented, which take into account the substrate concentration present in water samples. The properties of the three transformations are briefly discussed on the basis of examples from field studies.

Index words: Michaelis-Menten kinetics, linear transformations, natural communities, bacterioplankton, glucose assimilation.

## 1. INTRODUCTION

Monod (1942, 1949) introduced the Michaelis-Menten kinetic equation into microbial culture research to explain and simulate the observed active transport of substrates into cells. The equation is based on a biochemical theory describing an enzyme-mediated two-step process of substrate uptake across the cell cembrane (Cohen and Monod 1957, Pardee 1968), and has had widespread applications not only in microbial culture studies but also as a general biological growth model. It was not until Caperon (1967) presented his population growth model, however, that a satisfactory theoretical basis for wider applications was confirmed (Strickland 1971).

The use of the original kinetic equation in ecologically-orientated microbial studies has been somewhat hindered by its mathematical form, which is hyperbolic and requires such a detailed knowledge of substrate concentrations and growth rates that seldom can be obtained in research on natural populations. Wright and Hobbie (1965, 1966) presented the so-called Lineweaver-Burk linear transformation of the original Michaelis-Menten equation, which has proved to be operative in determining the kinetic parameters describing natural microbial communities. In the study of

1) Vaasa Water District, P.O. Box 262, SF-65101 Vaasa, Finland, present address: Tvärminne Zoological Station, University of Helsinki, SF-10850 Tvärminne, Finland.

laboratory cultures, some other linear transformations have also been used (Dowd and Riggs, 1965). In this paper, two other linear transformations for natural microbial community studies are presented in close analogy to those used in the culture studies. The properties of these transformations are briefly discussed in comparison with the traditional Lineweaver-Burk modification on the basis of some experimental data.

## 2. MATERIAL AND METHODS

The substrate used in the study was D-(6 − $^3$H) -glucose with a specific activity of 22.5 C·mmol$^{-1}$ (Radiochemical Centre, Amersham, England). The solution was autoclaved for 20 minutes at 115 °C after dilution. Carrier solutions of D-glucose (AnalaR, analytical reagent) were made in distilled water.

The water sample originated from a brackish water area off Kaskinen in the Gulf of Bothnia. Incubations were performed in the dark and at *in situ* temperature. Incubation volumes were 50 ml. Glucose was added into subsamples in 26 concentrations ranging from 0.005 to 22.6 $\mu$g·l$^{-1}$ . Each concentration was added in triplicate and compared with a blank containing 0.5 ml 35 % formaldehyde. The incubation time was 2 hours and the incubation was terminated by the addition of 0.5 ml formaldehyde.

Samples were filtered on 0.45 $\mu$m membrane filters (Millipore, France) and radioactivities were measured with a liquid scintillation counter (UltroBeta, LKB-Wallac, Finland) by the external standard channel ratio method. The scintillation cocktail consisted of the moist filter, 1.0 ml of dioxan and 10.0 ml of PCS (Amersham). Results were calculated as disintegrations per minute (dpm).

## 3. LINEAR TRANSFORMATIONS IN CULTURE STUDIES

The Michaelis-Menten enzyme kinetic formula has usually been used in bacterial population studies in the form:

$$v = V \cdot \frac{S}{K + S} \qquad (1)$$

where
v = substrate uptake rate
V = maximal substrate uptake rate
S = substrate concentration
K = half-saturation constant

Of the parameters in equation (1), the uptake rate (v) and the substrate concentration (S) are usually assayed experimentally in the case of laboratory cultivations. With the aid of these parameters it is possible to calculate the parameters V (maximal uptake rate) and K (half-saturation constant) describing the population.

Because eq. (1) takes the form of a hyperbola (Fig. 1), attempts have been made to transform the function to linear form. Dowd and Riggs (1965) presented three alternative methods

$$v = V - \frac{K \cdot v}{S} \qquad (v \text{ vs. } v/S) \qquad (2a)$$

$$S/v = \frac{K}{V} + \frac{S}{V} \qquad (S/v \text{ vs. } S) \qquad (2b)$$

$$1/v = \frac{1}{V} + \frac{K}{V \cdot S} \qquad (1/v \text{ vs. } 1/S) \qquad (2c)$$

Of these equations the last (2c) is known as the Lineweaver-Burk transformation (or double reciprocal plot), and has most frequently been used in biochemical research and in laboratory investigations of bacterial cultivations (Dowd and Riggs

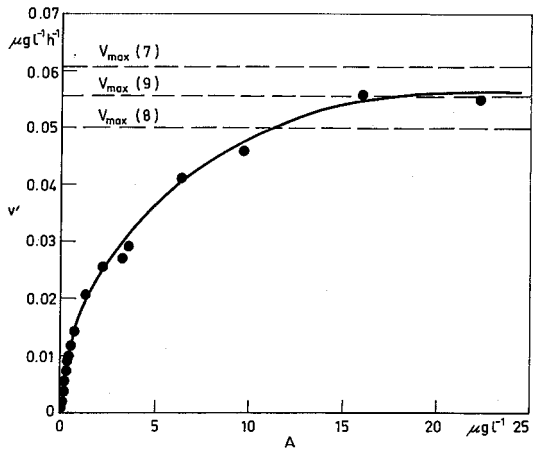


Fig. 1. Saturation curve of uptake rate calculated from the Kaskinen data. The curve corresponds to the hyperbola produced by equation (1), but the uptake rate is relative (v' = A/T, calculated according to the addition concentration). The calculated (theoretical) maximal uptake rates obtained using equations (7), (8) and (9) are shown.

1965, Lehninger 1975). Earlier, however, Wilkinson (1961) has presented some statistical weaknesses of the double reciprocal plot. Because the measured parameters (v and S) appear in the equation in inverse form, in particular their small values (large reciprocal values) strongly govern the slope of the regression line. Wilkinson compared equations (2b) and (2c) and recommended the rejection of the double reciprocal plot.

Dowd and Riggs (1965) examined all of the aforementioned linear transformations with the aid of computer simulation. If the measured parameters were errorless, the transformations would all yield approximately the same result. However, in normal experimental practice errors almost always occur in the determination of uptake rate (v). By simulating different errors of the parameter (v) in their "experiments" (= computer runs), Dowd and Riggs showed that the double reciprocal plot was considerably weaker than the other linear transformations. The simulated errors were of both constant size and constant ratio types. Even small errors in the values of uptake rate, particularly in the case of low rates, caused considerable misalignment of the regression line.

Paradoxically, the double reciprocal plot gives the best fit (smallest sum of squares), although the parameters (V and K) obtained with the line are the least reliable (Dowd and Riggs 1965). In all probability this good apparent reliability has been one factor leading to the wide acceptance of this transformation. Equation (2a) (the Eadie-Hofstee transformation) is the most sensitive to give warning of the departure of the data from linearity, upon which the standard enzyme kinetic analysis is dependent (Dowd and Riggs 1965, Lehninger 1975).

## 4. APPLICATIONS TO NATURAL COMMUNITIES RESEARCH

The enzyme kinetic equations used in the analysis of microbial laboratory cultures are not directly applicable to investigations of natural communities. Natural aquatic environments usually contain a very low, unknown concentration of utilizable substrate. For this reason the concentration parameter S of equation (1) must be corrected to the form:

$$S = S_n + A \qquad (3)$$

where

$S_n$ = substrate concentration in the natural water

A = concentration of substrate added in the experiment

In most cases the natural concentration must be treated as an unknown constant.

By definition the substrate uptake rate (v) is a function of substrate concentration and utilization time (turnover time T):

$$v = \frac{S_n + A}{T} \qquad (4)$$

where

T = time in which the population utilizes the concentration ($S_n + A$) to exhaustion if no supply occured

The turnover time T can easily be determined, using radioactively labelled substrates, from the equation

$$T = \frac{C \cdot t}{c} \qquad (5)$$

where

C = radioactivity added to the sample

t = time of experiment

c = radioactivity taken up by the micro-organisms during the experiment

Parsons and Strickland (1962) were the first to apply Michaelis-Menten kinetics to the analysis of substrate uptake by bacterioplankton. They calculated the substrate uptake rate by developing equations (4) and (5) to the form:

$$v = \frac{c \cdot f \cdot (S_n + A)}{C} \qquad (6)$$

where

f = isotopic discrimination factor for $^{14}C$ (1.05)

After adding radioactively labelled substrate to the divided water sample in different concentrations, Parsons and Strickland (1962) also calculated the concentration sum ($K + S_n$) with the aid of a linear function having double reciprocal form. Wright and Hobbie (1965, 1966) adopted the following equation, which they called the Lineweaver-Burk transformation:

$$T = \frac{K + S_n}{V} + \frac{A}{V} \qquad (T \text{ vs. } A) \qquad (7)$$

which was later widely adopted in aquatic-microbiological investigations. Using this equation it is possible on the basis of measurement results (A and T) to determine the kinetic parameters

$(y = a + bx;\ x = A;\ y = T)$

— maximum uptake rate $V = \frac{1}{b}$

— concentration sum $K + S_n = \frac{a}{b}$

— kinetic turnover time $T = a$

The graphic determination of these parameters is presented in Figure 2. The data for Kaskinen are presented in Figures 3a, 3b and 3c according to equation (7).

Equation (7) is not in fact a true Lineweaver-Burk transformation, but rather it resembles the transformation in equation (2b), to which are added equations (3) and (4). Other linear transformations suitable for investigations of natural populations include the equations presented by Tamminen (1980):

$$\frac{A}{T} = V - \frac{K + S_n}{T} \qquad (A/T \text{ vs. } 1/T) \qquad (8)$$

which has the form $y = a + bx$ ($x = 1/T$, $y = A/T$), from which we obtain

$V = a$

$K + S_n = -b$

$T = b/a$

or its reciprocal form:

$$\frac{1}{T} = \frac{V}{K + S_n} - \frac{A}{(K + S_n) \cdot T} \qquad (1/T \text{ vs. } A/T) \qquad (9)$$

which has the form $y = a + bs$ ($x = A/T$, $y = 1/T$), from which we obtain

$V = a/b$

$K + S_n = -1/b$

$T = 1/a$

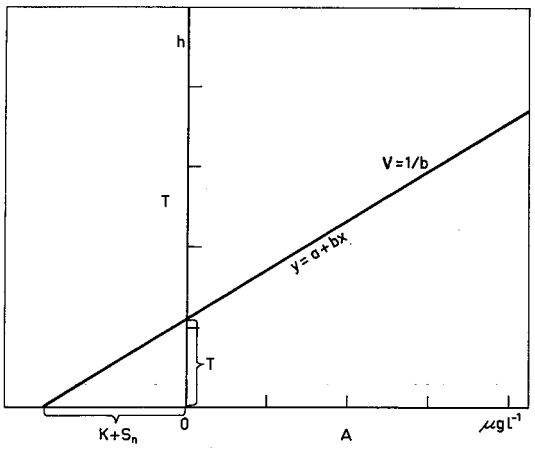


Fig. 2. Graphic determination of kinetic parameters.

The reciprocal turnover time (1/T) occurring in equations (8) and (9) is the measured substrate turnover rate ($h^{-1}$). A linear transformation corresponding to equation (8) has also been presented by Marxsen (1980). Because the dependent variable (A/T) of equation (8) can be interpreted as uptake rate (v', relative uptake rate,

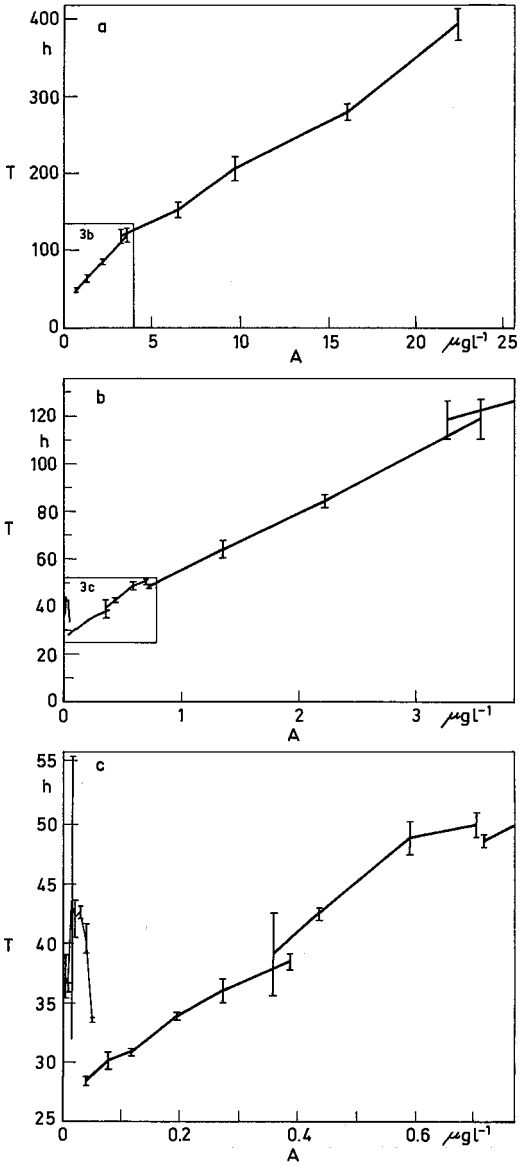


Fig. 3a, 3b and 3c. Turnover times (T) of the Kaskinen data as a function of the addition concentration (A) of glucose. The vertical lines indicate the standard deviations of three subsamples.

see Fig. 1), the equation closely resembles the Eadie—Hofstee plot (equation 2a):

$$v' = V - \frac{K + S_n}{T} \qquad (10)$$

All the linear transformations (eqs. 7, 8 and 9) have certain qualities calling for criticism. The determination of the regression line by the normal method of least squares (Model I regression) requires e.g. that the independent variable (x) is measured without error (see Sokal and Rohlf 1973). None of the equations completely fulfills this theoretical requirement, but the best situation is apparently obtained with equation (7). In the case of equation (9) the total experimental error (from the determination of both A and 1/T) loads the independent variable.

In equations (8) and (9) the same parameter (T) occurs on both sides of the equation, so that x and y are automatically correlated to some extent. This internal correlation, however, weakens the correlation observed between the parameters A/T and 1/T, because the relationship between them is negative (c.f. Dowd and Riggs 1965).

With regard to the parameter T it should be noted that its occurrence in reciprocal mode means that the measured parameter (c) occurs in the numerator (eq. 5). For this reason the critique of the weaknesses of measurement results having inverse form, as presented by Wilkinson (1961) and Dowd and Riggs (1965), applies to equation (7) but not to equations (8) and (9).

## 5. CONFIDENCE INTERVALS FOR PARAMETERS

The point estimation of kinetic parameters is usually not sufficient alone. Only with the aid of a given statistical confidence interval can a reliable picture be obtained of the applicability of the estimate. All the linear transformations (equations 7, 8 and 9) have the form $y = a + bx$. The kinetic parameters are functions of the constants a and b of the regression equation, and their confidence intervals are therefore calculated with the aid of the standard deviations of a and b. The calculation principles and a detailed procedure are presented in Appendix 1. Because the usual experimental procedure for the determination of kinetic parameters is the preparation of several replicate samples for each concentration of added substrate, equations for the calculation of the confidence intervals of the parameters are presented in this form. The method is based, with slight modifications, on the presentation of Hald (1967) and is directly applicable to calculations according to the commonest linear transformation (equation 7). If equations (8) or (9) are used, there are no duplicates in the calculation of the regression, and the calculations involved are therefore simpler.

The properties of different linear transformations were investigated using the data from Kaskinen. The linear regressions (equations 7, 8 and 9) calculated on the basis of this data are presented in Figures 4, 5 and 6. The confidence limits of the slopes were calculated as described in Appendix 1.

The most commonly used regression (equation 7) appears, on the basis of the location of points and the confidence interval of the regression line,

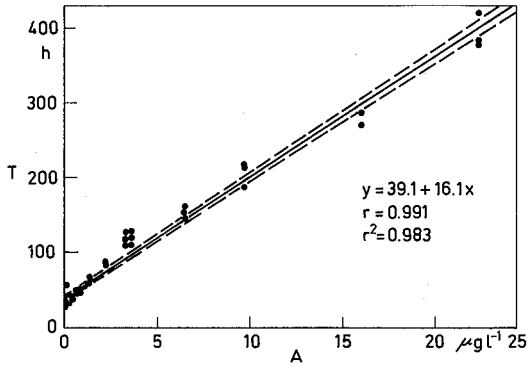


Fig. 4. The linear regression (equation 7) and its 95 % confidence interval of the Kaskinen data.

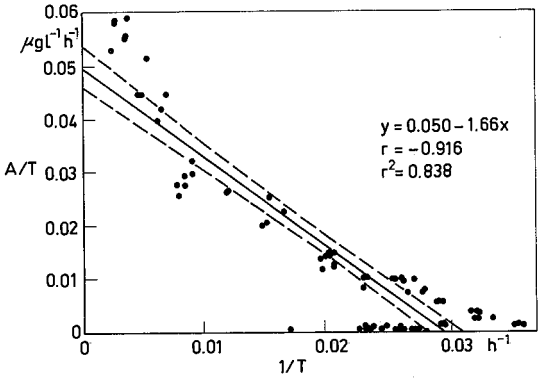


Fig. 5. The linear regression (equation 8) and its 95 % confidence interval of the Kaskinen data.

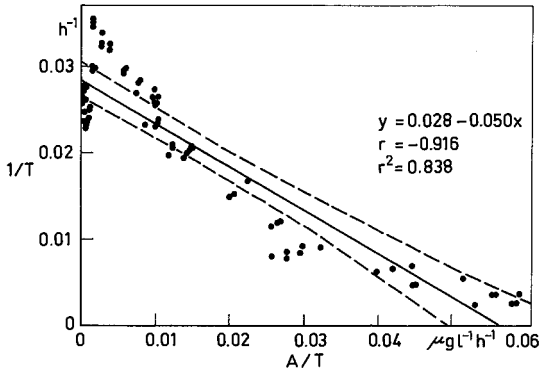


Fig. 6. The linear regression (equation 9) and its 95 % confidence interval of the Kaskinen data.

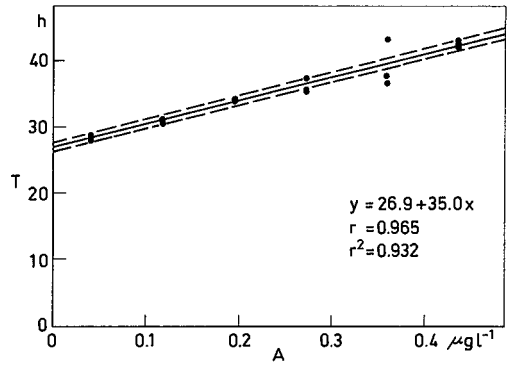


Fig. 7. The linear regression (equation 7) and its 95 % confidence interval of an equal-interval sample of the Kaskinen data.

to be clearly more linear than the other functions (Figs. 4—6). The correlation coefficient (r) and coefficient of determination ($r^2$) of the line are obviously the best. When the linearity was examined using the F-factor ($s_2^2/s_1^2$, in which $s_2^2$ is the variance about the regression line and $s_1^2$ the variance between replicates) this regression was, however, found to be highly significantly non-linear ($p = 0.01$). Although the correlation coefficients of the other regression lines also appear to be rather high, the data depart very clearly from linearity (see Figs. 5 and 6; it is not possible to apply the F-test to these data). Equation (8) behaved thus in the same manner as the standard Eadie-Hofstee transformation (2a), which emphasizes the possible nonlinearity of the data.

An investigation was made to determine to what extent the properties of the calculated regressions were dependent on the uneven distribution of the independent variable. The regressions calculated with the aid of a constant-interval sample from the data are presented in Figures 7, 8 and 9. The kinetic parameters (V, T and $K + S_n$), along with their confidence intervals and coefficients of variation, calculated with the equal-interval sample and for the whole data are shown in Table 1.

Quite different kinetic parameters are obtained for the same original data using the different linear transformations (Table 1). With fewer concentrations of added substrate, the differences between the confidence intervals of the kinetic parameters generated by the transformations increased, although the values of the parameters differed less in the sample than in the whole data. The relationship between the maximal uptake rates (from different transformations) and the relative uptake rates calculated from the data was presented in Figure 1.

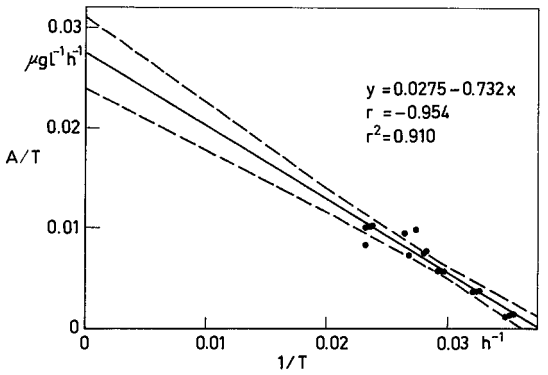


Fig. 8. The linear regression (equation 8) and its 95 % confidence interval of an equal-interval sample of the Kaskinen data.

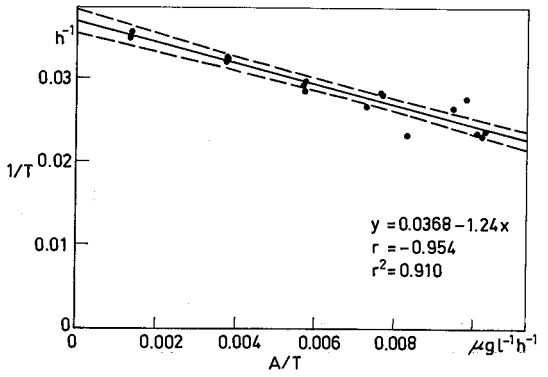


Fig. 9. The linear regression (equation 9) and its 95 % confidence interval of an equal-interval sample of the Kaskinen data.

Table 1. Kinetic parameters calculated for the complete data and for a constant-interval (six-addition) sample, their 95 % confidence intervals and coefficients of variation (CV % = $s/\bar{x} \cdot 100$ %).

| | Complete data | | | | Sample | | | |
|---|---|---|---|---|---|---|---|---|
| equation | parameter | | 95 % conf. limits | CV (%) | parameter | | 95 % conf. limits | CV(%) |
| 7 | V | = 0.062 ± | 0.002 $\mu g \cdot l^{-1} \cdot h^{-1}$ | 1.5 | V | = 0.029 ± | 0.000 $\mu g \cdot l^{-1} \cdot h^{-1}$ | 0.4 |
| | $K + S_n$ | = 2.42 ± | 0.19 $\mu g \cdot l^{-1}$ | 4.0 | $K + S_n$ | = 0.77 ± | 0.02 $\mu g \cdot l^{-1}$ | 1.3 |
| | T | = 39.1 ± | 2.9 h | 3.7 | T | = 26.9 ± | 0.7 h | 1.2 |
| 8 | V | = 0.050 ± | 0.004 $\mu g \cdot l^{-1} \cdot h^{-1}$ | 3.9 | V | = 0.028 ± | 0.004 $\mu g \cdot l^{-1} \cdot h^{-1}$ | 6.1 |
| | $K + S_n$ | = 1.66 ± | 0.17 $\mu g \cdot l^{-1}$ | 5.2 | $K + S_n$ | = 0.73 ± | 0.12 $\mu g \cdot l^{-1}$ | 7.9 |
| | T | = 33.4 ± | 4.3 h | 6.5 | T | = 26.6 ± | 5.6 h | 10.0 |
| 9 | V | = 0.056 ± | 0.011 $\mu g \cdot l^{-1} \cdot h^{-1}$ | 9.7 | V | = 0.030 ± | 0.004 $\mu g \cdot l^{-1} \cdot h^{-1}$ | 6.6 |
| | $K + S_n$ | = 1.99 ± | 0.011 $\mu g \cdot l^{-1}$ | 8.9 | $K + S_n$ | = 0.80 ± | 0.004 $\mu g \cdot l^{-1}$ | 7.9 |
| | T | = 35.2 ± | 2.6 h | 3.7 | T | = 27.1 ± | 1.1 h | 1.9 |

Examination of the coefficient of variation for the parameters indicates that the most frequently used regression (equation 7) gave the most reliable results for this data (smallest coefficient of variation). In the case of the actual kinetic parameters (V and $K + S_n$) the superiority of equation (7) in this respect was evident from the results obtained using both the sample and the whole data. In the case of the kinetic turnover time (T), however, the situation was less clear. Equation (9) generated, by a small margin, the most reliable turnover time when using the complete data, while in the case of the sample data the coefficients of variation obtained with equations (9) and (7) were very similar. Equation (8) gave results which were consistently poorer than those obtained with equation (7).

The linear regressions behaved thus in approximately the manner anticipated. The kinetic parameters were described most reliably by equation (7) in which there is no internal correlation between the dependent and independent variables to interfere with the overall correlation. Equation (8) clearly reacted more readily to departures from linearity within the data, and thus produced unreliable (linear) parameters. In practical investigations the most useful would therefore appear to be equation (7) if all the three kinetic parameters are to be measured. The turnover time can be measured with equal reliability using equation (9) according to this data.

As was shown by Down and Riggs (1965), the reliability of parameters determined using the double reciprocal plot (equation 2c) is however in some cases only apparent. As the actually measured parameter (turnover rate 1/T) in equation 7 occurs in reciprocal mode, the comments of Dowd and Riggs (1965) apply to this equation, particularly in the case of unevenly distributed data. Experimental procedures have often been presented in the literature in which substrate additions increased almost logarithmically, i.e. most of the additions took place over a small range of concentrations and a few or even a single large addition concentration dominated the slope of the regression line. This naturally increases the randomness in the fitting of the regression line, as the smallest measured turnover rates (1/T), producing highest turnover times (T), are strongly weighted in the procedure.

One result of this is that possible deviations from linearity within the data are hidden by a misleadingly high coefficient of determination of the regression line. However, it is of prime importance to observe deviations of the data from linearity, because the application of the standard enzyme kinetic analysis is dependent on the linearity of the data. Recently, the concept of multistep uptake kinetics of bacteria has been introduced to explain the often observed non-linearity in substrate uptake (see Fig. 5), when a wide range (several orders of magnitude) of substrate additions have been performed (Azam and Hodson 1981, Koch 1982).

Linearity can be monitored using equation (8) and also by calculating the F-value corresponding to equation (7). Some investigators have followed linearity only with the correlation coefficient corresponding equation (7), but as the present data demonstrated the correlation coefficient does not describe linearity with sufficient accuracy, especially if the data is distributed unevenly.

## 6. CONCLUSIONS

The uptake of substrate by heterotrophic microorganisms was monitored with the aid of Michaelis-Menten enzyme kinetic parameters, which were calculated by applying three different linear transformations to the same original data (measurement results). All three equations had the form $y = a + bx$:

$$x = A, \quad y = T \tag{7}$$
$$x = 1/T, \quad y = A/T \tag{8}$$
$$x = A/T, \quad y = 1/T \tag{9}$$

where
A = added substrate concentration
T = measured substrate turnover time (h)
1/T = measured substrate turnover rate ($h^{-1}$)

Equation (7), which is the frequently applied, so-called Lineweaver-Burk transformation, best approximates the requirement of the common (Model I) regression of errorless independent variable. However, in the equation (7) the measured parameter labelled substrate uptake is in the reciprocal mode, so that its small values strongly affect the slope of the regression line, thus increasing randomness in the determination of the kinetic parameters.

In equations (8) and (9) the same parameter (T) occurs on both sides of the equation, with the result that x and y are automatically correlated to a certain extent. This internal correlation, however, actually weakens the correlation observable between the variables A/T and 1/T, because the relationship between them is negative (c.f. Dowd and Riggs 1965).

The confidence intervals of the kinetic parameters calculated using the different linear transformations showed considerable differences. The kinetic parameters were determined with the greatest reliability using equation (7), in which there are no internal correlations between the dependent and independent variables to interfere with the overall correlation. Equation (8) reacted most sensitively to departures from linearity within the data and therefore produced the most unreliable (linear) parameters. In practical investigations the most utilizable of the equations studied woud appear to be equation (7), if all three kinetic parameters are to be determined. Turnover time may also be determined with equal reliability using equation (9).

The results also showed that the correlation coefficient did not describe the linearity of the data with sufficient accuracy, especially in the case of unevenly distributed data.

## LOPPUTIIVISTELMÄ

Planktisten heterotrofisten mikro-organismien substraatin ottoa seurattiin entsyymikineettisten muuttujien avulla, jotka laskettiin soveltaen kolmea eri Michaelis-Menten -kinetiikan lineaaritransformaatiota samaan perusaineistoon (mittaustuloksiin). Yhtälöt olivat muotoa $y = a + bx$

$$x = A, \quad y = T \tag{7}$$
$$x = 1/T, \quad y = A/T \tag{8}$$
$$x = A/T, \quad y = 1/T \tag{9}$$

joissa A = lisätty substraattipitoisuus, T = näytteestä mitattu substraatin kiertoaika (h) ja 1/T = näytteestä mitattu substraatin kiertonopeus ($h^{-1}$).

Yhtälö 7, joka on yleisesti käytetty nk. Lineweaver-Burk muunnos, täyttää parhaiten yleisimmin käytetyn (Model I) regressioanalyysin ehdon riippumattoman muuttujan virheettömyydestä. Sen sijaan yhtälöä rasittaa se, että mitattu muuttuja radioaktiivisen substraatin otto on käänteismuodossa, joten sen pienet arvot määräävät voimakkaasti regressiosuoran asettumisen. Tämä lisää satunnaisuutta kineettisten parametrien määrityksessä.

Yhtälöissä 8 ja 9 esiintyy sama tekijä (T) yhtälön kummallakin puolella, joten x ja y korreloivat väistämättä jonkin verran keskenään. Tämä sisäinen korrelaatio kuitenkin heikentää muuttujien A/T ja 1/T välillä havaittavaa korrelaatiota, koska näiden välinen suhde on negatiivinen (vrt. Down ja Riggs 1965).

Eri lineaarimuunnoksilla laskettujen kineettisten parametrien luottamusvälit poikkesivat selvästi toisistaan. Kineettiset parametrit määräytyvät luotettavimmin yhtälöllä 7, jossa riippuvan ja riippumattoman muuttujan välillä ei ole kokonaiskorrelaatiota häiritseviä sisäisiä korrelaatioita. Yhtälö 8 reagoi selvästi herkimmin aineiston poikkeamiin lineaarisuudesta, ja se tuotti näin epäluotettavimpia (lineaarisia) parametrejä. Käytännön tutkimuksissa käyttökelpoisimmalta vaikuttaa siis yhtälö 7, mikäli pyritään määräämään kaikki kineettiset parametrit. Kiertoaika voidaan määrittää yhtä luotettavasti myös yhtälöllä 9.

Tulokset osoittivat lisäksi sen, että korrelaatiokerroin ei kuvaa muuttujien välisen suhteen lineaarisuutta riittävän tarkasti, etenkään jos aineisto on jakautunut epätasaisesti.

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**Appendix 1.**
**Calculation of the standard deviations and confidence intervals of the kinetic on the basis of equation (7). The example data is a sample from the Kaskinen data with six concentrations of substrate addition. Each concentration was added in triplicate.**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A ($\mu g \cdot l^{-1}$) | ($x_i$) | 0.039 | 0.117 | 0.195 | 0.272 | 0.358 | 0.435 | $k = 6$ |
| | ($y_{i1}$) | 28.20 | 31.17 | 33.92 | 35.35 | 36.53 | 42.10 | $\bar{x} = 0.236$ |
| T (h) | ($y_{i2}$) | 28.53 | 30.69 | 34.16 | 32.27 | 37.71 | 43.03 | |
| | ($y_{i3}$) | 28.75 | 30.81 | 33.66 | 35.60 | 43.18 | 42.55 | |
| | $n_i$ | 3 | 3 | 3 | 3 | 3 | 3 | $\Sigma n_i = 18$ |
| | $\bar{y}_i$ | 28.49 | 30.89 | 33.91 | 36.07 | 39.14 | 42.56 | |
| | $\Sigma(y_{ij} - \bar{y}_i)^2$ | 0.153 | 0.125 | 0.125 | 2.179 | 25.18 | 0.433 | $\Sigma\Sigma(y_{ij} - \bar{y}_i)^2 = 28.19$ |
| | $Y_i$ | 28.28 | 31.01 | 33.74 | 36.44 | 39.47 | 42.14 | (y-values from regression) |
| | $n_i(\bar{y}_i - Y_i)^2$ | 0.132 | 0.043 | 0.087 | 0.411 | 0.327 | 0.529 | $\Sigma n_i(y_i - Y_i)^2 = 1.470$ |
| | $n_i(x_i - \bar{x})^2$ | 0.116 | 0.042 | 0.005 | 0.004 | 0.045 | 0.119 | $\Sigma n_i(x_i - \bar{x})^2 = 0.331$ |

regression: $y = a + bx$
$a = 26.92$
$b = 34.99$
$r = 0.965$
$r^2 = 0.931$

$$s_1^2 = \frac{\Sigma\Sigma (y_{ij} - \bar{y}_i)^2}{n - k} = 2.350$$ (variance between replicates)

$$s_2^2 = \frac{\Sigma n_i(\bar{y}_i - Y_i)^2}{k - 2} = 0.368$$ (variance about the regression line)

$$\text{F-value} = \frac{s_2^2}{s_1^2} = 0.156$$ ($n = 18, k = 6) \rightarrow F_{0.05} = 3.26$
i.e. the data is significantly linear

$$\text{pooled variance: } s^2 = \frac{\Sigma\Sigma (y_{ij} - \bar{y}_i)^2 + \Sigma n_i(y_i - Y_i)^2}{(n - k) + (k - 2)} = 1.854$$

Calculation of standard deviation of the regression constants a and b and of the regression line:

$$s_b = \frac{s}{\sqrt{n_i (x_i - \bar{x})^2}} = 0.133$$

$$s_a = \sqrt{\frac{s^2}{\Sigma n_i} + s_b^2 (\bar{x})^2} = 0.322$$

$$s_Y = s\sqrt{\frac{1}{\Sigma n_i} + \frac{(x - \bar{x})^2}{\Sigma n_i (x_i - \bar{x})^2}}$$ (standard deviation of the regression line)

From equation 7 we obtain: $V = 1/b$ ($= 0.029\ \mu g \cdot l^{-1}h^{-1}$)
$K + S_n = a/b$ ($= 0.770\ \mu g \cdot l^{-1}$)
$T = a$ ($= 26.92$ h)

from which the standard deviations of the parameters are determined on the basis of the above equations:

$$s_V = s_{1/b} = \frac{s_b}{b^2} = 0.0001\ \mu g\ l^{-1} \cdot h^{-1}$$

$$s_{K+S_n} = s_{a/b} = 1/b\sqrt{s_a^2 + \frac{a^2 \cdot s_b^2}{b^2}} = 0.0097\ \mu g\ l^{-1}$$

$$s_{T_k} = s_a = 0.32\ h$$

Confidence intervals (95 %) are calculated on the basis of standard deviations by the usual procedure

$V \quad (\pm t \cdot s_v) = 0.029 \pm 0.000(1)\ \mu g \cdot l^{-1}h^{-1}$
$K + S_n = 0.77 \pm 0.02\ \mu g \cdot l^{-1}$
$T = 26.9 \pm 0.7$ h