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SOME COMMENTS ON 'UREASE ACTIVITY AND ITS MICHAELIS CONSTANT FOR SOIL SYSTEMS', BY VIRAJ BERI *ET AL.**

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Key words

Soil urease

Summary

Recently Beri, Goswami and Brar made a very valuable contribution to our knowledge of soil urease activity. Unfortunately some errors crept into their experimental approach and the mathematical treatment of the experimental data. Rectification leads in some points to conclusions different from those of the authors.

Considerations

Approach I

Beri *et al.* used the following form of the integrated Michaelis-Menten equation to compute values of V_{max} and K_m:

$$(S_0 - S)/t = V_{max} + (K_m/t) \ln (S/S_0) \quad (1)$$

S_0 is the initial substrate concentration and S is the concentration after a certain incubation time t. But the authors substituted for S in Equation 1 their values of $(S_0 - S)$, thus leading to wrong values for V_{max} and K_m. Since their Table 2 gives urea hydrolysed (in m*M*) after 4, 8, 12 ..., 48 h, we can calculate from these data the proper S values to be used in Equation 1; see Table 1. Linear regression analysis of the plot of $(S_0 - S)/t$ versus $(1/t) \ln (S/S_0)$, leads according to eq. 1 to an intercept that equals V_{max} and a slope giving K_m. Using the data of Table 1, this gives the results shown in Table 2.

As can be seen from the correlation coefficients the correlations are rather poor, leading to large standard deviations for V_{max} and especially for K_m. What may be the reason for this?

At first sight the two italicized values in Table 1 strongly deviate from the trend. A closer look at the data suggests that a systematic error has been made; all the points calculated for t = 4 h strongly deviate from the regression lines. In connection with this, the decreases in urea concentrations during the first 4 h are larger than expected on the basis of V_{max}, K_m

* Viraj Beri, K. P. Goswami and S. S. Brar, Plant and Soil **49**, 105–115 (1978).

Table 1. Urea concentration S* (in m*M*) in soils incubated at 37°C and 50%, of the water holding capacity after various incubation periods

Soil type	Incubation period (h)									
	4	8	12	16	20	24	28	32	40	48
Gurdaspur silty loam	22.3	13.7	7.2	2.7						
Ludhiana silty clay loam	23.4	15.7	9.6	4.7	1.5					
Mangat silty loam	25.0	17.7	10.3	5.2	1.6					
Habowal silty clay loam	24.9	18.2	12.4	7.8	3.1	1.8	0.6			
Ludhiana loam	26.5	18.9	12.4	7.4	3.4	0.7				
Malerkotla sandy loam	28.3	21.1	14.7	9.7	4.7	0.9				
Malerkotla silty loam	27.6	(*19.8*)	17.3	13.0	9.4	6.1	3.6	1.6		
Amritsar silty loam	30.4	25.8	21.6	17.4	13.7	11.0	7.4	4.7	0.7	
Jamalpur sandy loam	36.0	31.0	26.3	22.0	(*20.4*)	15.4		8.4	3.9	(0.9)

* S at t = 0 is 36.2 m*M* urea for all the soils except 40.3 m*M* for Malerkotla sandy loam and 45.3 m*M* for Jamalpur sandy loam.

Table 2. V_{max} and K_m values determined by linear regression of $(S_0 - S)/t$ on $(1/t) \ln (S/S_0)$

Soil type	Number of points	Coefficient of correlation	$V_{max} \pm$ S.D.† ($mM.h^{-1}$)	$V_{max} \pm$ S.D.† ($\mu g\ N.g^{-1}.h^{-1}$)	$K_m \pm$ S.D.† (mM)
Gurdaspur silty loam	4	0.83	6.2 ± 1.7	34 ± 9	26 ± 12
Ludhiana silty clay loam	5	0.74	4.6 ± 1.2*	26 ± 7	19 ± 10
Mangat silty loam	5	0.83	3.6 ± 0.6**	20 ± 3	12 ± 5
Habowal silty clay loam	7	0.78	3.8 ± 0.7**	21 ± 4	18 ± 6*
Ludhiana loam	6	0.88	2.9 ± 0.3***	16 ± 1.5	9.4 ± 2.6*
Malerkotla sandy loam	6	0.60	3.2 ± 0.7*	16 ± 4	10 ± 7
Malerkotla silty loam	8	0.47	2.7 ± 0.9	15 ± 5	16 ± 12
Amritsar silty loam	9	0.75	1.5 ± 0.1***	8.6 ± 0.8	7.3 ± 2.5*
Jamalpur sandy loam	9	0.30	2.0 ± 0.7*	8.8 ± 3.0	10 ± 12

† S.D. = standard deviation; *, ** and ***: significant at the 5%, 1% and 0.1% level, respectively.

and S_0. This may be explained in various ways: Perhaps the first analyses were not done after 4 h but after somewhat longer incubation times. Or the initial urea concentrations S_0 may have been lower than expected on the basis of added urea and water; an indication for this is that Beri *et al.* found lower S_0 values than expected, but without mentioning this in their Tables. Another possibility is that urea was not homogeneously mixed with the soils, therefore higher rates of hydrolysis may have occurred than with homogeneous distribution of urea, especially during the first hours. To eliminate the uncertainty about what happened during the first 4 h, we can start our calculations at $t = 4$, using the concentrations determined at this time as S_0 values. In this way the number of points in the regressions is lowered by one, giving the results shown in Table 3. The improvement of correlations and accuracies of V_{max} and K_m is rather considerable.

Before comparing V_{max} and K_m values between soils it should be noted that the maximum incubation times used vary from 16 to 48 h. When we calculate V_{max} and K_m using only the first 4 or 5 data from Table 1, we get the results shown in Table 4. Comparison of Tables 3 and 4 shows that shortening incubation time mostly increases V_{max} and especially K_m considerably. Thus the results are shown to depend on incubation time. The values of V_{max} in $mM.h^{-1}$ must be converted into units per unit of dry soil to make them independent of the moisture content used during incubation. This is done on the basis of the moisture contents given by Beri *et al.* and the results are included in the Tables 2, 3 and 4.

Table 3. V_{max} and K_m values determined by linear regression of $(S_0 - S)/t$ on $(1/t) \ln (S/S_0)$

Soil type	Number of points	Coefficient of correlation	$V_{max} \pm$ S.D. ($mM.h^{-1}$)	$V_{max} \pm$ S.D. ($\mu g\ N.g^{-1}.h^{-1}$)	$K_m \pm$ S.D. (mM)
Gurdaspur silty loam	3	0.986	3.25 ± 0.23*	18.2 ± 1.3	9.3 ± 1.6
Ludhiana silty clay loam	4	0.968	2.58 ± 0.18**	14.4 ± 1.0	7.2 ± 1.3*
Mangat silty loam	4	0.951	2.27 ± 0.14**	12.7 ± 0.8	4.6 ± 1.1
Habowal silty clay loam	6	0.952	2.25 ± 0.15***	12.6 ± 0.8	7.8 ± 1.2**
Ludhiana loam	5	0.933	2.30 ± 0.16***	12.9 ± 0.9	5.9 ± 1.3*
Malerkotla sandy loam	5	0.878	1.98 ± 0.13***	10.0 ± 0.7	3.8 ± 1.2*
Malerkotla silty loam	6	0.965	1.71 ± 0.08***	9.6 ± 0.4	8.0 ± 1.1**
Amritsar silty loam	8	0.898	1.27 ± 0.06***	7.1 ± 0.3	4.6 ± 0.9**
Jamalpur sandy loam	6	0.921	1.72 ± 0.14***	7.7 ± 0.6	14 ± 3

Table 4. V_{max} and K_m values determined by linear regression of $(S_0 - S)/t$ on $(1/t) \ln (S/S_0)$

Soil type	Number of points	Coefficient of correlation	$V_{max} \pm$ S.D. ($mM.h^{-1}$)	$V_{max} \pm$ S.D. ($\mu g\ N.g^{-1}.h^{-1}$)	$K_m \pm$ S.D. (mM)
Gurdaspur silty loam	3	0.986	3.25 ± 0.23*	18.2 ± 1.3	9.3 ± 1.6
Ludhiana silty clay loam	4	0.968	2.58 ± 0.18**	14.4 ± 1.0	7.2 ± 1.3*
Mangat silty loam	4	0.951	2.27 ± 0.14**	12.7 ± 0.8	4.6 ± 1.1
Habowal silty clay loam	4	0.886	2.04 ± 0.20**	11.4 ± 1.1	5.5 ± 2.0
Ludhiana loam	4	0.981	2.75 ± 0.15**	15.4 ± 0.8	10.4 ± 1,4*
Malerkotla sandy loam	4	0.925	2.36 ± 0.21**	11.9 ± 1.1	8.1 ± 2.4
Malerkotla silty loam	3	0.999	2.27 ± 0.02**	12.7 ± 0.1	16.8 ± 0.3**
Amritsar silty loam	4	0.952	1.57 ± 0.11**	8.8 ± 0.6	10.6 ± 2.4*
Jamalpur sandy loam	3	0.998	2.10 ± 0.06*	9.4 ± 0.3	23 ± 1.5*

Relationship between V_{max} ($\mu g\ N.g^{-1}.h^{-1}$) and organic carbon content (%) of the soils. Linear regression of V_{max} on org. C, based on the data of the Tables 2, 3 and 4, respectively, leads to the relationships 2a, 2b and 2c:

$$V_{max} = 7.7 + (27 \pm 4) \text{ org. C with } r = 0.92^{***} \quad (2a)$$
$$V_{max} = 7.4 + (11 \pm 2) \text{ org. C with } r = 0.86^{**} \quad (2b)$$
$$V_{max} = 10 + (7 \pm 3) \text{ org. C with } r = 0.65 \quad (2c)$$

Although the equations 2a and 2b show significant correlations between V_{max} and org. C, their regression coefficients for org. C are very different; Equation 2c, based on only 4 or 5 data per soil, does not show a significant correlation. This shows that the results clearly depend on the experimental approach and on the mathematical treatment of the experimental data.

Relationship between K_m (in mM) and V_{max} ($\mu g\ N.g^{-1}.h^{-1}$) of the soils. Linear regression of K_m on V_{max}, based on the data of the Tables 2, 3 and 4, respectively, leads to the relationships 3a, 3b and 3c:

$$K_m = 2.2 + (0.64 \pm 0.12)\ V_{max} \text{ with } r = 0.90^{***} \quad (3a)$$
$$K_m = 7.4 + (0.01 \pm 0.34)\ V_{max} \text{ with } r = 0.01 \quad (3b)$$
$$K_m = 19 - (0.65 \pm 0.70)\ V_{max} \text{ with } r = 0.33 \quad (3c)$$

These results show that calculations based on expected S_0 values (Table 2) will lead to quite contrary conclusions than calculations based on S_0 values determined by analysis (Tables 3 and 4). Where Equation 3a shows a significant correlation between K_m and V_{max}, Equations 3b and 3c show that K_m and V_{max} are not correlated.

Use of $t_{\frac{1}{2}}$, the time needed to hydrolyse 50% of added urea, as a measure of urease activity. Beri *et al.* determined $t_{\frac{1}{2}}$ for each soil from the best fitting equation of urea hydrolysed as a certain quadratic function of time. Then $t_{\frac{1}{2}}$ was calculated as the time at which S had reached 50% of S_0, using this quadratic equation.

Using Equation 1 with substitution of $S = S_0/2$, we can rearrange to

$$t_{\frac{1}{2}} = (1/V_{max}) \cdot (S_0/2 + K_m \ln 2) \quad (4)$$

From Equation 4 it follows that $t_{\frac{1}{2}}$ depends on S_0. Since Beri *et al.* used various S_0 values to determine $t_{\frac{1}{2}}$, it is incorrect to compare these values directly. To determine $t_{\frac{1}{2}}$ for comparison between soils, one should start with equal initial concentrations and also use the same moisture content for every soil. Equation 4 shows that $t_{\frac{1}{2}}$ is inversely proportional to V_{max}; its usefulness is limited, however, because it depends also on K_m and on S_0 used.

Approach II

Beri *et al.* also calculated V_{max} and K_m from the following transformation of the Michaelis-Menten equation to a straight line:

$$(S_0/v_0) = K_m/V_{max} + (1/V_{max})S_0 \quad (5)$$

where v_0 is the initial velocity of hydrolysis $-(dS/dt)$ at $t = 0$ and urea concentration $S = S_0$; see Fig. 1.

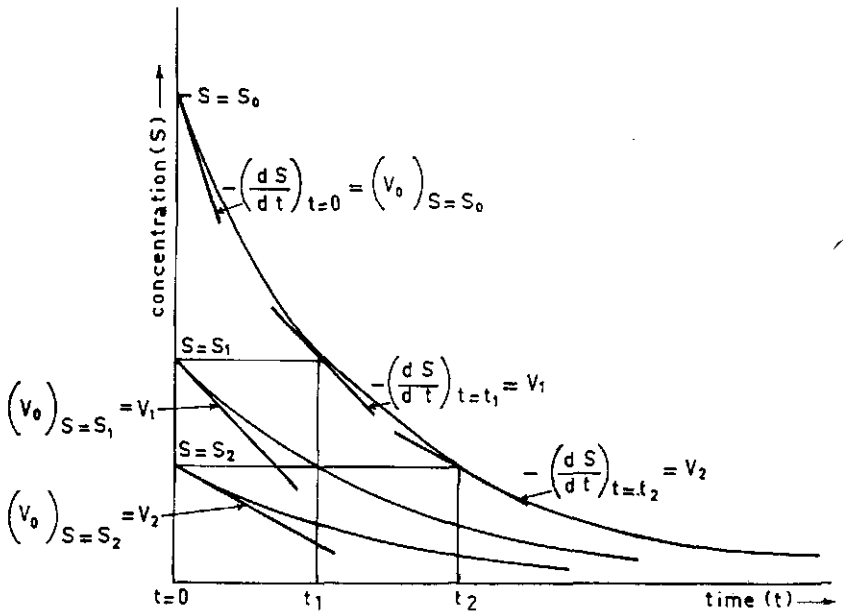

Fig. 1. Change of substrate concentration as a function of time.

Using various initial urea concentrations S_0, they determined the mean rate of hydrolysis (v) over 5 h incubation time, instead of v_0. Then they substituted their value of v into eq. 5. From Fig. 1 it can be seen that this mean rate does not equal v_0, corresponding with S_0, since $(S_5 - S_0)/t$ is not equal to $-(dS/dt)$ at $t = 0$.

In fact, the values of v determined by Beri *et al.* are lower than v_0 and their values of S_0/v higher than S_0/v_0. We tried to find the values of V_{max} and K_m that should result i f v_0 had been correctly applied in Eq. 5. Using the data plots of Fig. 1 from Beri *et al.* we calculated the values of v at the various values of S_0, and from these $(S_0 - S_5)$ and S_5/S_0. Then we applied again Eq. 1 with $t = 5$ to calculate V_{max} and K_m. The results of this recalculation are shown in Table 5. For Gurdaspur silty loam we could not recalculate V_{max} and K_m from the data plot of Beri *et al.* with sufficient accuracy. The recalculated V_{max} values are almost equal to those of Beri *et al.*; the K_m values after recalculation are lower than those of Beri *et al.* The rather high correlation coefficients indicate that also when S_0 is varied at a constant incubation time of 5 h, Michaelis-Menten kinetics are followed.

To compare this approach with Approach I, we have to keep in mind the experimental differences between them: the solution was buffered at pH = 7.2, toluene was added and the 1:1 soil buffer suspension was shaken during incubation only in Approach II. A further complication is that in Approach II either higher concentrations are used (Mangat silty loam) or lower concentrations (Jamalpur sandy loam) than in Approach I. To compare the V_{max} and K_m values of Table 5 (5 h incubation time) with the corresponding

Table 5. V_{max} and K_m values determined by linear regression of $(S_0 - S)/t$ on $(1/t) \ln (S/S_0)$; Approach II

Soil type	Number of points	Coefficient of correlation	$V_{max} \pm$ S.D.† ($mM.h^{-1}$)	$V_{max} \pm$ S.D. ($\mu g\ N.g^{-1}.h^{-1}$)	$K_m \pm$ S.D. (mM)
Mangat silty loam	10	0.952	24.8 ± 1.4	139 ± 8***	24 ± 3***
Jamalpur sandy loam	7	0.988	1.35 ± 0.04	6.0 ± 0.2***	1.7 ± 0.1***

† Recalculated for the same moisture content as in Table 4.

values in Table 4 (16 and 12 h incubation time), we calculated initial velocities v_0, according to Eq. 5, in the range of S_0 between 2 and 80 mM. We found that v_0 is always much higher in the case of Approach II than in Approach I with Mangat soil. For Jamalpur soil we found that v_0 is higher in Approach II than in Approach I when $S_0 < 35.5$ mM, while at higher concentrations the opposite is true. Before valid conclusions can be drawn from these differences between both approaches, more investigations are needed with different soils and over a wide range of concentrations.

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