Conductivity Method for Estimation of Urease Activity

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An accurate and rapid method has been developed for the estimation of urease activity, based on the difference in electric conductivity of urea and ammonium carbonate produced from urea by urease in the solution. Examples are given in detail.

Urease may be produced by many microorganisms and higher plants. It plays a predominant role in the catalytic or biological hydrolysis of urea. A suitable method for measuring its activity will greatly enhance studies dealing with the biological functions of urease and transformations of urea in plants and soil.

The classic methods for the estimation of urease activity are based on the determination of NH_3 or CO_2 evolved from the conversion of urea by urease in solution (*4*, *6*). Because of complexity of the procedures, it is often rather difficult to obtain accurate values at a specified reaction time. The conductivity method presented here eliminates this difficulty. Principles on which this method is based have been discussed in detail (*1*).

Experimental

Apparatus and Reagents. Conductivity bridge and cell.

Urea solution, 1000 p.p.m.

Urease solution (Table III), 25 p.p.m. (Nutritional Biochemical Corp.)

Preparation of Standard Curve. Standard curves may be constructed directly by using the data given in Table I. These curves are straight lines and are obtained by plotting $L_{ae} \times 10^4$ against their corresponding urea concentrations, where L_{ae} represents the conductivity of ammonium carbonate produced from urea by urease (*1*).

Determination of Conductivity. The urease activity in plants or in commercial products may be estimated by measuring the changes in specific conductivities, L's, of the sample-urea solution at a constant temperature. The following specific conductivities are to be measured (*1*, *2*).

L_o. Pipet 10.0 ml. of sample solution into a conductivity cell. Add 10.0 ml. of water, mix gently, and measure the resistance, R_o. Calculate its specific conductivity, L_o, and express as $L_o \times 10^4$.

L_t. Using the same amount of sample solution as for L_o, add 10.0 ml. of a 1000-p.p.m. urea solution 3 to 4 seconds before recording time. Mix gently and record time, t, and resistance, R_t, for several intervals during the first 10 minutes after the addition of urea solution. Calculate the conductivities, L_t, and express as $L_t \times 10^4$.

L_{ae}. Calculate the specific conductivity of ammonium carbonate, L_{ae}, at time t by subtracting L_o from L_t. Express L_{ae} in $L_{ae} \times 10^4$.

The reaction rate, k, is calculated from the urea concentrations associated with $L_{ae} \times 10^4$ values on the standard curve at different reaction times; the urease activity is calculated from the reaction rate.

Estimation of Urease Activity of Plants. In most plants, the urease content is too low to be estimated directly. In that case, it may be concentrated before measurements are made (*5*). In this experiment, 9-day-old, water-cultured soybeans and English peas were used. Samples were prepared by grinding 1.0 gram of plant material in a mortar and filtering after dilution to 40.0 ml. with water. The method for determining the amount of urea hydrolyzed has been described (*1*). The pH of the testing sample solution

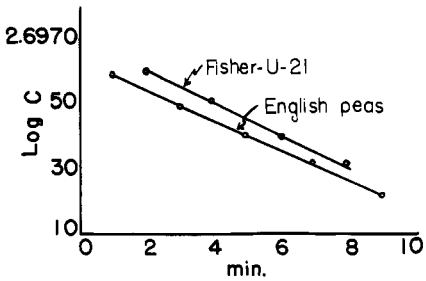

Figure 1. Kinetics of urea hydrolysis by diluted Fisher-U-21 urease and English pea solutions

Table I. Data for Standard Curves

	Urea, P.P.M.	
	0–40–90	80–400
	$L_{ae} \times 10^4$	
24–25° C.	0–1.016–2.075	1.861–8.004
26–27° C.	0–1.063–2.166	1.955–8.329

Table II. Kinetics of Urea Hydrolysis of Soybeans

(Cell constant = 0.32184 cm.$^{-1}$)

t, Sec.	R, Ohms	$L \times 10^4$ Ohm^{-1} Cm.$^{-1}$		Urea Hydrolyzed, P.P.M.	C, Urea Remaining, P.P.M.	Log C
		L_t	L_{ae}			
0	3600[a]	0.8940[b]	...	0.0	500.0	2.6990
45	1480	2.1746	1.2806	50.0	450.0	2.6532
195	720	4.4700	3.5760	162.0	338.0	2.5289
315	558	5.7640	4.8700	228.0	272.0	2.4330
435	471	6.8340	5.9500	281.0	219.0	2.3404
585	408	7.8820	6.9942	334.0	166.0	2.2201

[a] R_o; *all other values are* R_t. [b] L_o; *all other values are* L_t.

Table III. Urease Activity Estimation by Two Procedures

P.P.M. of Urea Hydrolyzed at t (Min.) by a Diluted Urease Soln.					
2	4	6	8	$k \times 10^4$	U./G.
		Conductivity Method			
4.6	8.0	10.4	12.5	2.7252	136.26
4.8	8.0	10.2	12.6	2.6485	132.43
4.5	7.9	9.8	12.5	2.7252	136.26
4.5	8.0	9.8	12.6	2.7055	135.28
				Av.	135.11
				Std. dev.	2.41
				Std. err.	1.39
		Direct Nesslerization (*3*)			
5.5	7.6	9.5	10.5	2.0151	100.76
6.2	8.5	11.5	13.0	2.6484	132.42
6.0	7.5	10.5	14.2	2.2454	112.27
4.0	7.5	10.0	14.0	3.0515	152.51
				Av.	124.51
				Std. dev.	22.84
				Std. err.	13.20

was about 7. Results for soybeans and English peas are shown in Table II and Figure 1, respectively. Data were obtained under the following conditions:

Initial concentration of urea solution, 500 p.p.m.
Temperature. 27° C.
Unit. Let 100 p.p.m. of urea per 20.0 ml. per minute = 1 unit (*U*).
Specific activity, *U* per gram of sample.

When log *C* is plotted against *t*, as shown in Figure 1, a straight line is obtained. The reaction rate may be calculated according to the first-order reaction (*2*)—i.e., $k = \frac{2.303}{585-45} \times (2.6532 - 2.2201) \times 60 = 0.1108$ per minute. The rate of urea hydrolysis in this sample-urea solution is 55.4100 p.p.m. per 20.0 ml. per minute. If 1 unit represents 100.0 p.p.m. per 20.0 ml. per minute, the urease activity of the testing sample solution will be 0.5541 unit, and the specific activity in the original plant is 4.4328 units per gram of sample. Urease activity of English peas was measured and calculated by the same procedure. The specific urease activity was 0.1678 unit per gram of sample.

Estimation of Urease Activity of Commercial Product. The testing sample solution of this experiment was prepared by diluting 5.0 ml. of a urease solution, having a concentration of 2.0 grams of Fisher-U-21 urease powder per liter, to 250 ml. with water. Results are summarized in Figure 1. The specific urease activity was calculated as 266.2500 units per gram of sample.

The proportions of these specific urease activities (estimated) are: soybeans : English peas : Fisher-U-21 = 4.4328 : 0.1678 : 266.2500 = 26.4 : 1.0 : 1586.7.

Discussion

Because of its simplicity and rapidity, the method presented is well suited for routine measurements. The preparation of new solutions for the estimation of urease activity at each reaction time is eliminated, which constitutes a great advantage over older methods. This procedure is, furthermore, directly applicable to plant materials. Similar recoveries of known amounts of urea by commercial and plant urease showed that colored impurities and other substances present in plant materials had no significant influence on the urease activity estimation. A comparison with an established method is shown in Table III.

The influence of a pH range from 6 to 8 on urea hydrolysis by urease was found to be nonsignificant (*1*). However, for accurate estimation of urease activity, it is advisable to adjust the pH's of samples from different sources to the same pH value, especially when the pH's of the sample solutions vary from less than 6 to more than 8. A pH of 7 is considered to be optimum for urease activity.

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