# THE TIME-COURSE OF SPORE GERMINATION*

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Investigations on the mechanism of spore germination involve a comparison of the effects of different agents on the germination rate. The decrease in optical density (OD) of the spore suspension has been used as a measure of the extent of germination (Powell, 1950). Germination rates have been expressed as the percentage decrease in OD during a constant time interval (Rode and Foster, 1962) or as the slope of the straight line portion of a semilogarithmic plot of the per cent of ungerminated spores vs. time (Hachisuka et al., 1955; Woese, et al., 1958; O'Connor and Halvorson, 1961). It was reported by Woese et al. (1958) that a plot of log $\frac{OD_t - OD_f}{OD_i - OD_f}$ vs t (where $OD_t$ = OD at time t, $OD_i$ = initial OD and $OD_f$ = OD at 100% germination) yields a linear function, after an initial lag, from which the germination rate can be calculated.

Analysis of a large number of germination curves obtained by continuously recording the decrease in OD during germination demonstrated that the straight line portion of the semilogarithmic plot spans a relatively short period

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and that, as germination proceeds, each curve deviates from linearity. These analyses have led to the finding that a plot of lnln $\frac{1}{Y}$ vs ln t (where $Y = \frac{OD_i - OD_t}{OD_i - OD_f}$, the fraction of the total reaction completed in time t) results in a linear relationship throughout the course of every germination curve analyzed. Thus

$$\ln \ln \frac{1}{Y} = -c \ln t + \ln \ln \frac{1}{Y_o} \qquad (1)$$

where -c equals the slope of the line and $\ln \ln \frac{1}{Y_o}$ equals $\ln \ln \frac{1}{Y}$ at t = 1. From equation (1) the following equation describing spore germination can be derived:

$$Y = e^{-kt^{-c}} \qquad (2)$$

where k equals $\ln \frac{1}{Y_o}$ (or $2.303 \log \frac{1}{Y_o}$). Since $Y = \frac{OD_i - OD_t}{OD_i - OD_f}$ then

$$OD_t = OD_i - (OD_i - OD_f)\, e^{-kt^{-c}} \qquad (3)$$

Two typical germination curves are presented in Fig. 1. The fit between experimental values and those predicted by equations (4) and (5) is readily apparent. Fig. 2 shows a plot of the data according to equation (1) from which the values of the constants c and k are determined. The equation describing germination curve A is

$$OD_t = .436 - .170\, e^{-14.0\, t^{-1.80}} \qquad (4)$$

and that describing curve B is

$$OD_t = .478 - .266\, e^{-6.50\, t^{-1.73}} \qquad (5)$$

The concentration of L-alanine used to induce germination appears to effect the value of k but not of c. It has been noted that a change in other variables such as tem-

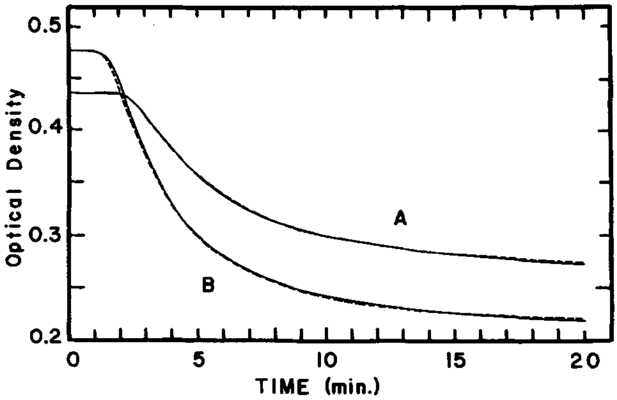

Fig. 1. Decrease in optical density during germination as a function of time. Spores of Bacillus cereus were prepared as described by Church et al. (1954). An aqueous suspension of clean, washed spores was activated by heating in a water bath at 65° for 4 hrs., cooled, centrifuged and resuspended in 0.05 M tris(hydroxymethyl)aminomethane buffer, pH 8.5. Germination was carried out at 30° in a Beckman DK-2 recording spectrophotometer at 625 mμ. The reaction was initiated by the addition of L-alanine to a buffered spore suspension contained in a 3 ml cuvette. The germination shown in curve A was initiated by the addition of L-alanine to a final concentration of 0.001 M and that in curve B by 0.10 M L-alanine. The solid lines represent the continuous recording and the dashed lines represent values calculated from equations (4) and (5). The $OD_f$ values were determined after 100 min.; $OD_f$ for curve A = .266, $OD_f$ for curve B = .212.

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perature, duration of prior heat activation or the nature of the germination inducer, gives rise to families of approximately parallel lines as in Fig. 2, but with different values for c than noted above. The effect of changes in these variables on the constants k and c is currently under investigation.

A complete derivation of equation (2) will be presented elsewhere together with applications and use of the equation in determining limiting (equilibrium) values of reactions and in calculating reaction rates.

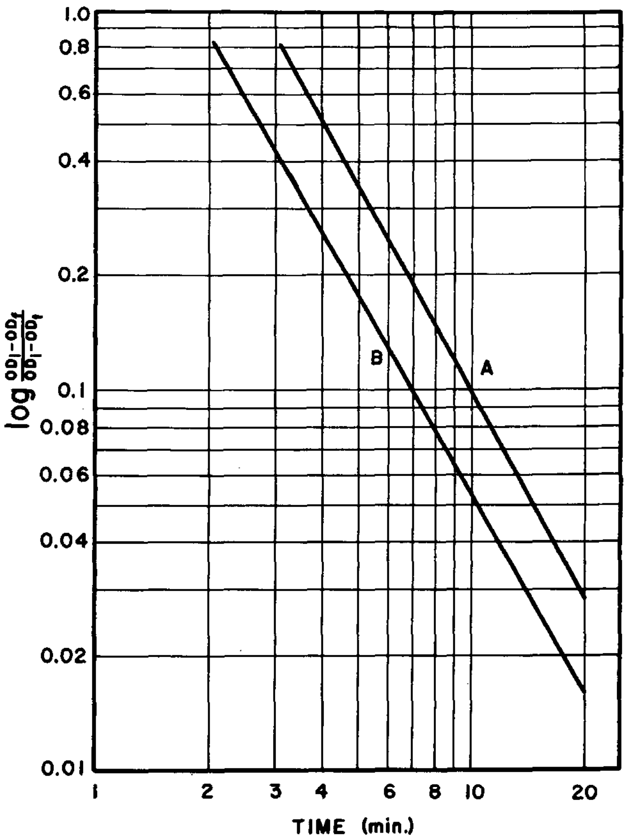

Fig. 2. Plot of log log $\frac{OD_i - OD_f}{OD_i - OD_t}$ vs log t

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