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Correction for the Inherent Error in Optical Density Readings

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Except at very low levels, uncorrected photometric determinations of bacterial cell densities show a decreasing proportionality to actual cell density or dry weight. A standard curve was prepared to convert photometric readings to truly proportional optical density values. With one dry weight determination, optical density values may be converted to absolute dry weight values.

The photometric determination of bacterial concentrations depends primarily on light scattering rather than light absorption. Yet, a useful relationship similar to the Beer-Lambert law of absorption exists; optical density (OD) = $\log I_0/I = kc$ (3). This proportionality is true only for a limited range (2-4) (Fig. 1), due to secondary scattering as the concentration of particles increases (2). Consequently, dilutions and/or standardizations are necessary for accurate determination of culture densities. From the literature, it seems that few authors consider this limitation important enough to even mention any standardization or corrections. Correction of the deviation from the ideal Beer-Lambert relationship can be achieved by the use of an equation (4); however, one equation will not be sufficient for all instruments. Indeed, Koch (2) showed that the equation will differ not only for different instruments, but even for different apertures, slit widths, and wavelengths in the same instrument. Since a separate corrective equation must be derived for each particular instrument and setting, it may be desirable to forgo the sophistication of equations and use a calibration curve instead. Our graphical method can be used to convert percent transmission (T) readings on cell suspensions to true OD values or to obtain absolute values, such as dry weights.

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Starting with a dense suspension of *Escherichia coli*, careful doubling dilutions were made to obtain a series of cell suspensions of accurately known relative density (RD). The percent transmission of each of the cell suspensions was read in the photometer (Bausch and Lomb Spectronic 20) and the uncorrected OD of each was calculated (OD = $2\text{-}\log_{10}T$). It is only at low values that these uncorrected ODs are proportional to the known RD values; the increasingly greater underestimation of ODs at the higher levels is readily apparent (Table 1). Since uncorrected OD values of the more dilute cell suspensions are very nearly proportional to their known RD values, it was taken as a working assumption that the uncorrected OD of the most dilute suspension (0.041) is the true OD. Multiplying each of the RD values by this basic or "unit" OD value should give true OD values for all the cell suspensions. To minimize the effect of experimental error, it would be beneficial to derive the unit OD value from as many photometer readings as possible. When all the uncorrected OD values are divided by their respective RD values, it is seen that the quotients for the three most dilute cell suspensions are essentially constant; the others decrease progressively. Accordingly, the average of the three lowest quotients (0.041) was taken as the unit OD value. The RD values were then multiplied by it to obtain true ODs for all the cell suspensions. Using these corrected data, a standard curve was plotted (Fig. 2), from which

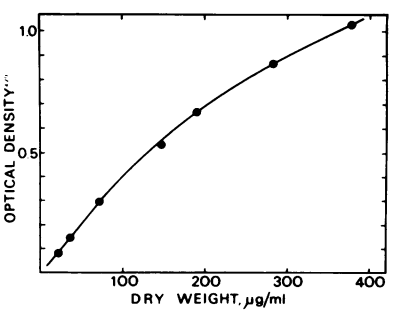

FIG. 1. *The relationship of uncorrected optical density at 420 nm to dry weight in cell suspensions of E. coli. Cells were grown in 1% yeast extract - 1% peptone broth at room temperature for 15 h in a gyratory shaker, washed, suspended, and diluted in distilled water. Aliquots were dried at 85°C to constant weight.*

TABLE 1. *Cell suspensions of known relative density: conversion of photometric readings to optical density and calculations of true optical density values.*

Dilution of cell suspension	RD[a]	Percent transmission	Uncorrected OD[b]	OD/RD	True OD[c]
None	64	5.5	1.260	0.020	2.624
1:2	32	12.6	0.900	0.028	1.312
1:4	16	28	0.553	0.035	0.656
1:8	8	50	0.301	0.038	0.328
1:16	4	69	0.161	0.040	0.164
1:32	2	82	0.086	0.043	0.082
1:64	1	91	0.041	0.041	0.041
				0.124[d]	
				0.041[e]	

[a] Individual dilution/highest dilution.
[b] OD = 2 − log T.
[c] True OD = unit OD × RD.
[d] Sum of underlined OD/RD ratios.
[e] Average (unit OD).

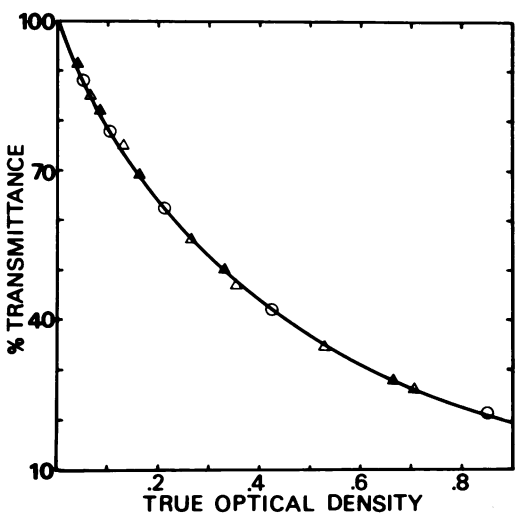

FIG. 2. *Standard curve for the conversion of instrumental readings of percent transmission to proportional or true OD values, by methods explained in the text and in Table 1. The curve was derived from three experiments (△,▲,○). Conditions were the same as for Fig. 1, except that 1% yeast extract − 1% peptone broth was substituted for the distilled water and T of the dilutions was read at 525 nm against a blank of 1% yeast extract − 1% peptone broth.*

the true ODs of unknown bacterial cell suspensions can be read after determining the T.

The necessity of such correction in determining the density of any but the most dilute cell suspension is shown by measurements made on a growing culture (Fig. 3). The two growth curves for an *E. coli* culture were determined by calculating the uncorrected OD from T readings, and then by reading true OD from the standard curve. Since the ordinate of Fig. 3 is exponential, the extent of real difference between the two curves is not readily apparent. However, the bar graph shows that around the modest OD level of 0.5, one-fourth of the culture's productivity would go undetected if corrections were not made, and the percentage of error becomes considerably greater at higher culture densities. Also, the two curves necessarily indicate different growth rates. Use of uncorrected OD values could be the cause of failure to obtain (5) the direct proportionality of maximum cell yield to concentration of a limiting nutrient that is expected (1) in bacterial growth.

When true OD values are plotted against actual dry weights of cells (Fig. 4), a straight line relationship is obtained, supporting the validity of this method of correcting the experimental readings. (Compare with Fig. 1, uncorrected OD against dry weight.) In an effort to simplify this procedure, a careful dry weight determination of the most concentrated cell

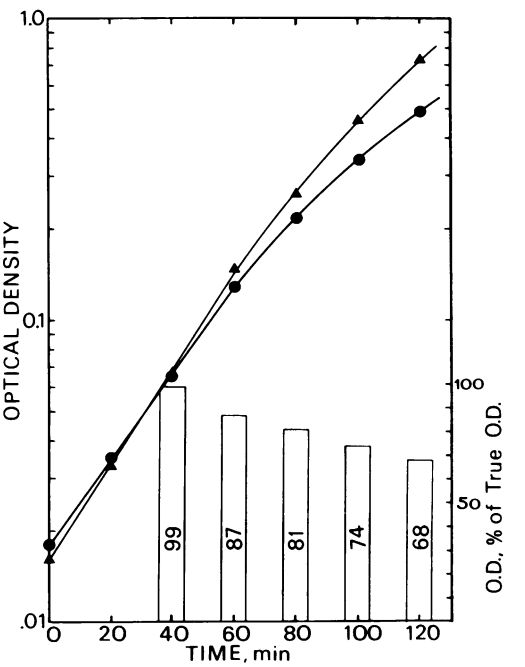

FIG. 3. *Growth curves of a single culture of E. coli plotted from uncorrected OD and true OD values. The discrepancy between the two curves is emphasized by the bar graph, where uncorrected OD values are expressed as percent of the true OD values. The shaken culture was growing in 1% yeast extract-1% peptone broth in side arm flasks at 37°C. Readings were taken as for Fig. 2 and converted to uncorrected OD (●) and true OD (▲).*

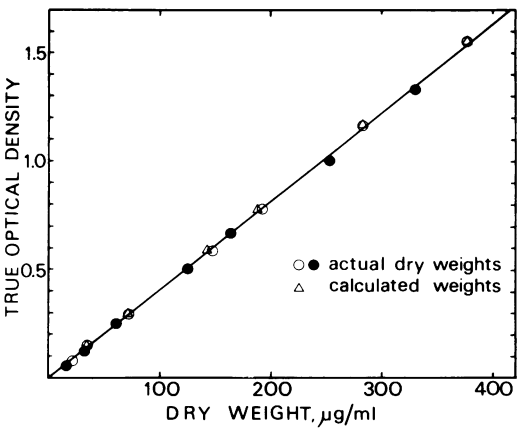

FIG. 4. *The relationship of true OD to dry weight in cell suspensions of E. coli. Experimental conditions were the same as for Fig. 1. Actual dry weights were determined for each cell suspension of two separate cultures (○,●). The triangle points were derived by determining the dry weight of only the most concentrated suspension and calculating dry weights for the other cell suspensions from the RD values.*

suspension was made, and dry weights of the diluted suspensions were calculated according to the RD values. Plotting these dry weight values against the corresponding true OD values also gave a straight line (triangle points, Fig. 4), identical to the line obtained when dry weight determinations were done on each cell suspension. Thus, standardization for the determination of absolute dry weights from OD readings need not be unduly laborious.

Toennies and Gallant (4) were fortunate in being able to use the same standardization equation for two different models of Coleman instruments, but they could not use it for the Klett-Summerson instrument. Our results with a number of different instruments (data not shown) agree with Koch's (2) and emphasize the need for preparation of a separate standard curve for each combination of instrument, wavelength, and type of cuvette. However, variations due to bacterial strain and culture medium, as well as any other indeterminate or unknown ones, are corrected for by use of the standard curve.

We emphasize again that large errors are introduced into photometric determinations made on bacterial cultures unless suitable corrections are applied before use of the basic instrumental readings.

ACKNOWLEDGMENTS

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