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Estimating the Size and Concentration of Unicellular Microorganisms by Light Scattering

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The optical density of suspensions of microorganisms was measured at two different positions in a spectrophotometer cuvette compartment. From these two measurements, the particle size and the particle concentration could be estimated. Estimates of particle numbers were not significantly different from those obtained by direct microscopic counting.

In contrast to the amount of experimental data in the literature on light scattering by inert particles, polymers, and mitochondria, few experimental data are available on the measurement of cell size and numbers by light scattering alone. In practice, turbidimetric measurements are calibrated for a specific microorganism by independent volumetric, dry weight, protein nitrogen, or direct microscopic counting determinations. Oster (6) and Lanni and Campbell (5) observed spectral scattering by bacteria but did not present quantitative data. Koch (2, 3) discussed the theoretical aspects of light scattering by bacteria and the application of the Mie and Rayleigh-Gahs theories. The experimental data (2) are limited to a study of the wavelength dependence of turbidity of *Escherichia coli*; the slope of a log-log plot of A versus wavelength was found to be 2.28 and in agreement with the above mentioned theories. Fikhman (1) presented similar data for cocci of various diameters; the slopes varied from 0.94 to 2.11 and were dependent on the cell size. More recently, Koch and Ehrenfeld (4) tested the theoretical predictions made by Koch (2, 3). A modified Zeiss PMQ II spectrophotometer was used to measure the angular light scattering from two different populations of *E. coli*. According to the author's best estimates, the average cell volumes of the two different populations were 0.37 and 2.57 μm^3. They concluded that the Rayleigh-Gahs approximation was applicable to these cells and further suggested that cell size could be estimated by using their modified instrument and measuring the light scattered at 5° and 30°.

This report describes experiments performed with a more generally available and unmodified spectrophotometer for estimating the particle size and number of a greater diversity of cell types and sizes.

MATERIALS AND METHODS

Eleven cultures were obtained from various sources, principally the American Type Culture Collection (identification was not verified). All isolates conformed to published descriptions of the cell size and morphology. The isolate identified as NMFA was pleomorphic and belonged in either *Arthrobacter* or *Mycoccocus*; the cells used in this study, however, were all coccoidal, singly or in pairs. The cells of the *Bacillus* sp. used appeared to be only vegetative cells in phase-contrast examination. When refractile spores could not be detected by this means, it was assumed that even if spores were present, they would not have influenced light scattering. All cells were grown on slants of Trypticase Soy Broth (BBL). Cells were removed from the surface of a slant and suspended in distilled water. The suspensions were mixed on a vortex mixer initially, but only shaken by hand on further dilutions to minimize further breaking of clumps. Cell counts were made from an appropriate dilution in a Petroff-Hausser counting chamber and then were calculated for all other dilutions. Cells were measured with a calibrated ocular micrometer at a 1,250-fold magnification by use of phase contrast.

Cell suspensions were placed in a quartz cuvette with a 1-cm light path. Optical densities (OD) at 600 nm were measured in a Beckman model B spectrophotometer. The slit width was 0.4 mm. To obtain a dyssymmetry value, the cuvette was first placed in a position next to the detector window and then moved to a position adjacent to the light source window. The distance between the center of the cuvette and the detector window was 6 and 70 mm for the respective positions. In the position adjacent to the light source

window, the turbidity was due to light scattering at angles greater than 13°, and the reading was arbitrarily accepted as the total OD (OD_T). In the position adjacent to the detector window, the turbidity was due to light scattered at angles greater than 52°, and the resulting lower OD was recorded as the OD due to wide-angle scattering (OD_W). Figure 1 illustrates the geometry.

Although the samples in the position adjacent to the detector window may have increased the sensitivity to absorption by allowing more of the scattered light to enter the detector, the choice of the wavelength should have minimized any absorption phenomena. No absorption peaks were observed when the cells were scanned in this wavelength region.

RESULTS AND DISCUSSION

Relationship of OD_T and OD_W values to cell size. An experiment was first performed to determine the relationship of the OD_T and OD_W values for small, medium, and large cells at various concentrations. *E. intermedia* was selected as a small cell, *Micrococcus lysodeikticus* as a medium-sized cell, and a *Rhodotorula* sp. as a large cell; *M. lysodeikticus* and *Rhodotorula* sp. were pigmented cells. With the spectrophotometer used, the ratio of OD_T to OD_W was not constant and varied with cell concentration (Table 1). Since it seemed necessary to obtain a constant ratio of OD_T to OD_W for each species in order to determine the cell size at different concentrations, an empirical adjustment was made for the OD_W value. The OD_W value was selected for this adjustment because the OD_T value was linear with concentration and the OD_W value was not. The most direct empirical adjustment to obtain a constant ratio of OD_T to OD_W, with only the experimentally derived values of OD_T and OD_W, was tested in the following form: adjusted $OD_W^* = OD_W + (OD_W \times OD_T)$. This calculation increased the value of OD_W in direct proportion to the value of OD_T. The ratios of OD_T to OD_W^* listed in Table 1 are not constant because of experimental errors, but the different cell sizes are clearly distinguishable at all concentrations used.

TABLE 1. *Values of OD_T and OD_W for different concentrations of three different species*

Species	OD_T	OD_W	OD_T/OD_W	Adjusted OD_W^*	OD_T/OD_W^*
E. intermedia	0.130	0.095	1.37	0.107	1.21
	0.205	0.14	1.46	0.169	1.21
	0.27	0.18	1.50	0.228	1.18
	0.36	0.23	1.56	0.313	1.15
M. lysodeikticus	0.12	0.08	1.50	0.090	1.33
	0.16	0.10	1.60	0.116	1.38
	0.27	0.15	1.80	0.190	1.42
	0.47	0.24	1.96	0.353	1.33
Rhodotorula sp.	0.175	0.06	2.91	0.70	2.50
	0.21	0.07	3.00	0.085	2.47
	0.28	0.09	3.11	0.115	2.43
	0.42	0.12	3.50	0.170	2.47

TABLE 2. *OD_T/OD_W^* values and estimated volumes of various cells*[a]

Cell	Average size, diameter, or width × length (μm)	Estimated volume (μ^3)	OD_T/OD_W^*
E. intermedia.....	0.8 × 1.2	0.6	1.19
M. cerolyticus.....	1.3	1.4	1.27
M. lysodeikticus...	1.4	2.2	1.36
Bacillus sp.....	1.8 × 4.5	11.4	2.00
Rhodotorula sp....	3.2	17.2	2.47
S. lutea.....	2.6	17.5	2.49

[a] Rods were assumed to be cylinders. *Rhodotorula* sp. and cocci were assumed to be spheres. The percentage of paired cocci was determined microscopically and their volume was estimated as 2 × V; the estimated volume per particle was adjusted according to the percentage of paired cocci. The light-scattering particle of *S. lutea* was assumed to be the octet cube.

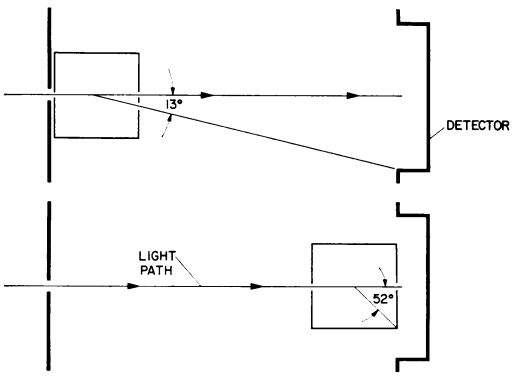

FIG. 1. *Geometry of cuvettes in spectrophotometer compartment.*

Ratios for the three microorganisms listed above and three additional microorganisms, their sizes, and their estimated volumes are listed in Table 2. The ratio of OD_T to OD_W^* appeared to be related linearly to the estimated volume per particle (Fig. 2).

Relationship of OD_T and OD_T/OD_W^* to the number of particles. At a concentration of 2.2×10^7 particles/ml, the *Sarcina lutea* particles gave an OD_T reading of 0.36. Consequently, the total particle volume required for each 0.01 unit of OD_T was calculated from:

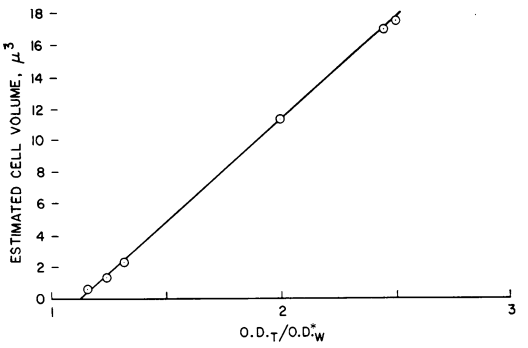

FIG. 2. *Estimated average particle volume of six different microorganisms versus* OD_T/OD_W^* *values.*

total particle volume 0.01 OD_T

$$= (17.5\ \mu^3 \times 2.2 \times 10^7)/36 \quad (1)$$
$$= 1.06 \times 10^7\ \mu m^3$$

The number of particles was estimated by:

number of particles

$$= \text{total volume}/(\text{volume/particle}) \quad (2)$$
$$= [100\ (OD_T) \times 1.06 \times 10^7]/(\text{volume/particle})$$

Then, from Fig. 2, the slope and intercept were used to determine volume per particle by:

$$\text{volume/particle} = 13\ (OD_T/OD_W^* - 1.13) \quad (3)$$

Substituting equation (3) in equation (2) then gave

number of particles

$$= [100\ (OD_T) \times 1.06 \times 10^7]/[13\ (OD_T/OD_W^* - 1.13)] \quad (4)$$
$$= (OD_T \times 8.15 \times 10^7)/(OD_T/OD_W^* - 1.13)$$

TABLE 3. *Estimates of number of cells by light scattering and direct counting*

Cell	OD_T	OD_W	OD_T/OD_W^*	Cell count/ml $\times 10^{-7}$	
				Equation 4	Direct count
Corynebacterium insidiosum	0.27	0.175	1.22	24	27
NMFA	0.42	0.145	2.04	3.8	4.6
Chlorella sp.	0.46	0.130	2.42	2.9	2.4
Serratia marcescens	0.20	0.145	1.15	81	90

The use of this formula was then compared to direct microscopic cell counts with four microorganisms that had not been used previously. (Table 3). A P value between 0.1 and 0.2 was obtained when paired data were used in a t test.

Although the experimental errors, which could probably be reduced, make this technique unacceptable for certain purposes, it may be useful if standard curves obtained by other types of analysis are not feasible or convenient.

LITERATURE CITED

1. Fikhman, B. A. 1963. Light diffusion of bacterial suspensions in the visible field of the spectrum. Biophysics USSR (English Transl.) 8:441–447.
2. Koch, A. L. 1961. Some calculations on the turbidity of mitochondria and bacteria. Biochim. Biophys Acta 51:429–441.
3. Koch, A. L. 1968. Theory of the angular dependence of light scattered by bacteria and similar-sized biological objects. J. Theoret. Biol. 18:133–156.
4. Koch, A. L., and E. Ehrenfeld. 1968. The size and shape of bacteria by light scattering measurements. Biochim. Biophys. Acta 165:262–273.
5. Lanni, F., and D. H. Campbell. 1948. Higher order tyndall spectra with bacterial suspensions. J. Colloid Sci. 3:273–274.
6. Oster, G. 1955. Light scattering, p. 51–71. *In* G. Oster and A. W. Pollister (ed.), Physical techniques in biological research, vol. 1, Academic Press Inc., New York.