An Evaluation of Optical Density to Estimate Fungal Spore Concentrations in Water Suspensions

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ABSTRACT

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Optical density (or turbidity) was used to estimate a wide range of spore concentrations of *Penicillium digitatum, Penicillium italicum,* and *Geotrichum candidum.* Four strains of each were used, and for each organism, optical density was closely related to spore concentration determined by haemocytometer count.

Additional key words: Enumeration of spore concentration.

Optical density (or turbidity) has been used to predict concentration and mass of bacterial suspensions (5, 6, 7), fungal growth in nutrient broths (as dry matter) (10, 12) and in studying numbers of cells of *Geotrichum candidum* over the range 0.5 to 8×10^6 cells/ml (12). In investigations with spore suspensions Johnson and Boyer (4) used optical density to measure rust uredospore concentrations and Tsai and Erwin (11) used optical density to measure concentration of *Verticillium microsclerotia,* but neither study involved an evaluation of the technique. Calaam (3) has questioned the use of turbidity in the study of fungal concentrations because of the presence of hyphae, a problem also noted by Wells and Spalding (12). While this objection applies for broth cultures or hyphal suspensions of fungi, it is of negligible significance in measurement of spore suspensions prepared with minimal hyphal contamination.

Spore suspensions are used widely as inocula in phytopathological experiments. Usually it is desirable to determine spore concentration as it may affect the experimental result (9). Counting spores by haemocytometer (1, 2, 8, 13) is slow and tedious, particularly if large numbers of suspensions are involved. However, with a calibration curve between optical density and spore concentration, it should be possible to estimate a spore concentration in a fraction of the time required for counting by the haemocytometer method.

In their discussions of the use of turbidity to predict numbers of bacterial cells, Koch (6) and Koga and Fujita (7) note the importance of uniform light scattering for accurate estimates. Uniform scattering is not possible if cells occur in clumps or chains, so it is necessary to prepare inocula with a uniform distribution of discrete spores and minimal amounts of hyphae when using the turbidity method. Koch (6) also showed that at longer wavelengths (about 500-600 nm and greater) the effects of variation in cell sizes and different spectrophotometers is minimized. A wavelength of 700 nm or greater would also reduce the effects of any absorption due to pigments produced by the spores.

For *Geotrichum candidum,* Wells and Spalding (12) found a linear relation between optical density and cell number over the range 0.5 to 8×10^6 cells/ml. In our study, optical density was assessed over a wider range of concentrations (about 10^6 to 10^8 spores/ml) for three fungi, each represented by four different strains.

MATERIALS AND METHODS

The fungi used in this investigation were *Penicillium digitatum* Sacc. (one strain resistant and three strains sensitive to benzimidazole), *P. italicum* Wehmer (four strains), and *Geotrichum candidum* Lk. ex Pers. (four strains), all isolated from citrus fruit obtained from local orchards. Each culture, when used, was at least a second-generation single-spore isolate.

The spores used in these experiments were grown either on oranges [two *P. italicum* strains (Pi1, Pi2), one resistant strain (Pd3), and one sensitive strain (Pd2) of *P. digitatum*] or on PDA plates [two *P. italicum* strains (Pi3, Pi4), all *G. candidum* strains (Gc1-4), and two sensitive strains of *P. digitatum* (Pd1, Pd4)].

The suspensions were prepared from fruit 7-10 days after inoculation, by gently brushing spores from the fruit surface with a small test-tube brush into about 500 ml of distilled water containing 0.05% of the wetting agent Lissapol LD®, (ICI Australia Ltd., 69 Macquarie St., Sydney, Australia), (Lauryl alcohol sulphate in the presence of acetic anhydride); or from plates 7-10 days

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after inoculation, by gently brushing spores from the surface of the plate with a fine paintbrush into 5-10 ml distilled water containing 0.05% Lissapol. Each suspension was stirred vigorously for 1 min to break up spore aggregates. Hyphae were removed by filtering the spore suspension through glass wool. Although not usually necessary for the organisms used in this work, such filtering may be essential to obtain a satisfactory spore suspension for other organisms.

For each suspension, spore concentration was determined by haemocytometer count (HC) and optical density (OD) was determined using a Varian Techtron Model 635 Spectrophotometer (700 nm, slit width 0.5 nm). Two samples were taken from each suspension for a HC and two for an OD reading; the mean of each pair was used in the calibration of OD with HC. Whereas OD duplicates were consistent over the whole range of OD values, HC duplicates were more variable for very high concentrations (above 10^8 spores/ml), when many spores lay on boundary lines between squares of the haemocytometer.

Since OD readings depend on the spore concentration of the solution being measured, a regression equation should be derived using HC as the independent variable. The equation is then inverted to predict HC from OD (14). This approach is at variance with those of other workers (3, 5, 6, 7, 12) who have taken OD as the independent variable to directly estimate microbial concentration or mass.

RESULTS AND DISCUSSION

For each fungus, OD was best related to HC over the full range of data by a fourth degree polynomial in the square root of HC, passing through the origin. However, for ease of inversion and because of the doubtful accuracy of very high HC values, we restricted HC values to those within the range 4×10^6 to 1.35×10^8 spores/ml. Over this range, OD was satisfactorily related to the square root of HC by a second-degree polynomial, not constrained to pass through the origin.

The OD measurements related closely ($P < 0.001$) to HC for all three fungal species (Fig. 1) (Table 1).

Spore size appeared to be an important factor in the average slope of the regression curve. For *P. digitatum,* with spores averaging 7.5 μm $\times$ 5.0 μm, the curve had a steeper slope than for *P. italicum,* which had smaller spores averaging 5.5 μm $\times$ 3.5 μm. For these two fungi we found that over most of the range of HC values common to both, the ratio of estimated OD values was almost constant and close to the square root of the ratio of their average cross sectional areas. This suggests the existence of a basic relation between OD and HC, the average slope of which varies in proportion to spore size.

The strains of *G. candidum* produced two distinct spore sizes: a larger, single-celled spore averaging 11 μm $\times$ 8 μm and a smaller double-celled spore averaging 5.5 μm $\times$ 3.5 μm, occurring in a 1 : 2 ratio. The regression curve for *G. candidum* appears to represent a composite of two

TABLE 1. Estimation of spore concentrations in suspension by optical density measurement. Equations for estimating HC from OD values, with the OD range over which they apply

Species	Equation	OD range
Penicillium italicum	$HC = 619.4 - 358.0\sqrt{2.97 - OD} - 94.5\ OD$	0.2 to 2.4
Penicillium digitatum	$HC = 771.6 - 376.6\sqrt{4.18 - OD} - 86.3\ OD$	0.3 to 3.3
Geotrichum candidum	$HC = 327.7 - 206.4\sqrt{2.49 - OD} - 59.0\ OD$	0.3 to 2.4

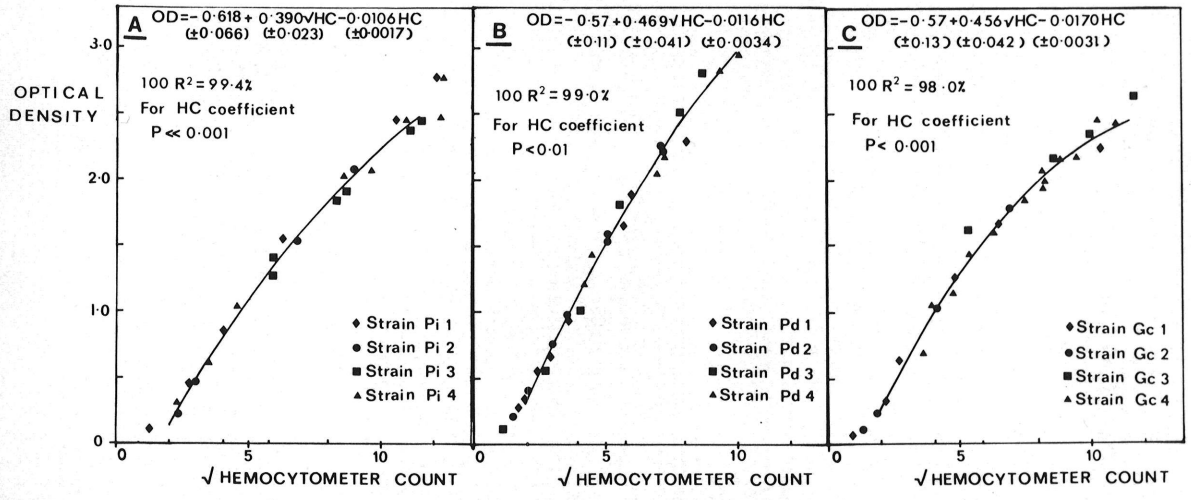

Fig. 1-(A to C). Relation between optical density (OD) and the square root of haemocytometer spore count (HC) for aqueous suspensions of spores of **A)** *Penicillium italicum,* **B)** *Penicillium digitatum,* and **C)** *Geotrichum candidum.* Counts are $\times 10^6$/ml; values in parentheses under constants indicate their standard errors.

curves, one for each spore size. Estimated OD values were similar to those for *P. digitatum* at low, and for *P. italicum* at high HC values. Apparently the main influence on OD gradually changes from the larger to the smaller spores as concentration increases, an effect similar to that observed for bacteria by Koch (6) and Koga and Fujita (7). For fungi with more than one spore size, such as the *G. candidum* strains we used, this effect implies that there would not be the same proportionality between average spore size and average slope as was suggested for fungi with a single spore size.

These results show, for the three fungi studied, optical density can be used to estimate spore concentrations. Since the organisms we used varied in size and type of spore and were produced on differing growth media, the same result may apply to other fungi for which suspensions can be prepared with minimal hyphal contamination and with an even distribution of discrete spores.

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