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SPECTROPHOTOMETRIC DETERMINATION OF MYCELIAL BIOMASS

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ABSTRACT

The optical density (450 nm) of samples of homogenized fungal biomass correlated linearly with the dry weight of the biomass in the samples. As shown for broths of the filamentous microfungus *Neurospora sitophila*, the sensitivity of the technique depended on the extent of fragmentation of fungal hyphae during homogenization: increased fragmentation increased sensitivity. The method applied during all phases of growth, was as accurate as the conventional dry weight technique and permitted rapid and simple measurement of biomass concentration.

INTRODUCTION

Of the many variables which are routinely followed during the course of a fermentation, the cell mass concentration is perhaps the most basic and often one of the most essential. Even when biomass itself is not the desired product, the overall yield of the process is biomass-dependent. Many derived parameters (*e.g.*, specific oxygen uptake rate, yield coefficients) require a knowledge of the biomass concentration. Several techniques are available for measurement of cell mass concentration. In media free of non-microbial solids, spectrophotometric turbidity measurements are very popular; however, this rapid and simple method, as conventionally practised, is not suitable for mycelial fungi and filamentous bacteria. Here we briefly review the techniques available for mycelial and filamentous biomass measurements, and evaluate an alternative, simple and rapid, procedure based on spectrophotometric measurement of optical density of pretreated fermentation broth.

Of all the biomass measurement techniques the dry weight method is the longest established and very accurate; however, this method is tedious and the results are not immediately available. As such, the method is not applicable to large-scale, multi-variable, multi-replicate experimentation (Pirt, 1975); nevertheless, dry weight measurement is a benchmark technique for comparing all other methods.

Other physical techniques, such as those based on measurements of liquid flow through filter cakes of biomass (Nestaas *et al.*, 1981) and on measurement of viscosity of broth (Perley *et al.*, 1979), are neither particularly simple nor very accurate; often the sensitivity of these measurements to biomass concentration is low and other factors (*e.g.*, temperature) may influence these techniques. Yet other biomass quantification methods, for example, fluorescein diacetate staining (Ingham and Klein, 1982), hyphal length and branching measurements (Trinci, 1974), and ergosterol (Seitz *et al.*, 1979) or chitin concentration-based (Sharma *et al.*, 1977) techniques have limited applicability for routine work.

Spectrophotometric determination of mycelial solids after fragmentation of mycelia by homogenization has been reported for the microfungi *Rhizopus oryzae*, *Trichophyton mentagrophytes*, *Sporothrix schenckii* (Granade *et al.*, 1985) and *Penicillium chrysogenum* (Nestaas *et al.*, 1981); however, important aspects of sensitivity of this method to homogenization pretreatment have not been discussed before, nor has the wider applicability of the technique to such fungi as *Neurospora sitophila* been established.

MATERIALS AND METHODS

The microfungus *Neurospora sitophila* (ATCC 36935) was maintained at 4°C on potato dextrose agar (PDA) slants. Test cultures were prepared in 250 mL conical flasks containing 50 mL medium of the following composition (per litre): glucose, 10.0 g; yeast extract (Diffco), 2.0 g; $(NH_4)_2SO_4$, 0.47 g; urea, 0.86 g; KH_2PO_4, 0.714 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2$, 0.2 g; $FeCl_3$, 3.2 mg; $ZnSO_4 \cdot 7H_2O$, 4.4 mg; H_3BO_3, 0.114 mg; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.48 mg; $CuSO_4 \cdot 5H_2O$, 0.78 mg; $MnCl_2 \cdot 4H_2O$, 0.144 mg. The pH was adjusted to pH 5.5 after sterilization at 121°C for 30-minutes. Flasks cooled to ambient were inoculated from PDA slants and incubated on a rotary shaker (35°C, 220 rpm, 2-days). The biomass produced was aseptically homogenized in a blender (Waring Commercial Blender 7011, Dynamics Corporation of America, New Hartford, CT) at "low intensity" setting for specified periods (2-6 minutes). The optical density of serially diluted, homogenized broth was measured (450 nm) spectrophotometrically against a blank of un-inoculated, sterile, medium. For dry weight measurements, a sample of diluted, homogenized broth was dried overnight at 95°C.

In separate experiments, the entire fermentation profile of *N. sitophila* was followed using dry weight and optical density techniques simultaneously. For these experiments, several shake flasks (250 mL) containing the earlier specified, sterilized medium, were inoculated (10% v/v homogenized biomass) and held on a rotary shaker (35°C, 200 rpm) until desired. At desired times, flasks were removed from the shaker in duplicate, rapidly cooled and stored at 4°C if necessary. One of the flasks was analyzed for total dry weight. In this case, non-homogenized broth was filtered under suction through a 25 μm pore "Nitex" nylon cloth (Thompson Co., Scarborough, Ontario), washed with several sample volumes of deionized water and dried overnight at 95°C. The contents of the second flask were measured spectrophotometrically as described above.

RESULTS AND DISCUSSION

The optical density (450 nm) of homogenized, serially diluted, *N. sitophila* broth is plotted against mycelial dry weight in Figure 1. Samples of broth homogenized for 2-, 4- and 6-minutes all displayed linear correlations between optical density and dry

weight (Figure 1); however, the sensitivity of the measurements, defined as change in dry weight per unit change in optical density, increased with increasing homogenization. As expected, at any fixed biomass concentration, increased homogenization reduced turbidity by reducing the size of the fungal hyphae. Quantitatively, under otherwise fixed conditions, the sensitivity of the measurement (S) correlated with the duration of homogenization (t) as follows

$$S = 0.011t + 1.453 \quad (1)$$

The correlation coefficient for Equation (1) was 0.96. Clearly, optical density measurement on homogenized fungal broth can be a simple, rapid and sensitive indicator of the biomass concentration so long as the sample pretreatment by homogenization is carried out under reproducible, standardized conditions.

The turbidimetric technique was applicable during all phases of fungal growth as illustrated in Figure 2 where the biomass concentration during the course of a *N. sitophila* fermentation was plotted as a function of fermentation time. The fermentation profiles determined with the conventional dry weight (non-homogenized broth) and by the spectrophotometric methods (6-minutes homogenization) compared closely as shown in Figure 2; the two measurement techniques agreed to within *ca.* ± 5% of each other (Figure 3). Because, under identical fragmentation conditions, different fungi may fragment or disrupt differently (Chisti and Moo-Young, 1986), the change in the sensitivity of the measurement to homogenization would be different for different cultures; however, the general direction of the change should be consistent with the observations reported here. This consideration applies also to any particular microorganism grown under different conditions; the sensitivity of cells and hyphae to disruption being dependent on conditions of growth (Chisti and Moo-Young, 1986). Other general limitations on spectrophotometric measurement methods apply also to

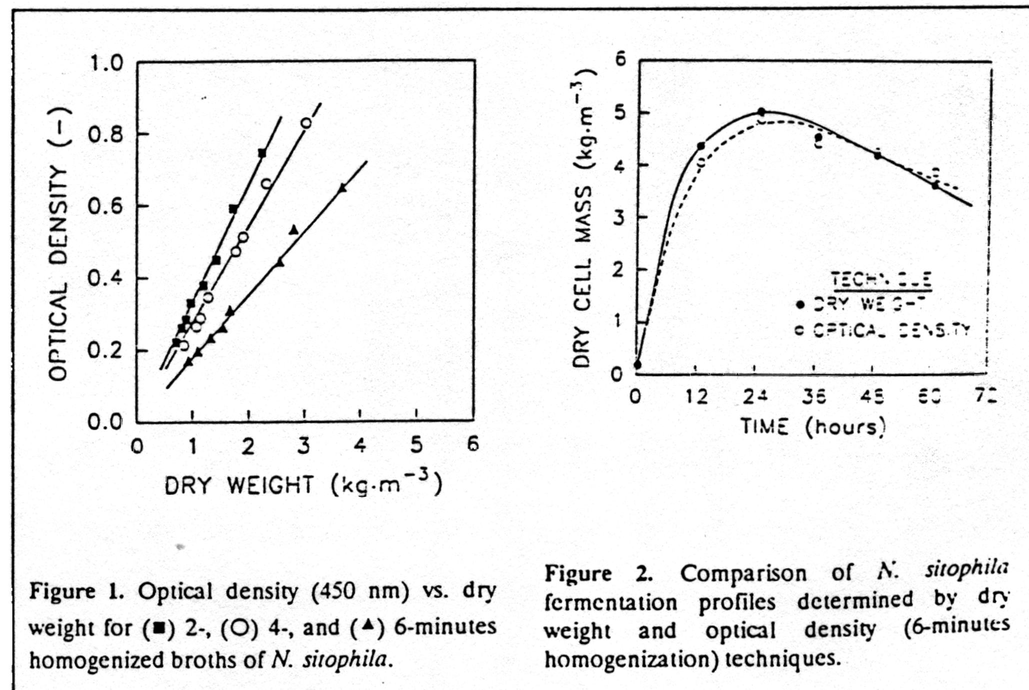

Figure 1. Optical density (450 nm) vs. dry weight for (■) 2-, (O) 4-, and (▲) 6-minutes homogenized broths of *N. sitophila*.

Figure 2. Comparison of *N. sitophila* fermentation profiles determined by dry weight and optical density (6-minutes homogenization) techniques.

fungal biomass measurements. Hence, consideration should be given to such factors as production of extracellular pigments and changes in coloration of the solids-free broth, in assessing the suitability of this technique for particular applications.

CONCLUSIONS

Estimation of the concentration of biomass in fermentation broths of mycelial fungi, filamentous bacteria or algae can be done by spectrophotometric turbidity measurements on homogenized samples of broths. Sensitivity of this method depends on the extent of hyphal fragmentation achieved during homogenization; short homogenization periods (≤ 6-minutes) are sufficient for the requisite sensitivity. However, pretreatment of the sample by homogenization must be carried out at standardized conditions. The accuracy of the spectrophotometric technique is comparable (± 5% of true value) to that of direct measurement of dry weight. In comparison with the latter, the spectrophotometric technique is much faster, the biomass concentration results being available within a few minutes of sampling.

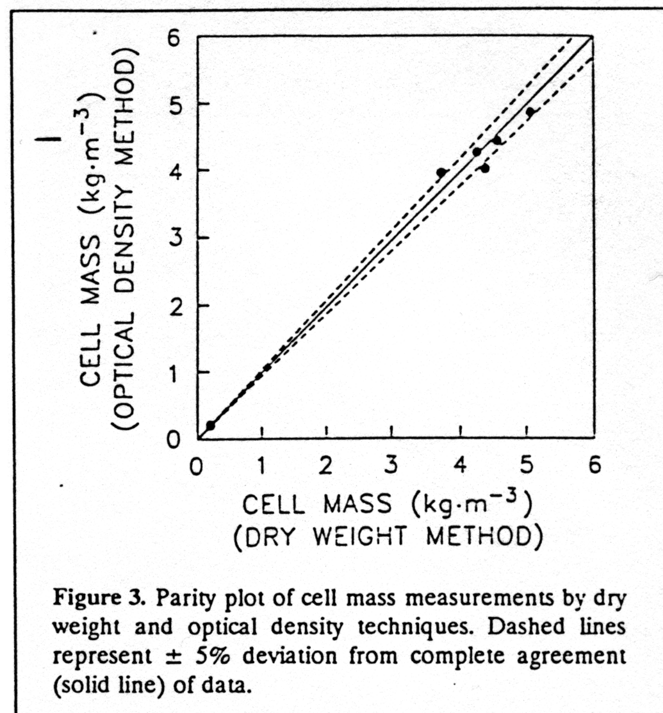

Figure 3. Parity plot of cell mass measurements by dry weight and optical density techniques. Dashed lines represent ± 5% deviation from complete agreement (solid line) of data.

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