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EFFECT OF Pseudomonas fluorescens (2-79) CULTURE AGE ON THE RELATIONSHIP BETWEEN OPTICAL DENSITY AND BIOMASS

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SUMMARY

The effect of cell-cell adhesion (clump formation) on the relationship between optical density and dry biomass was investigated for *Pseudomonas fluorescens* (2-79). Calibration curves were generated at the beginning, middle and end of the arithmetic growth phase, as well as during the decay phase. The results show that use of a single biomass-turbidimetric equation for the entire arithmetic growth phase may yield erroneous results.

INTRODUCTION

Off-line methods for microbial biomass determination have been reviewed by Sonnleitner et al. (1992). Apart from direct cell counting, the other classical way to determine biomass concentration is to harvest a known amount of the culture suspension, separate cells by centrifugation, wash the cells and dry them to constant weight at a few degrees above the boiling point of the solvent (usually 105°C). After gravimetric determination of the dry mass one can easily calculate the mass concentration in the fresh medium. This classical way of quantifying cell population density by mass can be sufficiently accurate and precise, but can also be laborious and time consuming. The turbidimetric technique, which is based on the reduction of light transmission as the density of the suspended matter increases, enjoys wide application because it is convenient and quick (Bailey and Ollis, 1977).

A linear calibration curve between exponential and/or arithmetic growth phase cell dry weight and optical density (absorbance) is established once. Other biomass samples are diluted as appropriate to provide readings in the region where optical density and dry cell mass concentration are linearly related. In this approach, it is assumed that microbial biomass is a homogeneous continuum, however this assumption may not be valid under certain conditions such as when there is cell-cell adhesion (cell clump formation) or cellsubstrate surface interactions (biofilm formation).

We are studying secondary metabolites and other compounds produced by *P. fluorescens* (2-79) and other fluorescent *Pseudomonas* spp. We have observed that strain 2-79 forms microscopic clumps during the last half of the arithmetic growth phase that become visible to the naked eye by the decay phase. Two kinds of hypotheses have been proposed to explain the mechanisms of cell adhesion. The two hypotheses are that cell adhesion depends upon physicochemical properties of surfaces (e.g. charge and hydrophobicity), or alternatively that all adhesion are basically receptor-ligand type interactions. Curtis and Lackie (1991) have suggested that the differences between these two theories are largely ones of scale, since receptor-ligand interactions must ultimately depend upon physical or chemical properties of the interacting molecules.

The objective of this study was to determine the extent to which cell clump formation affects the relationship between optical density and dry biomass.

MATERIALS AND METHODS

Microorganism and media. Stock cultures of *Pseudomonas fluorescens* 2-79 (NRRL-15132) were prepared as previously reported (Kisaalita et al., 1991). When this organism is cultured in a high-Fe(III) medium, it produces an antibiotic, phenazine 1-carboxylic acid (PCA). PCA is effective in inhibiting the growth of plant pathogenic fungi such as *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia solani*, and *Pythium* spp. (Grusiddaiah et al., 1986). Strain 2-79 also produces fluorescent yellow-green compounds, pyoverdines, when cultured in a low-Fe(III) medium [6 g K_2HPO_4, 3 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, 0.098 g $MgSO_4$, and 4 g succinic acid per liter and adjusted to pH 7.0 before sterilization (Meyer and Abdallah, 1978)].

Batch fermentation. Bacteria from slants served as inocula for 50 ml pre-cultures in two 125-ml flasks. Cell were harvested by centrifugation and washed three times with 5% NaCl solution prior to use as inocula for 200 ml low-Fe(III) medium in six 500-ml flasks. Both seed and experimental flasks were incubated at 25°C and agitated at 140 rpm. Optical density, pH and pyoverdine formation were monitored daily for each flask for five days. Dry biomass was measured from one of the six flasks every day.

Analyses. Samples for pyoverdine measurements were centrifuged to remove cells and 20 µl supernatant were added to a 3 ml cuvette before measurements were performed by a spectrofluorimeter set at optimal excitation (404 nm) and emission (463 nm) wavelengths. A six 5-ml sample was withdrawn from a designated flask each day, centrifuged (10,000 x g for 10 min), washed twice, and dried overnight at 98°C in pre-weighed aluminum cups. The cups with the dry biomass were cooled to room temperature and the dry biomass determined gravimetrically. Optical densities were determined at 620 nm. Samples were diluted as appropriate to provide absorption readings in the region where absorbance and dry cell mass concentrations are linearly related.

RESULTS AND DISCUSSION

A typical fermentation time-course is presented in Fig. 1. As shown, pyoverdine production is growth associated. Based on optical density, exponential and arithmetic growth phases occurred approximately between 50 - 60 and 60 - 100 hours, respectively. Exponential growth ended early probably due to low oxygen transfer rates, which is typical of shake flask fermentations. To asses the effect of cell clump formation on the relationship between optical density and dry biomass, calibration curves were generated at the 51, 76, 99, and 122 hours of incubation, representing the beginning-of-arithmetic, mid-arithmetic, end-of-arithmetic, and decay growth phases, respectively. As shown in Fig. 2, the 76 and 99 hour curves are almost identical. However, both the 51 and 122 hour curves were characterized by higher slopes. The high slope of the 51-hours curve can be explained by the absence of cell clumps. Also, the fact that cells at this stage are relatively smaller could have partly contributed to the relatively higher optical densities. The presence of cell debris during the decay phase was confirmed by microscopic examination. This debris was probably responsible for the unexpected high slope of the decay phase biomass-turbidimetric curve.

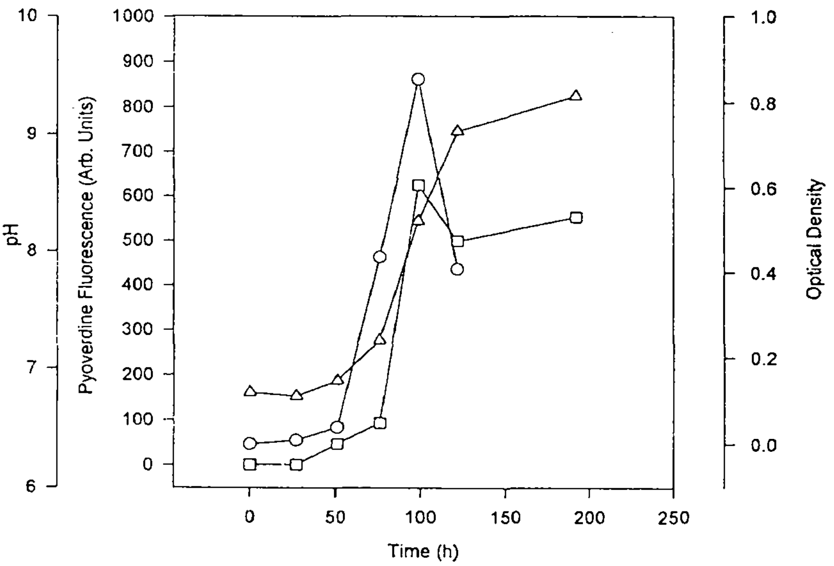

Fig. 1. Batch growth and pyoverdine formation by *Pseudomonas fluorescens* 2-79 (NRRL-15132) [fluorescence (□), pH (△), optical density (○)].

In conclusion, the use of a single biomass-turbidimetric equation for the entire arithmetic growth phase of cells that form clumps such as fluorescent pseudomonads can yield erroneous results. Attempts to generate an empirical equation including other independent batch fermentation process variables such as pH did not yield satisfactory results. It is therefore recommended that in establishing calibration curves for clump-forming cells, the biomass-turbidimetric equation should be verified over the entire arithmetic growth phase.

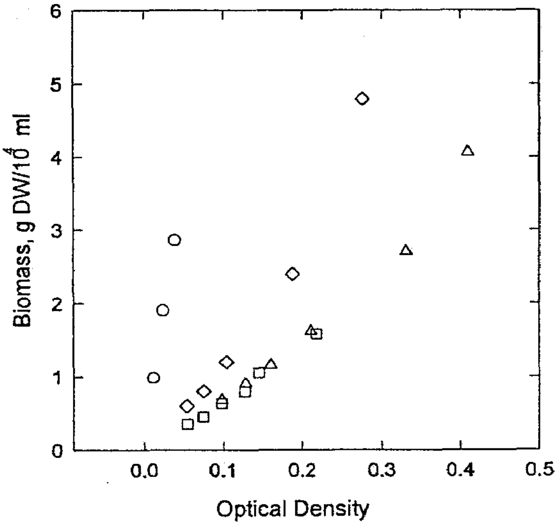

Figure 2. Biomass-optical density relationships for *Pseudomonas fluorescens* 2-79 after 51 (○), 76 (△), 99 (□), and 122 (◇) hours of batch fermentation.

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