APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1976, p. 640-641 Vol. 32, No. 4
 Printed in U.S.A.

Linear Transformation of Standard Curves for Yeast Turbidity

PHILIP B. BESTIC AND WILFRED N. ARNOLD*

Department of Biochemistry, University of Kansas Medical Center, Kansas City, Kansas 66103

Received for publication 24 May 1976

Within a very serviceable range, light extinction plotted against yeast concentration follows a rectangular hyperbola. A linear transformation, which facilitates the determination of yeast concentration, is described.

A common method of monitoring concentrations in liquid cultures of yeasts depends on turbidity, as estimated with a colorimeter or a spectrophotometer (see, for example, references 4 and 5). Instrument response (e.g., Klett reading or absorbance) is typically curvilinear with respect to standard concentrations (based on dry weight per unit volume). Thus, the convenience attending a series of optical measurements is somewhat offset by the necessity for tedious reference to a standard curve.

We have routinely used a Klett-Summerson photoelectric colorimeter (Klett Manufacturing Co., New York, N.Y.) in conjunction with cylindrical cuvettes, and also with matching cuvettes attached directly to culture flasks. We observed that standard curves for a variety of yeasts are rectangular hyperbolas which become linear on double-reciprocal coordinates. Accordingly, 1/Klett = slope × 1/concentration + intercept, which, for computational purposes, can be simplified to concentration = $1/[(p/\text{Klett}) - q]$, where p = 1/slope and q = intercept/slope. The numerical values for p and q can be evaluated either by graphical or computational methods. An example follows.

Saccharomyces rouxii (Boutroux) was grown in liquid culture as described by Arnold (1). At early stationary phase cells were harvested by centrifugation and then washed in 0.2 M sodium acetate buffer (pH 5.8) by repeated resuspension and recentrifugation. Stock suspensions were prepared in the same buffer and series of concentrations were generated by volumetric dilutions. The Klett no. 56 filter was used and Klett values were read against a buffer blank. The mass of cells in a known volume of stock suspension was determined by filtration onto a tared membrane filter. Filter and cells were then washed with distilled water and dried to constant weight at 95°C. Other concentrations were computed according to the dilution used. Figure 1 depicts the traditional standard curve together with a double reciprocal plot of the same data. In this example p = 376 and q = 0.425 and within the range 0.1 to 5.0 mg (dry weight/ml) calculated concentrations showed less than 2% deviation from corresponding values based on gravimetric measurements. At the upper limit of concentration (in this case 8.08 mg/ml) the deviation for computed concentration was about 3%.

Standard curves have also been generated with *Saccharomyces cerevisiae* and *Candida albicans* which were grown on a variety of media and harvested at different stages of growth. We also tested a variety of Klett filters. In our experience all of the standard curves could be satisfactorily transformed into straight lines by plotting the reciprocal of Klett value against reciprocal of concentration within the range of 0.01 to 10.0 mg (dry weight/ml). A spectrophotometer (Zeiss PM6) which was operated at 560 nm with 1-cm cuvettes was more sensitive than the Klett colorimeter; the standard curve was a rectangular hyperbola, but the useful range for the linear transformation was restricted to 0.01 to 0.5 mg (dry weight/ml).

In converting a number of Klett readings to concentrations we have found that the computational method involves about one-tenth the time required in reading a graph of Klett versus concentration. For a large series of determinations the computational method is quicker, less tiring, and less prone to operator error. Obviously standard data must be generated for a particular yeast species which is monitored with defined optical instrumentation, and the values of p and q given here are used only as an example.

Koch (2) used a second-degree polynomial to describe standard curves of *Escherichia coli*, and Pringle and Mor (5) gave a log-log plot as an example with yeast. In our hands these transformations are far less useful for yeasts than is the double reciprocal plot described herein. Koga and Fujita (3) studied light scattering by single-celled microorganisms (including *S. cerevisiae*) and fitted some of their data

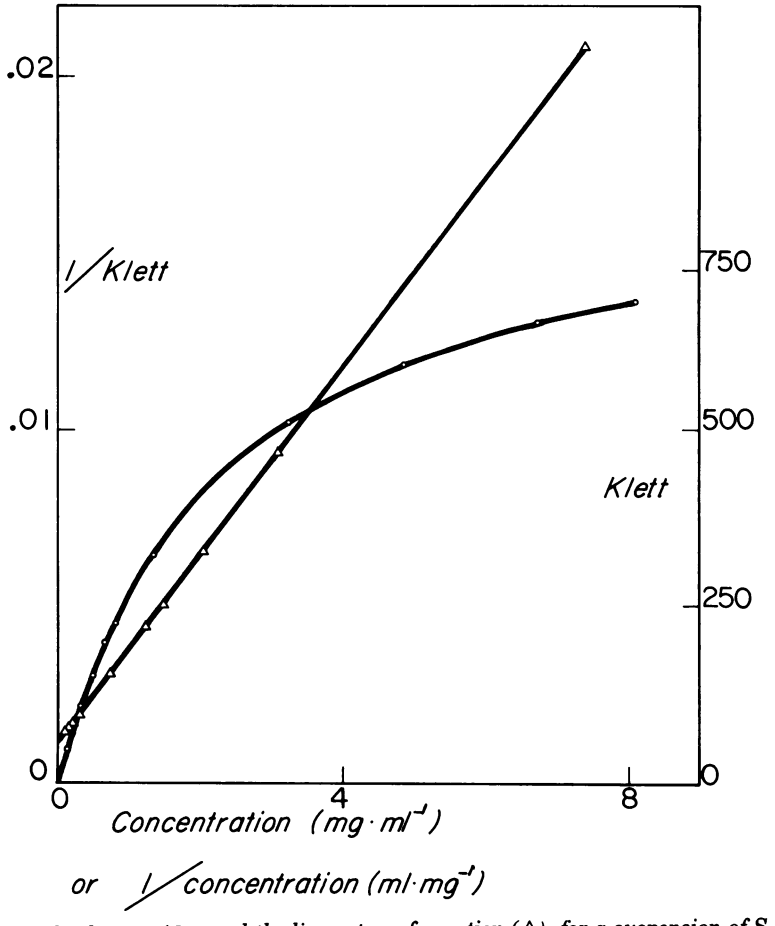

FIG. 1. *Standard curve (○), and the linear transformation (△), for a suspension of S. rouxii.*

to a hyperbolic function. However, the inference that a rectangular hyperbola lends itself to linear transformation has apparently not been adopted by workers in the yeast field, and the computational method we describe is offered as a potentially useful tool in the routine monitoring of yeast growth.

This work was supported by a grant from the Research Corporation.

LITERATURE CITED

1. **Arnold, W. N.** 1974. Expression of cryptic β-fructofuranosidase in *Saccharomyces rouxii*. J. Bacteriol. 120:886-894.
2. **Koch, A. L.** 1970. Turbidity measurements of bacterial cultures in some available commercial instruments. Anal. Biochem. 38:252-259.
3. **Koga, S., and T. Fujita.** 1960. Total cross section for optical scattering by spherical cells in suspension. J. Gen. Appl. Microbiol. 6:101-107.
4. **Morris, E. O.** 1958. Yeast growth, p. 251-321. *In* A. H. Cook (ed.), The chemistry and biology of yeasts. Academic Press Inc., New York.
5. **Pringle, J. R., and J. Mor.** 1975. Methods for monitoring the growth of yeast cultures and for dealing with the clumping problem, p. 131-168. *In* D. M. Prescott (ed.), Methods in cell biology, vol XI. Academic Press Inc., New York.