Procedure for measurement of logarithmic growth

H. J. Colvin  
University of Wisconsin

K. D. Munkres  
University of Wisconsin

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Abstract
Measurement of logarithmic growth

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is expedited by the use of small flasks and small terminal volumes of medium. With small cultures, however, one must use microanalytical techniques for the measurement of growth. Moreover, the measurement of growth throughout the cycle requires the accurate collection and weighing of conidio and germinated conidio as well as mycelia. Finally, since the drying of the cells may be detrimental to cell functions such as respiration or enzyme activities, one may wish to cultivate replicate flasks some for dry weight measurements and others for measurements of other cell functions. Hence, the cell dry weights from replicate flasks should be highly reproducible.

We shall describe a procedure which appears to meet all of the above criteria. Small flasks (25 ml) containing 10 ml of the medium are used. With a New Brunswick shaker, 108 flasks can be used in one experiment. To insure that the mycelial growth will be dispersed rather than "clumped", we remove mycelial fragments from the conidial inoculum and treat the inner surface of the flasks with a silicone reagent to prevent the measurement and adjustment of optical density. Adequate aeration is achieved by rotary shaking of the cultures and by the use of loose cotton plugs on the flasks rather than cotton plugs. The entire cell mass in a flask is collected, dried, and weighed on a tared filter. This sampling technique, unlike previously described ones (Davis and de Serres 1970), does not require additional handling of the cells after they are collected.

Materials and methods: Media; Either minimal medium (Fries (Davis and de Serres 1970)) or on enriched medium (YEGCE-Howell, Zuiden and Munkres 1977) J. Cell Biol. 50: 721) were supplemented with 2% glucose. There medio were solidified with 1.5% agar. Growth; Conidio of wild-type N. crassa (RL 1256A, a derivative of 74A) were obtained from 7-14 day-old slants. Conidio were washed from the slants with liquid medium and filtered through glass wool. The optical density of the conidial suspension at 550 nm was adjusted to 0.3-0.4 by dilution with the medium. Ten ml of conidial suspension was added to each of a series of sterile siliconized 25 ml Erlenmeyer flasks. The flasks were incubated at 30°C in a new Brunswick rotary shaker (Model G-25) at 270 rpm on a platform No. AG-50 (The platform would accommodate 108 flasks.) Measurements of oxygen tension in the culture medium with an oxygen electrode indicated that aeration was adequate. During the early logarithmic growth phase the medium was fully saturated with oxygen and at least 50% saturated in late log and early stationary phase.

Growth flasks; Erlenmeyer flasks (25 ml) were treated twice with a 1% solution of dimethyl-dichlorosilane in benzene at 60°C (Sigma Chemical Co.). The flasks were dried at 90°C for 30 min after each treatment with this solution. Metal caps (22 mm 0. D.) (Motheron Scientific Co., Cat. No. 61756-45) with a 1/4 inch thick cotton layer in the top were used as aseptic covers for the flasks. Filtration; Cells were harvested by vacuum filtration with an apparatus consisting of a Gooch porcelain crucible (Coor’s No. 27002), a paper filter, a 125 ml vacuum flask, and a crucible holder. The latter was constructed by cutting a 1/4 in. hole in a serum battle stopper (5/B in. dia.) and inserting a 1.5 in. piece of 10 mm glass tubing in the smaller end of the stopper. The crucibles were washed serially in nitric acid, top water, and distilled water and dried. The filter papers used were either Whatman no. 1 or 41 paper. The former was used for the collection of cells during the first four hours of growth and the latter for all subsequent timer. Filter papers of 17 mm dia. were cut with the aid of a device resembling a cork borer. Filter papers were placed in the crucibles and both were dried overnight in an oven at 90°C. The tared weight of crucible and paper was determined to within ± 0.1 mg.

All of the cells from a growth flask were collected with the filtration device and washed on the filter with distilled water. Cells, filter paper and crucible were dried in oven overnight at 90°C, cooled to room temperature, and weighed with a Mettler H-16 balance to within ± 0.1 mg. Net cell dry weight was calculated as the difference between gross and tare weights.

Special care in the collection of conidio is required. The use of Whatman #1 paper rather than #41 prevents the conidio from passing through the filter. #41 paper, however, was more suitable for the collection of mycelia than #1 because the mycelia clog the pores in the latter and the filtration proceeds more slowly. Even with the #1 paper, however, the conidial suspension tends to flow over the edge and under the filter pad. This problem can be avoided by slowly pipetting the conidial suspension to the center of the pad with a Pasteur pipette.

Results and Discussion: The results of two typical experiments are shown in Figure 1. Logarithmic growth occurs for about 5 hr with a mass doubling time of 2 hr. The coefficient of variation of dry weights from replicate flasks was 10% during the first 4 hr and 1-3% thereafter. The latter approaches the variance inherent in replicate measure-
ments of one sample. Greater reproducibility could probably be achieved by the use of a microbalance. Serious deviations from logarithmic growth rates occurred if the mycelia clumped rather than remaining dispersed. Dry weights in the former case were as much a 20-40% lower than in the latter. The clumping pattern of growth was avoided by the removal of mycelial fragments from the conidial inoculum and by coating the inner surface of the culture flask with dimethyl-di-chlorosilane.

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Eight-day cultures, grown on Vogel's arginine agar medium (1.5% agar) with arginine (50 mg/100 ml medium) was washed down with a stream of Vogel's arginine SSP medium (Vogel's arginine medium plus 2% sucrose, 10% sorbose, Penicillin G Sodium 100U/ml of medium, dihydrostreptomycin sulphate 100pg/ml of medium) with a sterile Pasteur pipette. The milky liquid was transferred to a sterile tube and centrifuged 15 minutes at 2500 rpm.

The supernatant was discarded and the residue resuspended in the freezing fluid (Vogel's arginine SSP plus dimethyl sulfoxide, 4:1), until a four-fold dilution of this suspension gave an absorbance reading of 1.0 at 280 nm on a Beckman spectrophotometer. 2 ml of the concentrated suspension was placed in sterile vials, sealed and quick-frozen, using liquid nitrogen. The vials were stored at -70°C until used. To date they have been held up to three months, but longer periods of holding time are being tested. Thawing was done in warm water and the contents of the vial were emptied into a filter of Vogel's medium plus arginine and antibiotics (Penicillin and Streptomycin in the concentrations noted above). No retardation of growth has been noted when the frozen and thawed cultures have been compared to others maintained by continual passage.

Griffiths, A. J. F. and B. Sivak. UV transmission through various clear films in mutation experiments.

To reduce the risk of contamination in long, or student-operated UV exposures, it is desirable to use some form of cover on the irradiated sample. Traditionally quartz has been used for this purpose. This report indicates that some cheaper materials are just as good. The materials tested were: plastic Petri plates (from a/s Nokra plast, DK 4690, Naslev, Denmark); "Saran Wrap" (from Dow Chemicals, Ltd., 122 Arrow Road, Weston, Ontario); "Look Roasting Film" (from Look Film Associates, Scarborough, Ontario); and "Baggies" (from Colgate-Palmolive Co., Ltd., New York, N. Y.).

Strip of the clear materials from various sources were fitted into the sample cuvette of a Unicorn SP-800 UV spectrophotometer, so that the beam passed at right angles through one thickness. The behavior of the various films is shown in the Figure, in which the base line follows the 100% transmittance line. Most of the commonly-used UV tubes (e.g., Hanovia BBA-45, Osram HNS 12) emit at 254 millimicrons. It can be seen that of those materials tested, Baggies provide the only material which will transmit most radiation of this wavelength.


This technique basically follows that of Perkins (Neurospora News. 9: 11) with the following modifications: (1) Crosses are made on filter paper strips in tubes containing liquid Westergaard medium. The medium contains 0.2% sucrose, compared to the usual 2%. This drastically therefore makes the use of fluffy unnecessary. Crosses are initiated by the simultaneous introduction of each parent as a drop or two of conidial suspension. (This technique was introduced to A. J. F. G. by F. J. de Serres). (2) Low conidiation and the use of filter paper permit the removal from the cross tube of all the perithecia. The paper can be cut up and placed on slides which are held mounted on adjustable platforms over the agar collection slabs. Two models of platform have been used. (See figures on following page).

Model I has been used extensively for the routine collection of hundreds of asci. It consists of two tubing clamps (a), mounted on a plastic stand (b) with rapid-hardening epoxy glue. The inverted slide bearing the perithecia is placed across the top, and the slide bearing the agar collection block is placed across the two adjustable arms and racked up into close proximity to the dehiscing perithecia. Two such devices may be mounted back-to-back on each stand.

Model II is a more recent design and permits adjustment in two dimensions by the use of sliding plastic shelves (c). The shelves