A Turbidimetric Method for Determining the Effect of 2,4-D Upon the Growth of Yeast

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A considerable amount of work has been reported concerning the effect of 2,4-dichlorophenoxyacetic acid (2,4-D) upon the development or growth of plant tissues. The subject has been reviewed recently by Mitchell and Marth (*1*). In view of the widespread use of this substance as a herbicidal or herbistatic agent, a study of the mechanism of its action on plant cells seemed desirable.

TABLE 1

TURBIDITY MEASUREMENTS OF YEAST CULTURES AT VARIOUS CONCENTRATIONS OF 2,4-D

Date	Concentrations of 2,4-D/ flask (ppm)	Per cent transmission			
		Flasks			Average
		1	2	3	
10–10–47	Control*	19	19	18	19
10–25–47	Control	18	20	22	20
"	0.001	20	23	21	21
"	0.01	22	22	21	22
"	0.1	22	21	21	21
"	1.0	22	21	22	22
10–10–47	1.0	18	18	20	19
"	10	22	23	23	23
"	100	46	46	47	46
"	1,000	82	81	82	82

* Control contains no 2,4-D.

Since growth and respiration are generally closely allied, it seemed plausible to use respiration as a means of studying the underlying mechanism of 2,4-D. In making such a study, it was thought desirable to run preliminary experiments on living yeast cells. This study could then serve as groundwork toward establishing the most effective concentration of 2,4-D to be used in experiments on mechanism. Yeast cells were selected (1) because of their ease of growth and manipulation and (2) because it is assumed that cell reactions of plants and animals, regardless of phylogenetic rank, generally are the same. In this particular phase of the work to be reported, the effect of 2,4-D on the growth of yeast is measured, by determining the turbidity of the medium, using a Cenco Photelometer.[1]

Cultures of *S. cerevisae* were grown on fort agar (*2*) slants at a pH of 4.8 and incubated for 48 hrs at 30° C. These were stored at 4° C. To 500 ml of a Czapek's solution were added 25 gm of glucose and 0.12 γ of biotin. Twenty-five ml of this solution was put into 15 125-ml Erlenmeyer flasks which were autoclaved at 15 lbs pressure for 15 min.

[1] Cenco-Sheard-Sanford "Photelometer," Type C-5, Central Scientific Company, Chicago, Illinois.

A stock solution of 2,4-D was prepared at a concentration of 1,000 ppm, and from this, concentrations of 100, 10, and 1 ppm were prepared. All four concentrations were then autoclaved at 15 lbs pressure for 15 min.

The 15 sterile flasks containing the Czapek's solution were divided into 5 groups of 3 each. The first group served as the control (no 2,4-D). Each flask was then inoculated with yeast cultures and incubated at 30° C. At the end of 7 days, growth was determined by the turbidimetric method. A red filter was used throughout.

The results, as shown in Table 1, indicate that concentrations of 2,4-D from 10 to 1,000 ppm have the most effect upon the growth of yeast. Concentrations of 1 ppm have practically no effect. This finding was further substantiated when another batch of yeast was subjected to 2,4-D in which the concentration ranged from 0.001 to 1.0 ppm. The same procedure was used as described above. With the exception of one aberrant value, the results were similar, *i.e.* the same as the control.

The test described above, which is based on the assumption that growth is directly proportional to turbidity, is relatively simple and can be run in a comparatively short time.

References

1. MITCHELL, J. W., and MARTH, P. C. *Growth regulators.* Chicago: Univ. Chicago Press, 1947.
2. PARFITT, E. H. *J. dairy Sci.*, 1933, **16**, 141.

A Simple Adaptation of the Mercury Calibration of Warburg Manometer Sets to Insure Interchangeability

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Almost anyone who has worked with the Warburg technique has experienced the irritation and delay which follows the breaking of a flask. Even with reasonable care, vessels do get broken, and unless one has extra sets, runs may be delayed until the flask has been repaired and recalibrated. This can be avoided by the use of flasks and manometers with interchangeable joints and an extra set of flasks. In this laboratory the most commonly used flask is the simple double-side-arm type, and extra flasks were procured to use either with a set of four Summerson manometers or as replacements on the other manometers. In planning the calibration of the glassware it was realized that, if the usual calibration methods were used, it would be necessary to calibrate each of these vessels both with its Summerson manometer and with its intended replacement manometer and that without a great deal of extra calibration interchangeability was strictly limited. To increase the interchangeability of flasks and manometers the following scheme was worked out.

Any flask in the replacement set is weighed empty and then filled, according to the method of Burris (*1*), with

enough mercury to rise about 1 cm above the flask into the manometer arm. The flask is placed on the dry joint of the first manometer of any one of the sets and seated. A line is scratched with a diamond point at the top of the mercury column. The flask is removed and the temperature of the mercury recorded immediately. As quickly as possible, and making sure that the temperature of the mercury does not change and that no mercury is spilled or left on the manometer joint, one repeats this procedure with the remaining manometers, marking the height of the mercury with a diamond point on each. If the temperature of the mercury changes considerably during handling, it may be found advisable to record it after each manometer is marked, for future calculation. We found it simpler in the long run in such cases to wait until the temperature returned to the initial level. The flask and mercury are then weighed and the manometers calibrated from the reference mark in the usual manner.

From this point on it is only necessary to calibrate any flask to the reference mark on any one manometer to obtain the Vg for that flask in combination with any of the manometers. Complete interchangeability then becomes a simple matter of calculation of kO_2 or kCO_2 values from known Vg values for all combinations. Furthermore, broken flasks after mending or new types of flasks may be readily fitted into the scheme by a quick calibration against any one manometer.

Reference

1. Umbreit, W. W., Burris, R. H., and Stauffer, J. F. *Manometric techniques and related methods for the study of tissue metabolism.* Minneapolis: Burgess, 1945. P. 50.

A Simplified Apparatus for One-dimensional Paper Partition Chromatography

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In carrying out one-dimensional paper partition chromatography, Consden, *et al.* (*1*) make use of glass troughs of cylindrical shape which, held in a horizontal position, serve as reservoirs for the developing solvents into which the filter paper strip chromatograms dip. Longenecker (*2*) has recently described a way of making such troughs.

The present note describes a *circular* trough which is easily and cheaply made from the two halves of an ordinary Pyrex Petri dish, 3.5″ in diameter. The centers of the two halves, with their lips facing upward, are connected by fusing them to a solid glass rod ⅜″ in diameter and 16.0″ long. This can be done readily at low cost by any competent glass blower.

In the accompanying diagram (Fig. 1) the double trough so obtained is shown in place in a glass humidifying chamber. The bottom trough serves to contain water or any aqueous solution which provides the chamber with the necessary humid atmosphere and also serves to provide a base for support. Absorbent cotton is wrapped around the solid glass rod and is wet with the same water phase present in the trough at its base. This cotton wick provides for the rapid restoration of equilibrium when it is necessary to open the humidifying chamber from time to time. The organic solvent used for development is placed in the upper trough. The paper strip chromatograms, suitably folded to dip into the organic solvent and to hang down from the trough, are held in place by a thin, solid, glass rod bent in a great arc. One end of the rod is turned perpendicularly to the plane of the arc and serves as a handle.

Around the circumference of the upper trough is placed a wire guard to hold the paper strips away from the side

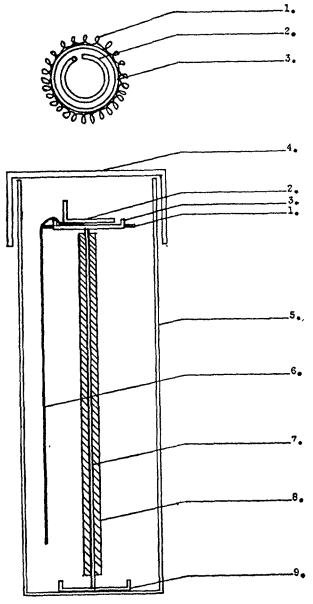

Fig. 1. Diagram of apparatus: (1) stainless-steel wire guard, (2) glass retaining rod, (3) Petri dish, (4) crystallizing dish, (5) cylindrical glass jar, (6) chromatogram strip, (7) glass rod, (8) absorbent cotton, (9) Petri dish.

of the trough over which they hang. This wire guard is made by bending a stainless-steel wire to form a scalloped circle (see Fig. 1) which has sufficient spring tension to grip the sides of the Petri dish trough so that no other means of attachment is necessary.

As a humidifying chamber, one may conveniently use a stock cylindrical glass jar, 18″ high and 6″ in diameter, covered by an inverted crystallizing dish (6.5″ in diameter). The closure may be made gas-tight by applying a starch glycerine paste to the edge of the jar.

The apparatus described, which can be used in developing 8 or more strip chromatograms at one time, depending on their width, has the advantage of cheapness and ease of manipulation.

References

1. Consden, R., Gordon, A. H., and Martin, A. J. P. *Biochem. J.*, 1944, **38**, 224.
2. Longenecker, W. H. *Science*, 1948, **107**, 23.