Agar Dish Isopiestic Equilibration Method for Controlling the Water Potential of Solid Substrates

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Maintenance of substrate water potential in petri dishes is achieved by using vapor-pressure controlling, solute-amended agar gel discs attached to the inside of the top halves of the dishes.

The most widely used method for controlling the water status of a solid microbial substrate is to bring the substrate into water vapor equilibrium with a solution of known water potential (Fig. 1A). The time required for isopiestic equilibration of the substrate to the desired water potential can be reduced appreciably by initial adjustment of the substrate water content according to the moisture retention characteristics of the substrate (4).

This note describes a compact isopiestic equilibration system for controlling substrate water potential, in which the vapor pressure is controlled by a solute-amended agar gel of known water potential located in close proximity to the solid substrate. An experimentally convenient system is to place the substrate in the bottom of a glass petri dish, solidify appropriate solute-amended agar in a plastic petri dish (88 mm in diameter), and transfer the whole of this agar aseptically to the inside of the glass petri dish top (97 mm in diameter) as shown in Fig. 1B. The vapor pressure-controlling agar gels are made by incorporating salts such as NaCl or KCl into

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2% water-agar. The gels possess the desired properties since (i) osmotic coefficients and thus water potentials of such salts are available over a wide range of solution concentrations and temperatures (2, 3), providing a range of water potentials down to the minimum for microbial growth; (ii) low molecular size, monovalent ions move readily in agar gels, thereby preventing ionic gradients in the gels during substrate equilibration; and (iii) the water potential of 2% water-agar (Difco) is negligible (4; unpublished data). Plastic petri dishes of salt-amended agar can be prepared in bulk and stored in sealed, moisture-proof plastic bags indefinitely before use for equilibration.

The advantages of the agar dish isopiestic equilibration system are a reflection largely of the general advantages associated with the use of petri dish systems for microbial investigations. Experimental scope is increased because of the compactness and mobility of the system, and the feasibility of long-term pure culture studies is increased since contamination possibilities during incubation and sampling are minimized. In addition, because of the close proximity of the vapor pressure-controlling agar and the solid substrate, equilibration time is reduced and the opportunity for temperature gradients to occur within the system is diminished.

For the initial adjustment of the substrate to the desired water potential, the relationship between substrate water content and water potential, i.e., the moisture retention characteristics of the substrate, must be known. This can be determined readily by equilibrating a removable substrate sample (Fig. 1C) in an appropriate agar plate system until constant sample weight is attained, replacing the solute-amended agar lid intermittently to ensure final equilibration at the correct water potential. Equilibration rate will vary according to the nature of the substrate but can be increased by addition of water to a level near that at which the substrate will finally equilibrate, bearing in mind the necessity of achieving final equilibration of all samples of one set by a wetting or by a drying process because of the hysteresis phenomenon (4).

The moisture retention curves obtained for three texturally different soils by using the agar plate equilibration method (Fig. 2) are consistent with those obtained by other methods. The steep slope of the curves at potentials lower than $-5$ to $-10$ bars emphasizes the necessity of maintaining substrate water potential isopiestically rather than attempting direct gravimetric control of the water status. Psychrometric measurement (1) of the water potential of soils equilibrated by using the agar plate method showed that soil water potential could be maintained indefinitely within 1 to 2 bars of the vapor-pressure controlling, solute-amended agar.

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