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Aerating Liquids by Agitating on a Mechanical Shaker

Kluyver and Perquin's *Schüttelkulturmethode (Biochem. Z.,* 1933, 266, 68) has become a popular method of studying the physiology of molds. This method involves continuous agitation of submerged cultures on shaking machines and assures a more uniform supply of nutrients and oxygen to all cells as well as a more uniform removal of gaseous waste products than does the surface culture method. During studies on the metabolism of *Penicillium chrysogenum,* generously supported by the Bristol Laboratories, it became desirable to determine how much greater the potential supply of oxygen is in shaken media than in media kept stationary. Since certain media foam quite vigorously when shaken, the question of how seriously such foams interfere with the diffusion of oxygen also needed consideration.

Experiments to measure the rate at which oxygen diffuses into media would be most significant from a physiological point of view when performed with media that contain respiring cells. Unfortunately such experiments are also the most laborious to set up. For example, one could suspend varying amounts of cells in shake-flasks and observe the rate at which they take up oxygen. A graph of "volume of oxygen absorbed per unit time" plotted against "amount of cells" would indicate a direct proportion between the two until a further increase in cells no longer gave an increase in respiration. This would mean, provided that the supply of oxidizable substrate is adequate, that the rate of respiration has become so high that it is limited by the rate of oxygen diffusion into the medium. The rate of oxygen uptake corresponding to the horizontal portion of the curve would then be a measure of the rate at which oxygen diffuses into the medium. (Cf. Umbreit, Burris, and Stauffer. *Manometric techniques.* Minneapolis: Burgess, 1945, p. 9.)

One could, of course, determine the rate of diffusion more simply in the absence of respiring cells. These determinations unfortunately are subject to the criticism that any conclusions which they may suggest are not necessarily applicable to living systems. However, since at least preliminary information can be obtained in this manner, such determinations were made as follows: 150 ml of freshly boiled and rapidly cooled distilled water, sometimes containing added substances, were placed in 500-ml Erlenmeyer flasks, which were then plugged with cotton, and agitated at 28° C on a reciprocating shaker, having a 4-in stroke and shaking at a rate of 85 strokes per min. At zero time and at intervals thereafter, the amount of oxygen dissolved was determined by the Winkler method (American Public Health Association, *Standard methods for the examination of water and sewage,* New York, 1946). All determinations were made at least in duplicate.

Fig. 1 gives some typical absorption curves. Oxygen dissolved in agitated liquids very rapidly, nearly saturating the liquid within a few minutes. Diffusion into still water proceeded slowly, as expected. In all cases the rate of absorption was most rapid at the beginning of the experiment, and slowed down as saturation was being reached. Assuming the ideal case in which oxygen is used by the organisms as fast at it is furnished, one can estimate the potential oxygen supply from these high initial rates. For example, under our conditions oxygen diffused into shaken distilled water during the first 30 sec at a rate of about 30 ml of oxygen per hr per 150 ml of distilled water, which would theoretically support the growth of 1 g (dry weight) of cells having

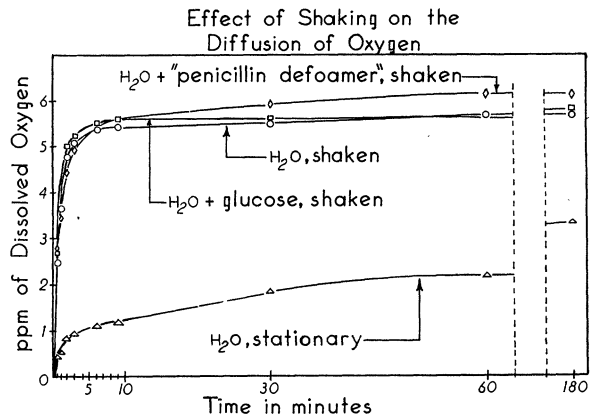

FIG. 1. The rate at which oxygen diffused during the first 30 sec, in ppm per hr per 150 ml of liquid was: 300 for shaken distilled water; 50 for stationary distilled water; 330 for shaken distilled water containing 0.05 ml penicillin defoamer; and 320 for distilled water containing 0.5% glucose.

a Q_{O_2} of about 30. This estimate is made with reservations, since the presence of high concentrations of nutrients and of cells will reduce the solubility of oxygen.

Not only did oxygen diffuse six times more rapidly into agitated water than into quiet water, but the total amount of oxygen present after 3 hr was 70% greater in the shaken than in the undisturbed water. Furthermore, it also seems likely that until the still water becomes completely saturated with oxygen there is an uneven distribution of oxygen; however, no determinations were made to test the validity of this assumption. The addition of 0.05 ml of Swift's "penicillin defoamer," an antifoam agent used in the manufacture of penicillin, did not appear to have a significant effect on the diffusion of oxygen; neither did the addition of 0.5% glucose.

It was at first difficult to find an artificial system that might be comparable to a medium covered by a foam, because the various foams tested in preliminary ex-

periments (such as beaten egg white, foams produced from solutions of corn steep liquor, Rinso, and Tide) either interfered with the Winkler method or were unstable. However, when nitrogen was bubbled from a Pyrex gas dispersion tube with fritted cylinder through a 2.5% solution of gum arabic, a thick foam developed which completely covered the solution and in spite of agitation stayed intact for about an hour. Fig. 2 shows

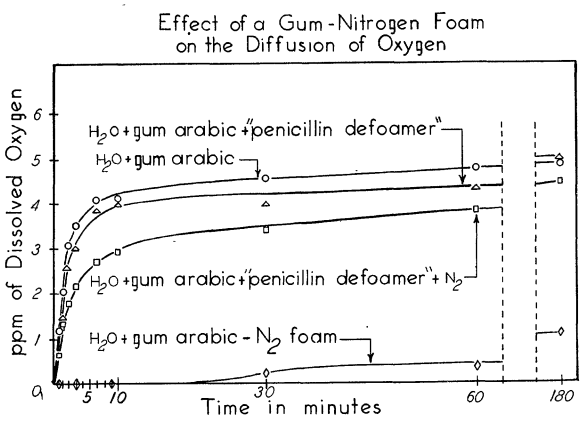

FIG. 2. The rate at which oxygen diffused during the first 30 sec, in ppm per hr per 150 ml of liquid was: 140 for the gum arabic solution; 150 for the gum arabic solution containing 0.05 ml of penicillin defoamer; 80 for the gum arabic solution that contained 0.05 ml of defoamer and through which nitrogen was bubbled; 0 for the gum arabic solution covered with a gum arabic-nitrogen foam.

that, as long as this foam persisted, the diffusion of oxygen was almost completely prevented. The possibility that the flushing with nitrogen removed enough oxygen from the overlying atmosphere to decrease the rate of diffusion was examined by repeating the experiment in the presence of 0.05 ml "penicillin defoamer." This antifoam agent prevented the foam, and diffusion proceeded at a fairly rapid rate. Flushing with nitrogen apparently did decrease the rate of diffusion somewhat, as can be seen from the absorption curve for the solution of gum arabic that contained the antifoam agent but through which no nitrogen was bubbled. The fact that oxygen diffused more slowly into the gum solution than into distilled water can probably be explained by an increase in the viscosity of the solution. Gum arabic did not interfere significantly with the Winkler titration. When gum concentration was increased in intervals of 0.5% from 0% to 3%, the diffusion rate decreased markedly while the titration blanks at zero time were about the same for all levels.

These results do not necessarily imply that the foaming that occurs during actual shake-flask fermentations interferes as seriously with aeration as did the gum arabic-nitrogen foam in our experiments. They do, however, draw attention to the need for further studies on the aeration of microbial cultures.

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Only Qualified Praise of Chisholm's "Social Responsibility"

George Brock Chisholm's article (*Science*, January 14, p. 27) received the unqualified approval of Rollin G. Myers in your March 11th Comments and Communications section (p. 264), and it appears justified to express in these pages some criticism on a basic point of Dr. Chisholm's philosophy since it is hoped that almost everybody in this country, at least, will agree with Dr. Chisholm's aims and efforts in general.

The point in question is Dr. Chisholm's concept of "original sin." Dr. Chisholm says "The uncomfortable fact is that very few people indeed can love themselves in a healthy natural way which tolerantly accepts all their own human urges as normal and inevitable aspects of the healthily functioning man or woman. Most of us, by being civilized too early or too forcibly, have been driven to believe that our natural human urges are 'bad,' 'not nice,' 'wicked,' 'sinful,' or whatever the local equivalent may be. . . . Unfortunately, the concept of 'sin' is, under one name or another, very firmly entrenched throughout much of the world."

It would seem that Dr. Chisholm is unfamiliar with the history of the Christian concept of "original sin" and particularly with the attitudes of such outstanding contemporary theologians as Reinhold Niebuhr and Paul Tillich. Suffice it to quote from R. Niebuhr's *Beyond tragedy, essays on the Christian interpretation of history* in condensation as follows: "Sin is not so much a consequence of natural impulses which in animal life do not lead to sin as of the freedom of reason by which man is able to throw nature out of joint and to make fateful decisions in human history. . . . Sin lies at the juncture of spirit and nature. . . . The most basic need of the human spirit is the need for security. . . . The primary insecurity of human life arises from its finiteness and weakness. . . . When man looks at himself he finds himself to be only one of many creatures in creation. But when he looks at the world he finds his own mind the focusing center of the whole. When man acts he confuses these two visions of himself. He knows that he ought to act as to assume only his rightful place in the harmony of the whole. But his actual action is always informed by the ambition to make himself the centre of the whole. . . . When thought gives place to action, self intrudes itself into every ideal. . . . His sin is to turn creatureliness into infinity . . . when he centers his life about one particular impulse . . . tempted by his peculiar situation of being a finite and physical creature and yet gifted to survey eternity."

It is exactly, then, the recognition of being sinful, the concept of the ever-present danger of deceiving himself, which ennobles man and which offers a hope of overcoming the great difficulties of present international human relationships.

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