

LXVII.—*Studies in Fermentation. Part III. The Rôle of Diffusion in Fermentation by Yeast Cells.*

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WHEN a fermentable sugar is transformed into alcohol and carbon dioxide by the action of living yeast suspended in an aqueous solution of the sugar, it is generally accepted that the chemical action takes place within the yeast cell, and that the sugar has to diffuse into the latter before it is attacked by the enzyme. A consideration of the influence of various factors on the rate of alcoholic fermentation shows that under ordinary conditions diffusion supplies the yeast cells rapidly and efficiently with sugar. The high temperature-coefficient and the constancy of the velocity with different concentrations of the sugar constitute practically conclusive evidence on this point. It is, however, conceivable that under certain conditions, such as with very dilute sugar solutions, or with very active yeast cells, diffusion could not take place rapidly enough to supply the yeast cells with sugar to enable them to exert their maximum fermentative power. In the present investigation the limiting conditions have been examined under which diffusion alone would no longer be capable of supplying the yeast cells efficiently with sugar, and under which the apparent velocity of the reaction would thus be influenced by convection currents produced either owing to the evolution of gas in the liquid, or by external means.

In order to obtain an insight into the extent to which such convection currents (stirring) might conceivably affect the rate of fermentation by yeast cells, it is useful first to determine what are the maximum differences of concentration which might arise in a liquid in which no convection currents whatever take place, and in which uniformly distributed yeast cells are operative.

It will be readily conceded that if we place a single yeast cell in the centre of a spherical vessel containing a sugar solution, differences of concentration between the surface of the cell and the boundary of the vessel will arise, which will at first increase and become the greater the further away the boundary of the vessel is. These differences of concentration will attain their greatest value if no diminution of the concentration in the outermost layer is allowed to take place and if the yeast cell has been operative for an indefinite period of time, so that the "permanent" state has been attained. In the latter case the amount of sugar removed by the yeast cell per unit of time is the same as that flowing to it, owing to diffusion across any concentric spherical surface inside the sphere.

The amount of sugar  $F$  removed by each yeast cell per unit of time can be determined from experiments, the cell may be assumed to be of spherical shape, and a sufficiently accurate value assigned to its radius. It might at first sight be assumed from analogy to diffusion in a straight line (unidimensional) that a permanent state could only be attained after the concentration on the surface of the yeast cell had diminished to zero. That this is by no means the case has already been proved in the paper by one of us (Sand, *Proc. Roy. Soc.*, 1905, **74**, 356), in which a similar problem was treated. This problem was, however, rather different from that at present under discussion, and it will be convenient to deduce directly the formulæ required to suit the present case.

The first part of our problem thus consists in determining what is the concentration at the surface of a spherical yeast cell of radius  $R$  placed at the centre of a spherical vessel of infinite radius on the boundary of which the concentration  $C$  is maintained. The yeast cell removes a quantity of sugar  $F$  by chemical action per unit of time. Imagine the spherical vessel subdivided by a large number of concentric, spherical surfaces of radius  $x$  and surface  $4\pi x^2$ . Across each of these the amount of sugar  $F$  diffuses per unit of time. Indicating the diffusion-coefficient of the sugar by  $\kappa$  and the concentrations by  $C$ , we have:

$$F = 4\pi\kappa x^2 \frac{dc}{dx} \quad . \quad . \quad . \quad . \quad . \quad (1),$$

with the limiting condition:

$$c = C \text{ for } x = \infty \quad . \quad . \quad . \quad . \quad . \quad (1a).$$

The integral of the above equation under the condition 1a is:

$$C - c = \frac{F}{4\pi\kappa x} \quad . \quad . \quad . \quad . \quad . \quad (2),$$

and indicating the concentration of the sugar on the outside of the yeast cell by  $C_1$  and the radius of the latter by  $R$ , we have:

$$C - C_1 = \frac{F}{4\pi\kappa R} \quad . \quad . \quad . \quad . \quad . \quad (2a).$$

The equation 2a thus allows us to calculate the maximum conceivable difference of concentration that might arise between the surface of the yeast cell and other parts of the liquid if no stirring whatever takes place.

We now come to the consideration of the changes of concentration of the sugar solution which arise in the interior of the yeast cell owing to the combined influences of chemical action and diffusion. We may assume the enzyme to be uniformly distributed throughout the yeast cell, and we know the velocity of the reaction to be

independent of the concentration of the sugar within wide limits, so that this velocity may be assumed to be uniform throughout the whole cell (see Trans., 1906, **89**, 133). Indicating therefore by  $s$  the amount of sugar removed in the interior of the cell by chemical action per unit of volume and time, and employing the same notation as above, we have:

$$s = \frac{F}{\frac{4}{3}\pi R^3} \quad . \quad . \quad . \quad . \quad . \quad (3).$$

We proceed to calculate the concentration of the sugar  $c$  corresponding with any point in the yeast cell at a distance  $r$  from its centre after the permanent state has been attained. The latter, as can be seen from other considerations, is approached with exceedingly great rapidity (compare Sand, *loc. cit.*). We imagine the yeast cell divided into an infinite number of concentric, spherical shells of radius  $r$ , thickness  $dr$ , and thus of volume  $4\pi r^2 dr$ . If we indicate the amount of sugar flowing (owing to diffusion) into a shell towards its centre per unit of time at the radius  $r+dr$  by  $f_{(r+dr)}$ , and the amount flowing out at a radius  $r$  by  $f_{(r)}$ , and, further, the amount removed in the shell by chemical action by  $S$ , then, when the permanent state is established, we have:

$$S + f_{(r)} = f_{(r+dr)}$$

or

$$S = df \quad . \quad . \quad . \quad . \quad . \quad (4).$$

Further, we have:

$$S = 4\pi r^2 s dr \quad . \quad . \quad . \quad . \quad . \quad (5).$$

$$f = 4\pi \kappa r^2 \frac{dc}{dr} \quad . \quad . \quad . \quad . \quad . \quad (6).$$

( $f$ ,  $c$ , and  $r$  are variables)

Differentiating (6) and substituting the result and the value of  $S$  from equation (5) into equation (4), we find:

$$4\pi r^2 s dr = \left( 8\pi \kappa r \frac{dc}{dr} + 4\pi \kappa r^2 \frac{d^2c}{dr^2} \right) dr$$

or

$$\frac{s}{\kappa} = \frac{2}{r} \frac{dc}{dr} + \frac{d^2c}{dr^2} \quad . \quad . \quad . \quad . \quad . \quad (7),$$

the limiting conditions being  $f=F$  for  $r=R$ , and  $c=C_1$  for  $r=R$ .

The integral of equation (7) is:

$$c = \frac{\text{const.}_1}{r} + \frac{1}{6} \frac{s}{\kappa} r^2 + \text{const.}_2,$$

and the limiting conditions yield the values:

$$\text{const.}_1 = 0; \quad \text{const.}_2 = C_1 + \frac{1}{6} \frac{s}{\kappa} R^2,$$

which, in conjunction with (3), lead to:

$$C_1 - c = \frac{s}{6\kappa} (R^2 - r^2) = \frac{F'}{8\pi\kappa} \frac{R^2 - r^2}{R^3}.$$

From this equation we find for the concentration  $C_0$  at the centre of the cell ( $r=0$ ):

$$C_1 - C_0 = \frac{F'}{8\pi\kappa R} \quad . \quad . \quad . \quad . \quad . \quad (8).$$

This result represents the maximum difference of concentration to be found in the yeast cell; and, with maximum stirring of the liquid (during which  $C_1$  is the average concentration of the liquid), it also represents the maximum difference of concentration arising throughout the system. With minimum stirring of the liquid, we find by combination with equation 2a for the greatest difference in concentration to be met with in the system:

$$C - C_0 = \frac{3}{8} \frac{F'}{\pi\kappa R} \quad . \quad . \quad . \quad . \quad . \quad (9).$$

that is, a value three times as great as the foregoing.

The value of  $F'$  for brewery yeast was determined experimentally by means of the apparatus described in previous publications (Trans., 1906, **89**, 128; *J. Soc. Chem. Ind.*, 1908, **27**, 653). A solution of dextrose and a known quantity of yeast cells, the number of which was determined by means of a hæmacytometer, were introduced into the bottle of the apparatus and the velocity of fermentation measured on the manometer scale. An absolute value of the velocity was obtained after calibration of the apparatus, the latter operation being carried out by fermenting a known weight of dextrose and observing the corresponding change in pressure. From these measurements can be calculated the number of grams of dextrose fermented per second by a yeast cell of average activity. The results of four experiments with different samples of yeast are given in the following table:

*Temperature, 30°. Volume of liquid = 60 c.c.*

Concentration of dextrose = 5 grams per 100 c.c.

„ „ yeast = 1 gram of pressed yeast per 100 c.c. (approximately).

Number of yeast cells in 60 c.c.	Grams of dextrose fermented per second.	$F$ .
$2.10 \times 10^9$	$6.2 \times 10^{-5}$	$3.0 \times 10^{-14}$
$1.91 \times 10^9$	$6.2 \times 10^{-5}$	$3.2 \times 10^{-14}$
$1.79 \times 10^9$	$5.0 \times 10^{-5}$	$2.8 \times 10^{-14}$
$2.07 \times 10^9$	$6.0 \times 10^{-5}$	$3.0 \times 10^{-14}$

At this concentration of the uniformly distributed yeast, the average concentration of the sugar decreases so slowly that the

permanent state for which our formulæ have been deduced is, for practical purposes, reached so quickly, that during its attainment there is no appreciable variation of the outside concentration  $C$ . We may also express this by saying that the yeast cells do not appreciably interfere with each other's action.

For purposes of calculation, the value  $F = 3 \times 10^{-14}$  gm./sec. has been taken. The diameter of the yeast cells is approximately  $8\mu$ , giving  $R = 4 \times 10^{-4}$  cm. The diffusion-coefficient of dextrose,  $\kappa$ , is approximately  $7 \times 10^{-6}$  cm.<sup>2</sup>/sec.\*

Substituting the values in equations 8 and 9, we have:

$$C_1 - C_0 = \frac{3 \times 10^{-14}}{8 \times \pi \times 7 \times 10^{-6} \times 4 \times 10^{-4}} = 4.3 \times 10^{-7} \text{ gm./cm.}^3.$$

$$= 0.43 \text{ mg. per litre.}$$

$$\text{and} \quad C - C_0 = 1.29 \quad , \quad , \quad ,$$

The maximum difference of concentration brought about in the system with stationary uniformly distributed cells is therefore about 1.3 milligrams per litre. Two-thirds of this difference can be removed by stirring. If we make  $C_0 = 0$ , we obtain conditions under which there diffuses into the yeast cell just enough sugar as can be fermented by the cell. Diffusion therefore supplies the yeast cell efficiently with sugar unless the concentration falls below 1.3 milligrams per litre, or, with maximum stirring of the solution, a concentration a third as great will satisfy the condition. From equation 8 and 9 we can also calculate the activity of a yeast cell which could just entirely ferment the whole of the sugar diffusing into the cell. If the concentration of the sugar solution is 5 grams per 100 c.c. with maximum stirring of the solution,  $C_1 = 5 \times 10^{-2}$  gram/cm.<sup>3</sup>, and with minimum stirring,  $C = 5 \times 10^{-2}$  gram/cm.<sup>2</sup>. Further, making  $C_0 = 0$ , and denoting the number of grams of sugar fermented per second by  $F^1$ , we have, with maximum stirring,  $F^1 = 8\pi \times 7 \times 10^{-6} \times 4 \times 10^{-4} \times 5 \times 10^{-2} = 3.5 \times 10^{-9}$  gram/sec., and with minimum stirring,  $F^1 = 1.2 \times 10^{-9}$  gram/sec. Yeast which would fulfil this condition in the first case would have to be 120,000 times as reactive as brewery yeast, and in the second case 40,000 times as reactive as brewery yeast.

It is also evident that the greater the yeast cell the more slowly it is supplied by diffusion with sugar. From equations 8 and 9 we can calculate the size of a yeast cell which would just entirely ferment the whole of the sugar diffusing into the cell, assuming

\* So far as we are aware, the diffusion-coefficient of dextrose has not been determined, but as substances of about the same molecular weight have almost the same diffusion-coefficient, the value for dextrose cannot be greatly different from that of mannitol, which at  $30^\circ$  is  $4.4[1 + (30^\circ - 10^\circ)0.025] \times 10^{-6} = 6.6 \times 10^{-6}$ . It is assumed that the sugar diffuses in the yeast cell at the same rate as in water.

that this large cell is built up of material of the same activity as brewery yeast, that is, keeping  $F/R^3$  constant:

$$\frac{F}{R^3} = \frac{3 \times 10^{-14}}{(4 \times 10^{-4})^3} = 4.7 \times 10^{-4}.$$

Substituting in equation 8,  $C_1 = 5 \times 10^{-2}$ ,  $C_0 = 0$ ,

and  $F = R^3 \times 4.7 \times 10^{-4}$ ,

we obtain  $R^2 = 1.87 \times 10^{-2}$  or  $R = 0.14$  cm.

With maximum stirring of the solution, yeast cells of radius 1.4 mm. would therefore just be efficiently supplied by diffusion with dextrose from a solution containing 5 grams per 100 c.c. With minimum stirring, a value of the radius 0.8 mm. would satisfy the condition.

From these calculations we may conclude that during fermentation diffusion supplies the yeast cells very efficiently with sugar, and, further, it is unlikely that conditions could be experimentally realised under which diffusion becomes a controlling factor of the rate of fermentation.

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