act in alcohol or water quantitatively giving 5-bromouracil, free sulfur, cyanamide and hydrobromic acid.

2. We obtained no evidence of the formation of uracil-5-pseudothiourea.

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[CONTRIBUTION FROM THE FLEISCHMANN LABORATORIES, STANDARD BRANDS INCORPORATED]

Influence of Oxygen on the Fermentation of Maltose and Galactose[1]

BY ALFRED S. SCHULTZ, LAWRENCE ATKIN AND CHARLES N. FREY

Although bakers' yeast contains maltase, it customarily ferments maltose in pure solutions only after a rather long induction period. The addition of dextrose, in small amounts, remarkably shortens the induction period.[2,3] Further work has shown that oxygen, under appropriate conditions, shortens the induction period for maltose fermentation. The investigation was extended to galactose, the fermentation of which was aided by oxygen but not by dextrose.

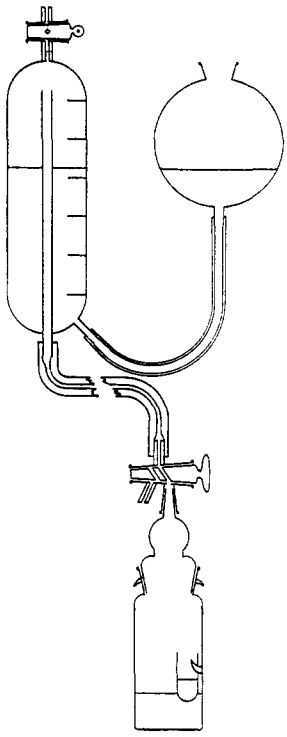

Fig. 1.—Apparatus for the measurement of fermentation under various gases.

Experimental

Apparatus.—The fermentation apparatus employed is a modification of one previously described[4] in which arrangement has been made for the use of a known atmosphere (see Fig. 1). The reaction bottles (vol. 250 ml.) were provided with glass hooks designed to hold oversize Keilin tubes containing the yeast suspension until the system had reached equilibrium. The system was filled with nitrogen or oxygen by evacuating the prepared reaction bottle and repeatedly flushing the gasometer with the gas by means of the stopcock on top and the inlet below. The evacuated reaction bottle was quickly connected with the filled gasometer, the connections flushed out by means of the three-way stopcock, and the gas admitted to the bottle. Excess gas was discharged through the top of the gasometer. All experiments were done at 30° and the bottles were shaken at 100 oscillations per minute.

Materials.—The final volume of the fermenting solution was 80 ml. and contained 0.8 g. of moist (compressed) bakers' yeast, a phosphate–citrate buffer of *p*H 5.4, 3 g. of the sugar under study, nicotinic acid (1 mg.), thiamin (0.05 mg.), and mineral salts including ammonium ions (200 mg. of ammonium sulfate).

Maltose Fermentation.—In pure nitrogen the initiation of maltose fermentation is very slow, as can be seen from Fig. 2. Air causes increased attack and oxygen still more. Dextrose in a nitrogen atmosphere causes a prompt fermentation of the maltose to occur. The initial hump in the latter curve is due to the fermentation of dextrose, the gas equivalent of the amount added being about 90 ml.

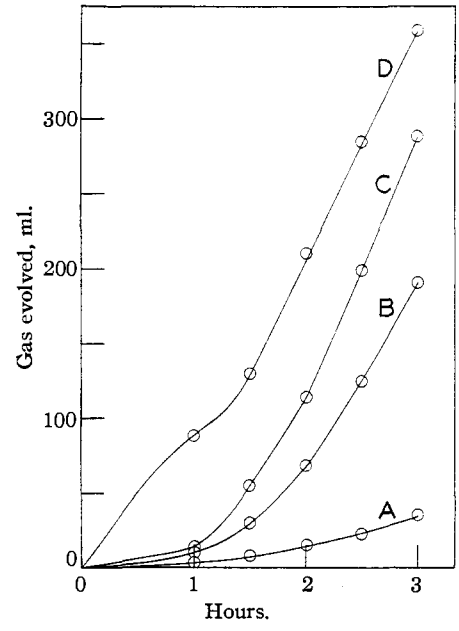

Fig. 2.—Maltose fermentation: Curve A, nitrogen atmosphere; Curve B, air atmosphere; Curve C, oxygen atmosphere; Curve D, nitrogen atmosphere plus 0.4 g. of dextrose in solution.

Dextrose Fermentation.—The fact that oxygen stimulates fermentation is contrary to the so-called Pasteur reaction wherein the reverse is supposed to occur. The explanation lies in the fact that our experimental conditions do not produce an extreme degree of aerobicity even when

(1) Presented before the Division of Biological Chemistry at the Boston meeting of the American Chemical Society, September, 1939.

(2) A. S. Schultz and L. Atkin, THIS JOURNAL, **61**, 291 (1939).

(3) J. Leibowitz and S. Hestrin, *Enzymologia*, **6**, 15 (1939).

(4) A. S. Schultz and Q. Landis, THIS JOURNAL, **54**, 211 (1932).

pure oxygen is the atmosphere. Comparison of the fermentation of dextrose in nitrogen, air and oxygen (Fig. 3) shows that although oxygen reduces the fermentation rate of dextrose, it is no lower than the rate of maltose fermentation in oxygen. Thus the stimulation of maltose fermentation by oxygen, under these conditions, actually implies no contradiction of the Pasteur reaction.

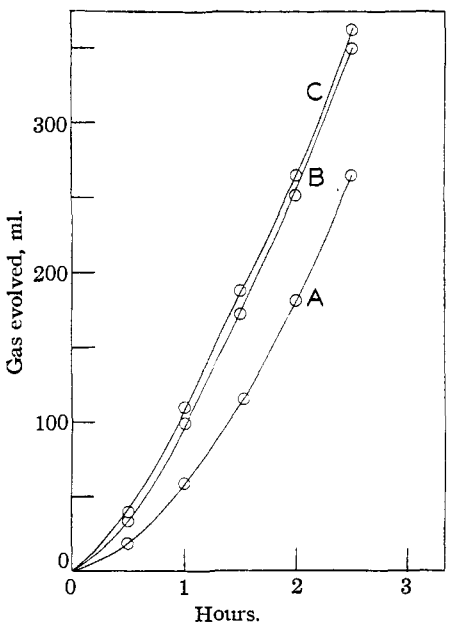

Fig. 3.—Dextrose fermentation: Curve A, oxygen atmosphere; Curve B, air; Curve C, nitrogen.

Galactose Fermentation.—Many yeasts have the property of fermenting galactose after a sufficient induction period. Under our conditions nitrogen extends the induction period almost indefinitely whereas oxygen materially shortens it (see Table I). Dextrose has no stimulating action. If the yeast, after it has acquired the ability to ferment galactose, is centrifuged off a satisfactory galac yeast is obtained. This method can be used in place of the method of Kirby and Atkin,[5] when more convenient.

TABLE I

GALACTOSE FERMENTATION IN OXYGEN AND NITROGEN, ML. OF CARBON DIOXIDE EVOLVED

Hours	3	7	8	9	10.5
In O_2 atm.	1	42	88	150	263
In N_2 atm.	0	0	3	3	5

Discussion

The classification of yeast species is based largely on sugar fermenting ability. The above data indicate that the physical conditions of the test must be considered before one states whether a yeast can ferment a given sugar. In this connection the question of direct fermentation arises. Some workers have compared the maltase content of yeast with the rate of maltose fermentation and from an observed disparity have considered that the disaccharide is fermented without preliminary hydrolysis. It has already been pointed out[2,3] that the maltase determinations are open to question. The present communication indicates that the method of making measurements of the rate of maltose fermentation also must be reëxamined.

The effect of oxygen on maltose fermentation will be masked if the maltose preparation contains sufficient dextrose as an impurity.[2] The fact that oxygen and dextrose are both initiators of maltose fermentation does not alter the theory that permeability changes may be responsible for the effect observed.

Summary

The presence of oxygen aids bakers' yeast to initiate the fermentation of maltose. The effect is similar to that of a small quantity of dextrose. The effect of these observations on the classification of yeasts and theories of disaccharide fermentation is briefly discussed. An atmosphere of oxygen stimulates the initiation of galactose fermentation but dextrose is without effect. A convenient method for the preparation of a galac yeast is suggested.

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(5) G. W. Kirby and L. Atkin, *J. Biol. Chem.*, **116**, 511 (1936).