

## COMPARATIVE STUDIES ON RESPIRATION.

### XII. A COMPARISON OF THE PRODUCTION OF CARBON DIOXIDE BY PENICILLIUM AND BY A SOLUTION OF DEXTROSE AND HYDROGEN PEROXIDE.

BY F. G. GUSTAFSON.

(From the Laboratory of Plant Physiology, Harvard University, Cambridge.)

(Received for publication, June 11, 1920.)

The writer has shown<sup>1</sup> that the production of CO<sub>2</sub> by *Penicillium chrysogenum* is increased by acid and decreased by alkalies. The next step is to investigate the mechanism by which CO<sub>2</sub> is produced. For this purpose it seems desirable to try to construct an artificial system which would imitate the action of the fungus.

In the experiments with *Penicillium* the oxidizable substance was for the greater part dextrose, as the fungus was kept in a 0.5 per cent solution of that substance. A 0.5 per cent dextrose solution was therefore chosen as the basis of the artificial system. From a number of available oxidizing agents hydrogen peroxide was chosen as being the most promising. Dakin<sup>2</sup> states that of the various oxidizing agents hydrogen peroxide comes nearest to bringing about the same reactions *in vitro* as normally take place within the organism. An artificial system was accordingly made which consisted of a 0.5 per cent solution of dextrose plus hydrogen peroxide.<sup>3</sup>

The experiments were performed in essentially the same manner as those already described<sup>1</sup> except that the artificial system was substituted for the fungus. In each experiment the hydrogen ion concentration was kept constant during the whole experiment and the rate of production of CO<sub>2</sub> was measured before and after the addition

<sup>1</sup> Gustafson, F. G., *J. Gen. Physiol.*, 1919-20, ii, 617.

<sup>2</sup> Dakin, H. D., *Oxidations and reductions in the animal body*, London, 1912.

<sup>3</sup> In these experiments "Dioxygen" was used, as it is nearly neutral and it is stated by the makers to contain only 0.04 per cent of substances other than water and hydrogen peroxide, no preservative being added.

of hydrogen peroxide, while in the experiments with the fungus the hydrogen ion concentration was varied during the experiment. The results are nevertheless comparable in the two cases.

In the final experiments the same bottle of "Dioxygen" was used throughout, so that the results would be exactly comparable. It was found that the contents of two bottles which titrated the same with permanganate did not give the same results in respect to the amount of  $\text{CO}_2$  produced. For this reason all the data here published were obtained by the use of hydrogen peroxide from the same bottle.

The time required to cause a standard change in the indicator tube, when the other tube contained 0.5 per cent dextrose but no hydrogen peroxide, was taken as 100 per cent. This change may have been caused by a leak in the apparatus or by the breaking down of the dextrose or by both. The source of the  $\text{CO}_2$  is of no importance for the measurement. It is only necessary that in the absence of hydrogen peroxide the rate of production of  $\text{CO}_2$  should be constant so that the rate found after the introduction of the hydrogen peroxide could be compared with it. For convenience the rate in the absence of hydrogen peroxide is called the normal rate; the rate after the addition of hydrogen peroxide is expressed in terms of the normal. This normal change was very slow and varied between 20 and 35 minutes.

In beginning an experiment the 0.5 per cent dextrose solution was made up to the desired hydrogen ion concentration; 60 cc. of this solution were put into the apparatus and the air was caused to circulate for 2 hours or more till all dissolved  $\text{CO}_2$  had been pumped out. Several readings were then taken to get the normal rate of change. Then 8 cc. of hydrogen peroxide were added by opening the flask holding the dextrose solution; 3 minutes were allowed to elapse before the first reading was taken so as to allow a thorough mixing of the solution. In the first few minutes the production of  $\text{CO}_2$  was very great, but as will be seen from Fig. 1, it fell off very rapidly in the solution having a pH of 1, while in the neutral solution the fall was not so rapid.

The experiments with the alkaline solutions were made with the direct method (without the apparatus for the circulation of air) as described in a previous paper.<sup>1</sup> Experiments were first made with neutral solutions. The dextrose solution was placed in a tube,

hydrogen peroxide was then added, and 4 minutes were allowed to elapse. The time required to produce a definite<sup>4</sup> amount of CO<sub>2</sub> was then measured. The time it took to produce the same amount of CO<sub>2</sub> in the alkaline solution was then compared with the time in the neutral solution.<sup>5</sup> In Fig. 1, the comparison is expressed by taking

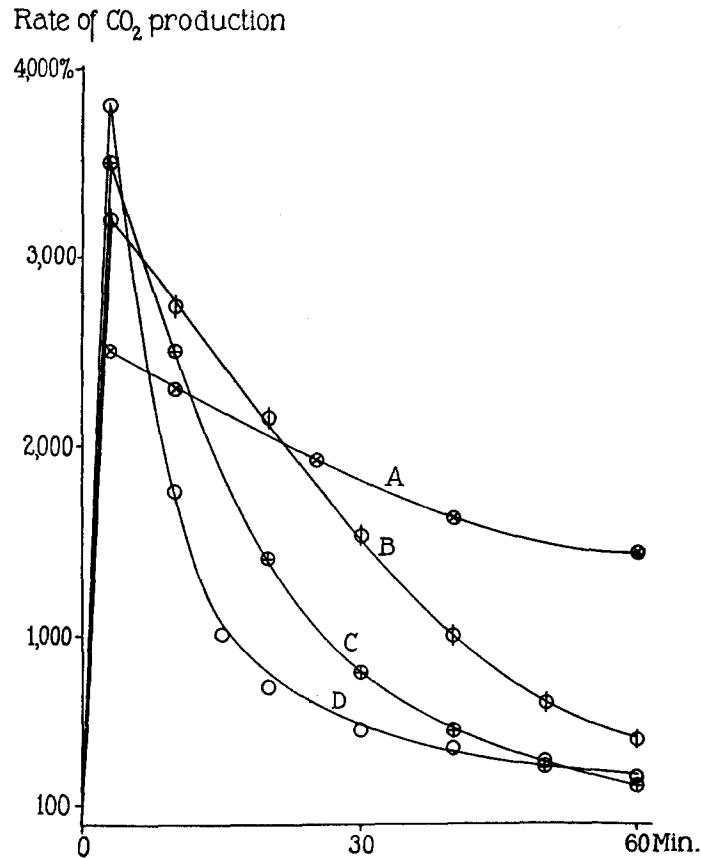


FIG. 1. Curves showing the rate of production of CO<sub>2</sub> by a solution of dextrose and hydrogen peroxide. Curve A shows the rate in a solution having a pH of 8.75, B in a solution of pH 7, C in a solution of pH 5, D in a solution of pH 1. The rate in the absence of hydrogen peroxide is taken as 100 per cent.

<sup>4</sup>This was ascertained by titrating with CO<sub>2</sub> as described in a former paper. (Gustafson, F. G., *J. Gen. Physiol.*, 1919-20, ii, 617.)

<sup>5</sup>The experiments with alkaline and neutral solutions were carried out almost simultaneously.

for the alkali curve a proportional part of the ordinate of the neutral curve obtained by the use of the apparatus. Thus if by the direct method the rate of production of  $\text{CO}_2$  in the alkaline solution was two-thirds as great as in the neutral solution it was assumed that this would also be the case if the experiment could be performed in

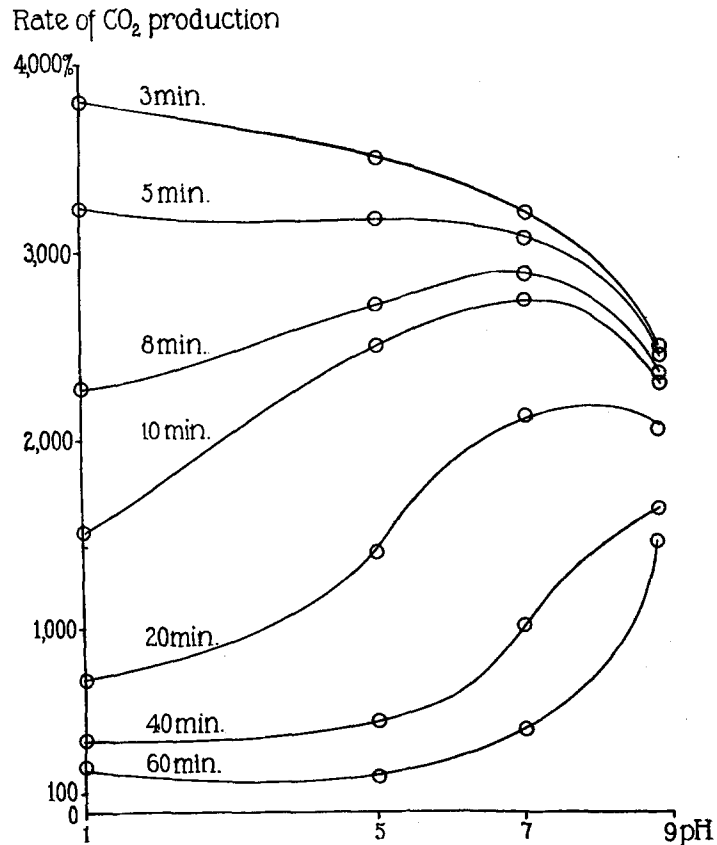


FIG. 2. Curves showing the rate of production of  $\text{CO}_2$  by a solution of dextrose and hydrogen peroxide as related to the acidity of the solution.

the apparatus and accordingly the ordinate of the alkali curve in Fig. 1 was made equal to two-thirds the ordinate of the neutral curve.

From these experiments it was found that the production of  $\text{CO}_2$  in the alkaline solution was at first below that at neutrality, but the fall in production was less rapid, and as will be seen from Figs. 1 and

2 the rate was higher at the end of an hour in the alkaline solution than in the neutral solution. The decrease in production of CO<sub>2</sub> in the alkaline solution is not so great as that shown by the curve, because in taking more than one reading the solution had to be made alkaline again by adding more NaOH, and this produced a buffer action, which increased with every reading.

It is evident that during the first part of the experiment the production of CO<sub>2</sub> resembles that of the organism in being greater in an acid than in a neutral medium, but less in an alkaline medium than at neutrality. It should be noted that the increase of rate in acid followed by a decrease is also observed in the case of *Penicillium*.

The falling off of the rate in the latter part of the experiment might be due to a decrease in the amount of some substance. Evidently this is not the sugar; it is doubtful whether it is the hydrogen peroxide, since titration with permanganate as well as the catalase test shows that the hydrogen peroxide has hardly decreased at all by the time the rate has fallen off. It might possibly be an active form of hydrogen peroxide or a catalyzer. It is of course possible that the falling off may be due to the formation of an inhibitory substance.

According to Spoehr<sup>6</sup> there is no reaction between glucose and hydrogen peroxide in a neutral solution, but in a 0.5 M KOH solution 2 gm. of glucose had been completely used up at the end of 3 days, forming CO<sub>2</sub>, formic acid, glycollic acid, and  $\alpha$ -hydroxymethyl-*D*-arabonic acid. As far as the writer is aware no work has been done with acid solutions.

It is probable that the reason why Spoehr did not notice any production of CO<sub>2</sub> in the neutral solution was because the amount is very small and the production lasts only a short time, while in the alkaline solutions the production continues for a longer time.

#### SUMMARY.

A neutral solution of dextrose and hydrogen peroxide acts like *Penicillium chrysogenum* in producing an increased amount of CO<sub>2</sub> upon the addition of acid, but not upon the addition of alkali.

<sup>6</sup> Spoehr, H. A., *Am. Chem. J.*, 1910, xliii, 227.