CLVIII. ENZYME SYSTEM OF *BACT. SUBOXYDANS*

II. EFFECT OF ACIDS AND pH

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It has been shown [Butlin, 1936, 1938] that the addition of chalk to maize wort agar used as growth medium for *Bact. suboxydans* has a striking effect on that organism's enzymic activity. This effect was ascribed to the neutralization by the chalk of the acid formed by the growing culture on the medium. To obtain further evidence for this, the direct action of acids on the highly oxidizing suspension derived from chalk-containing maize wort agar was examined.

EXPERIMENTAL

A suspension of *Bact. suboxydans* (dry weight 25 mg./ml.) was prepared as previously described [Butlin, 1936], after 2 days' growth at 30° on maize wort agar containing 2% chalk. 4 ml. portions of the main suspension were treated with 1 ml. acid of various strengths, left for $\frac{1}{2}$ hr., centrifuged and washed twice with sterile saline, and finally made up with 6 ml. sterile saline. The control received the same treatment except that water was substituted for acid. The O₂ uptake and CO₂ production with 0.3 ml. 0.1 *M* glucose were then determined in the Barcroft differential manometer by the usual methods, phosphate buffers of pH 6.0 and a temperature of 30° being used throughout.

Figs. 1 and 2 contain the O₂ uptake-time and CO₂ evolution-time curves with glucose as substrate given by suspensions treated with varying concentrations of HCl. The latter are expressed in terms of the actual strength of acid in contact with the bacterial cells during treatment. It should be emphasized that after treatment the cells were well washed and their action with glucose conducted at pH 6.0.

The results obtained bear a strong resemblance to those showing the effect of time of growth of the culture on the medium [Butlin, 1938], except that there was no initial increase in activity. The control suspension gave the normal behaviour of *Bact. suboxydans* with glucose at pH 6.0, i.e. rapid initial O₂ uptake, relatively slow initial evolution of CO₂, followed by an increase in the latter until its rate of evolution became equal to the rate of O₂ uptake. Treatment of the suspension with 0.001 *N* HCl had only slight effect, but with increasing concentration of acid the O₂ uptake and evolution of CO₂ decreased by approximately equal amounts until the latter entirely ceased, when the O₂ uptake (470 μ l.) was equivalent to slightly less than $1\frac{1}{2}$ atoms O for each molecule of glucose. This condition, in which the reaction with glucose was reduced to a simple oxidation process, was attained by treatment of the suspension with 0.006 *N* HCl. From this stage the effect of treatment with more acid was less intense and a concentration as great as 0.02 *N* was required to reduce the O₂ uptake to the equivalent of

1 atom O ($344\mu\text{l.}$). Despite the large decrease in total O_2 uptake and the total inhibition of CO_2 production, it is clear from Fig. 1 that the initial rate of O_2 absorption, up to about $350\mu\text{l.}$, is practically unaltered by treatment of the suspension with concentrations of HCl not greater than $0.02N$. From this it is

Fig. 1. O_2 uptakes, with $0.3\text{ ml. } 0.1 M$ glucose as substrate, of suspensions of *Bact. suboxydans* treated with different concentrations of HCl and then washed. Temp. 30° . pH 6.0.

Fig. 2. Evolution of CO_2 , with $0.3\text{ ml. } 0.1 M$ glucose as substrate, of suspensions of *Bact. suboxydans* treated with different concentrations of HCl and then washed. Temp. 30° . pH 6.0.

inferred that the rapid initial O_2 uptake is chiefly the result of a simple oxidation process. With concentrations of HCl greater than $0.02N$ the O_2 uptake was gradually inhibited until there was complete aerobic inactivation after treatment with $0.4N$ acid. The variation with different concentrations of acid of total O_2 uptake and CO_2 production given by the treated suspensions with glucose after 3 hr. in the manometer is shown in Fig. 3. The rapid fall in the curve at the beginning illustrates the relatively great effect of the smaller concentrations of

acid, which decrease O_2 uptake and CO_2 evolution by equal amounts, up to the point when the CO_2 production is completely inhibited ($0.006N$ HCl). After this stage there is much greater resistance to the effect of increasing acid concentration, shown by the much flatter portion of the curve at an O_2 uptake equivalent approximately to 1 atom O. The real effect is even greater than is apparent from Fig. 3 owing to the necessity for telescoping the scale for the larger acid concentrations.

Fig. 3. O_2 uptake and CO_2 evolution, after 3 hr. in manometer with 0.3 ml. $0.1 M$ glucose as substrate, of suspensions of *Bact. suboxydans* treated with different concentrations of HCl and then washed. Temp. 30° . pH 6.0.

These results indicate strongly a differential action of acid on the enzymic system of *Bact. suboxydans* and constitute further evidence for the conclusions reached in a previous paper [Butlin, 1938]. There it was suggested that *Bact. suboxydans* possesses an enzyme system containing at least two important components. One, the more sensitive to acid, catalyses a reaction in which the O_2 absorbed is equal to the CO_2 evolved. The rapidly falling portion of the curve in Fig. 3, where the O_2 uptake and CO_2 evolution decrease by equal amounts when the suspension has been treated with comparatively small concentrations of acid, is contributory evidence for the existence of this component. The other component catalyses a simple oxidation of glucose to gluconic acid in which 1 atom O for each molecule of glucose is absorbed and a partial further simple oxidation to 5-ketogluconic acid. This is represented by the flatter portion of the curve, which clearly illustrates its resistance to the action of acid.

These data were obtained with HCl. Gluconic acid behaved in a similar manner.

Influence of pH

The above results may be regarded not merely as the result of the action of acid on the enzyme system of *Bact. suboxydans* but also as the consequence of subjecting the suspensions to various pH levels for a certain period, the cells

being then washed and their action on glucose determined at pH 6.0. Fig. 4 gives the pH produced by the addition of increasing concentrations of HCl to the suspensions, which consisted of cells in physiological saline, and to saline alone. The buffering action of the cells is apparent from the figure.

Fig. 4.

Fig. 4. pH produced by different concentrations of HCl. (1) o—o On suspensions of *Bact. suboxydans* in saline. (2) •—• on saline alone.

Fig. 5.

Fig. 5. (1) O₂ uptake o—o; (2) CO₂ evolution ×—× and (3) "O₂ uptake unaccompanied by CO₂ evolution" after 1 hr. in manometer with 0.3 ml. 0.1 M glucose as substrate, given by suspensions of *Bact. suboxydans* at different pH's.

The results obtained by the action of acids gave evidence for the presence of two components in the enzyme system of *Bact. suboxydans*. Similar conclusions may be drawn from the values for O₂ uptake, CO₂ output and "O₂ uptake unaccompanied by CO₂ output" given with glucose by well washed suspensions of the organism at different pH after 1 hr. in the Barcroft manometer (Fig. 5). For pH 3.0, 4.0, 5.0 phthalate, for 6.0, 7.0 and 8.0 phosphate and for 9.0 and 10.0 borate buffers were used. A temperature of 30° was used throughout. The results show that, though there is little significant difference in CO₂ production or total O₂ uptake at pH 5.0, 6.0 and 7.0, there is a rapid decrease below 5.0 and over 7.0. On the other hand the values for "O₂ uptake unaccompanied by CO₂ output" remain practically constant between pH 4.0 and 8.0 at a value just above the equivalent of 1 atom O, whereas at pH 4.0 and 8.0 the CO₂ production is practically nil. Here again we have the phenomenon of CO₂ production ceasing when the reaction represented by "O₂ uptake unaccompanied by CO₂ output" is intact. This constitutes further evidence for the suggested two-component enzymic structure of *Bact. suboxydans* outlined above.

Cultures grown on other media

The evolution of CO₂ with glucose is not confined to suspensions derived from cultures grown on maize wort agar with or without chalk. In suitable conditions it has been observed with cultures from every other growth medium so far employed, e.g. glycerol-yeast agar with and without chalk, malt wort agar with and without chalk, bouillon agar with chalk, glycerol-yeast extract solution, 10 %

glucose-chalk-yeast extract solution and 10% sorbose-yeast extract solution. This property of *Bact. suboxydans* is therefore not specific to cultures grown on maize wort agar but is a normal characteristic of the organism.

DISCUSSION

Previous experimental results [Butlin, 1938] suggested that *Bact. suboxydans* possesses an enzyme system containing not only a component catalysing a simple oxidation process without production of CO_2 but another component which catalyses a reaction in which the O_2 absorbed is equal to the CO_2 evolved. The work described in the present paper presents confirmatory evidence for this suggestion, based on the differential effect of pH and of the direct action of acid on the enzyme system.

In ordinary liquid culture *Bact. suboxydans* is characterized by its slight intensity of oxidation as exemplified in such typical and almost quantitative transformations as glucose to gluconic acid, sorbitol to sorbose and glycerol to dihydroxyacetone. It is therefore surprising that it should possess a highly oxidizing factor in its enzymic equipment as shown by the action of washed suspensions of the organism with glucose under Barcroft conditions. It might be suggested that the strain used in the experiments had altered in character since its isolation. Similar results, however, have been obtained from 5 cultures from different sources. Moreover, these highly oxidizing suspensions when inoculated into liquid cultures show the modified oxidations characteristic of the organism. A more probable explanation is that *Bact. suboxydans* is unable appreciably to exert its more highly oxidizing function because of the conditions it creates in the liquid cultures usually employed. This function, which is sensitive to acid conditions, will be inhibited if acid is formed in sufficient quantity, leaving intact the more moderately oxidizing component. This explanation is readily applicable to the case where glucose is the substrate in suitable liquid medium of initial pH 6.0: the rapid formation of gluconic acid by the moderately oxidizing component would quickly inhibit the more drastic oxidation. At first sight it does not appear to explain the almost quantitative production of sorbose from sorbitol and dihydroxyacetone from glycerol, the chief final products being neutral, when suitable concentrations of the substrates in yeast water at pH 6.0 are inoculated with suspensions of *Bact. suboxydans*. In practice, however, it is found that owing to the formation of some unknown acid the pH of these solutions falls rapidly to 4.5 and often lower, and at these levels only the less actively oxidizing component is able to function. *Bact. suboxydans*, therefore, while possessing a highly oxidizing component in its enzyme system, is usually unable appreciably to utilize it and is chiefly noted for its property of restricted oxidation.

It is possible that the CO_2 production with its accompanying equal O_2 absorption is due to some anabolic process of the nature suggested by Barker [1936], Giesberger [1936] and Clifton [1937], in which the substrate is converted into cell materials simultaneously with the normal oxidative process. This possibility is being investigated.

SUMMARY

From further evidence based on the differential effect of pH and of the direct action of acid on the aerobic activity of washed suspensions, it is concluded that *Bact. suboxydans* possesses an enzyme system containing at least two important components. One, less sensitive to acid but more rapid in its action, catalyses a simple oxidation process. The other component catalyses a reaction in which the O_2 absorbed is equal to the CO_2 evolved.

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