LXVII. THE ENZYME SYSTEM OF *BACT. SUBOXYDANS*

I. VARIATION OF AEROBIC ACTIVITY WITH AGE OF CULTURE

By KENNETH RUPERT BUTLIN

*From the Microbiological Section, Chemical Research Laboratory,
Department of Scientific and Industrial Research, Teddington*

(Received 25 January 1938)

As described in a previous paper [Butlin, 1936], *Bact. suboxydans*, an acetic acid organism characterized by its property of restricted oxidation in aerobic conditions, was found to possess a strongly oxidizing enzymic system when grown on maize wort agar containing 2% chalk. In contrast, when grown for 3–4 days on maize wort agar without chalk, of initial pH 6.5, it appeared to possess only a simple and restricted power of oxidation, washed suspensions of the organism giving with glucose an O₂ uptake equivalent approximately to 1 atom O for each mol. substrate, with very little or no evolution of CO₂. It was considered probable that the difference in behaviour of the two types of suspension was due to the influence on cell activity of acid gradually formed on the growth medium. This suggested an examination of the influence of the period between inoculation of the medium and removal of the growth from the medium (hereafter called the growth period) on the aerobic enzymic activities of *Bact. suboxydans*. Recent work by Wooldridge *et al.* [1936; 1937] has shown that the aerobic and anaerobic activities of most bacterial dehydrogenases may vary considerably with the growth period. The present investigation shows that *Bact. suboxydans* exhibits similar variation. Moreover, the results obtained throw light on the enzymic structure of the organism.

EXPERIMENTAL

The culture of *Bact. suboxydans* used in these experiments was supplied by Prof. A. J. Kluyver of Delft. The media employed for growth consisted of (a) maize wort agar of pH 6.5, (b) maize wort agar with 2% chalk, contained in flat 16 oz. bottles. After inoculation they were incubated at 30°. One or more bottles of each were withdrawn at required intervals and suspensions prepared as previously described [Butlin, 1936]. The suspensions were made up only roughly to the same density, for it had been previously found that dilution within a wide range made no appreciable difference to aerobic activity, e.g. a suspension of dry weight 23 mg./ml. gave almost identical values for O₂ uptake and evolution of CO₂ with glucose when diluted to three times its volume. The O₂ absorption and CO₂ evolution by the suspensions with glucose were measured very soon after preparation by means of the Barcroft differential manometer, the usual technique being followed [Dixon, 1934]. CO₂ was measured by the direct method. Both flasks, which differed in volume by less than 50 μ l., contained 1 ml. well washed suspension and 1.7 ml. buffer: to the left was added 0.3 ml. water, to the right 0.3 ml. of 0.1 *M* glucose. The latter was contained in a Keilin tube during equilibration, after which it was dislodged and mixed with the other contents.

A temperature of 30° and phosphate buffers of pH 6.0 were used throughout. Theoretically 0.3 ml. of 0.1 *M* glucose takes up 334 μ l. oxygen at N.T.P. for each atom O absorbed by the glucose molecule.

Variation in aerobic enzymic activities of Bact. suboxydans grown on maize wort agar containing 2% chalk

Figs. 1 and 2 contain the O₂ uptake-time and CO₂ output-time curves with glucose as substrate given by suspensions of *Bact. suboxydans* grown for variable periods on maize wort agar containing 2% chalk. These curves show that the

Fig. 1.

Fig. 2.

Fig. 1. Oxygen uptakes, with 0.3 ml. 0.1 *M* glucose as substrate, of suspensions of *Bact. suboxydans* grown for different periods on maize wort agar containing 2% chalk.

Fig. 2. Evolution of CO₂, with 0.3 ml. 0.1 *M* glucose as substrate, of suspensions of *Bact. suboxydans* grown for different periods on maize wort agar containing 2% chalk.

Fig. 3. "O₂ uptake unaccompanied by CO₂ evolution", with 0.3 ml. 0.1 *M* glucose as substrate, of suspensions of *Bact. suboxydans* grown for different periods on maize wort agar containing 2% chalk.

activity increases at first, rises to a maximum after 2 days' growth and then gradually diminishes. The behaviour is in general similar to that reported by Wooldridge *et al.* [1936] for most dehydrogenases.

The characteristic feature of the action of all the suspensions with growth periods of 1-7 days is a rapid initial O_2 uptake and a relatively slow initial production of CO_2 . The rate of evolution of CO_2 increases and eventually becomes equal to the rate of O_2 absorption. This suggests an initial rapid oxidation accompanied by a slower separate reaction in which the evolution of CO_2 is equal in volume to the O_2 taken up by the glucose.

The 11-day-old culture gives a rapid initial O_2 uptake equivalent approximately to 1 atom O per mol. glucose, followed by very slow O_2 absorption and very small CO_2 evolution. After 19 days' growth there is an O_2 absorption approximately equivalent to 1 atom O but no evolution of CO_2 . There is still slight activity after a growth period of 37 days but none after 7 weeks.

If, as the experiments indicate, there is a definite reaction in which the O_2 absorbed is equal to the total CO_2 evolved, the difference in volume between total O_2 uptake and CO_2 output represents an absorption of O_2 without an accompanying production of CO_2 , i.e. some simple oxidation process. This is reflected in the rapid initial O_2 uptakes shown in Fig. 1 and is illustrated in Fig. 3, which gives the values for " O_2 uptake unaccompanied by CO_2 evolution" derived from Figs. 1 and 2. For cultures nineteen and more days old the curves are identical with those for O_2 uptake, there being no evolution of CO_2 . It will be seen that, except for the 37 days' growth period, the curves in Fig. 3 are confined within a narrow range, the final values falling between 330 and 405 μ l. Moreover, apart from the 1-day growth, these final figures show with lengthening period of growth a closer approximation to the theoretical figure for 1 atom O (334 μ l.). It is very probable that these curves represent, up to 334 μ l., the formation of gluconic acid, and beyond 334 μ l. a further partial simple oxidation to 5-ketogluconic acid. These figures of O_2 uptake without CO_2 evolution remain fairly constant during the growth periods, up to 19 days, in which the total O_2 uptake greatly diminishes and the evolution of CO_2 ceases altogether. This implies that the enzyme catalysing the simple oxidation to gluconic acid is less susceptible to adverse growth conditions than the enzyme responsible for the reaction involving CO_2 production.

It should further be mentioned that considerable acid formation takes place during the experimental growth period. The chalk dispersed throughout the maize wort agar gradually disappears and the growth becomes acid when the accessible chalk has been decomposed.

Variations in aerobic activities of Bact. suboxydans grown on maize wort agar without chalk

The results obtained for cultures on maize wort agar without chalk are summarized in Fig. 4, which gives the manometric values after 3 hr. with glucose as substrate for O_2 absorption, CO_2 evolution and " O_2 uptake unaccompanied by CO_2 evolution" for the various periods of growth.

The suspension made from a 1-day growth had highly oxidizing activities—characterized by rapid initial O_2 uptake, relatively slow initial CO_2 evolution, followed by an increase in the latter until its rate of production became equal to the rate of O_2 uptake—similar to those of the suspensions obtained from the chalk-containing medium after 1-7 days' growth, and was almost identical in activity with the 3 days' growth from that medium. The 3 days' growth gave very little CO_2 evolution and the O_2 uptake decreased to a value slightly in excess of the equivalent of 1 atom O. After a growth period of 4 days there was only slow O_2 absorption and no CO_2 production. Cultures 6 days old gave no sign of aerobic enzymic activity with glucose. This may be contrasted with the

behaviour of suspensions from the chalk medium which still showed slight activity after 37 days' growth.

It is clear too from Fig. 4 that, as with the suspensions from the chalk-containing medium, the value for "O₂ uptake unaccompanied by CO₂ evolution"

Fig. 4. (1) O₂ uptake, (2) CO₂ evolution and (3) "O₂ uptake unaccompanied by CO₂ evolution", after 3 hr. in manometer with 0.3 ml. 0.1 *M* glucose as substrate, of suspensions of *Bact. suboxydans* grown for different periods on maize wort agar without chalk.

remains fairly constant at a level approximately equivalent to 1 atom O, during a period in which the CO₂ evolution sinks to zero and the O₂ uptake greatly decreases. This again implies the greater resistance to adverse growth conditions of the enzyme catalysing the simple oxidation to gluconic acid.

It was observed that the bacterial growths on this medium became increasingly acid with age and required several washings before testing in the manometer.

DISCUSSION

In general, variations in the enzymic activity of bacterial cultures depend on the availability of food materials, the nature of the metabolic products and on such factors as temperature, moisture, pH and access to O₂. In the case under consideration it is at once clear that the chief factor is the acid formed by the organism. The variations have been followed using maize wort agar, with and without chalk, as growth media. The contrast between the growths on the two media is very striking. There is an initial similarity after 1 day's growth, both media giving highly oxidizing suspensions. With increasing growth period there is then a rapid fall in activity of the suspensions from the medium without chalk, until after 6 days aerobic activity is completely lost, whereas suspensions made from the chalk-containing medium show activity even after 37 days' growth. Where acid formed by the organism comes into direct contact with the bacterial cells it is apparent that it has a profound effect on the enzymic activity of the organism. On the chalk-containing medium the acid is neutralized as soon as formed and any decrease in activity of the culture must be due to some or all of the other factors.

It is not surprising that the direct action of acid on bacterial cells should strongly influence enzymic activity. The significance of the experimental data presented in this paper is in their bearing on the enzymic system of *Bact. suboxydans*. Owing to the differential effect of the acid, it is possible to conclude that this system possesses at least two important components. One, less sensitive to acid and more rapid in its catalytic action, is responsible for the oxidation of

glucose probably to gluconic acid and partially to 5-ketogluconic acid, reactions in which no CO_2 is evolved. The second component of the enzymic system catalyses the slower action of O_2 on glucose which results in an evolution of 1 mol. CO_2 per mol. O_2 absorbed. This is probably accompanied by the complete breakdown of the glucose molecule, but there is the possibility that the action involves the synthesis of carbohydrates for storage in the bacterial cells [Clifton, 1937]. This second component is much more sensitive than the first to the increasingly adverse conditions, acid and otherwise, which arise as the growth period lengthens. Thus, in the case of suspensions from the chalk maize wort agar the simpler oxidative component persists intact after 19 days' growth, whereas the evolution of CO_2 is almost nil after a growth period of 11 days. Similarly, suspensions from the non-chalk medium maintain their simple oxidative enzymic component intact after 3 days' growth, during which period the highly oxidizing factor is rapidly and almost completely inactivated.

It is surprising that *Bact. suboxydans*, which in ordinary liquid culture is characterized by its slight intensity of oxidation, should possess a highly oxidizing factor in its enzymic equipment. A possible explanation of this and further evidence for the enzymic structure outlined above will be presented in a future paper.

SUMMARY

1. Washed suspensions of *Bact. suboxydans* vary considerably in aerobic enzymic activity according to the period of growth on maize wort agar, with and without chalk.

2. One-day-old cultures on both media give highly oxidizing suspensions, but whereas 37-day-old cultures on the chalk-containing medium still show slight activity, suspensions of 6-day-old cultures from maize wort agar without chalk possess no aerobic enzymic action. This difference is attributed mainly to the formation of acid by the organism and its direct action on the cell if not neutralized.

3. Experimental results suggest that normally *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.

The writer wishes to express his sincere thanks to Dr A. C. Thaysen for his interest and advice, and to Mr W. H. D. Wince for technical assistance. This work was carried out as part of the programme of the Chemistry Research Board and is published by permission of the Director of Chemical Research in the Department of Scientific and Industrial Research.

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