OXIDATIVE ASSIMILATION BY BACILLUS SUBTILIS[1]

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It is a common observation that the oxidation of many substrates is not carried to completion by various species of microorganisms and that instead a portion of the substrate is assimilated by the cells. In those species possessing a high level of endogenous respiration and in which the rate of respiration is not increased markedly following the addition of a utilizable substrate, oxygen consumptions greater than the theoretical amounts for complete combustion are observed frequently before the rate of respiration levels off to near that of the endogenous control. We have observed this behavior repeatedly with species of the genera *Bacillus* and *Mycobacterium*. This paper summarizes observations on oxidative assimilation by a strain of *Bacillus subtilis* which consumed with different substrates amounts of oxygen ranging from less than 100 per cent of the amounts required for complete combustion to percentages over 200.

METHODS

Cells of *Bacillus subtilis*, strain D-63, for the experiments on assimilation were grown on nutrient agar containing one per cent glucose and were harvested from cultures incubated for 18 hours at 30 C. The cells were suspended and washed in saline and finally were resuspended in M/15 phosphate buffer of pH 7. All suspensions were adjusted to the same turbidity, a 1–10 dilution of the suspension reading 100 in a Klett-Summerson colorimeter equipped with a green filter. Oxygen uptake and carbon dioxide production in the manometric studies were determined by standard Warburg methods. The carbon content of the cells was determined by the method of Van Slyke and Folch (1940), analyses being carried out on cells from 10 ml samples of well aerated suspensions. The use of these larger samples of cells led to more accurate results than were obtained when smaller samples from the Warburg experiments were analyzed for carbon content. The cells were centrifuged from the suspension, the supernatant fluid was discarded, and the cells were washed with saline, centrifuged again, and the fluid removed by aspiration. Carbon dioxide production, determined following absorption in baryta, served as a measure of the amount of substrate oxidized on the assumption that the same ratio of substrate oxidized to carbon dioxide produced held in these aerated suspensions as that determined from the manometric data. Attempts were made to determine directly changes in carbon content of the suspension medium, but the experimental error involved in these determinations and the small amount of change actually observed in the small samples required for the estimation made it necessary to doubt the validity of these data. It might be mentioned, however, that the values obtained were in general agreement with the results calculated on the basis of the manometric data.

Assimilation during growth of the cells in a synthetic medium (Siegel and Clifton, 1950) was followed in the same manner as outlined above. Relatively large inocula were employed to provide sufficient cells for the carbon determinations. This, together with the fact that the growth rate of this strain is not high under the conditions employed, lowers the probable accuracy of the results obtained in the growth experiments.

RESULTS

The values obtained with the Warburg technique for oxygen consumption during 10 minute intervals by washed cells in the presence of various concentrations of glucose are cumulatively summed and graphed as a function of time in figure 1. The typical behavior is noted; oxygen

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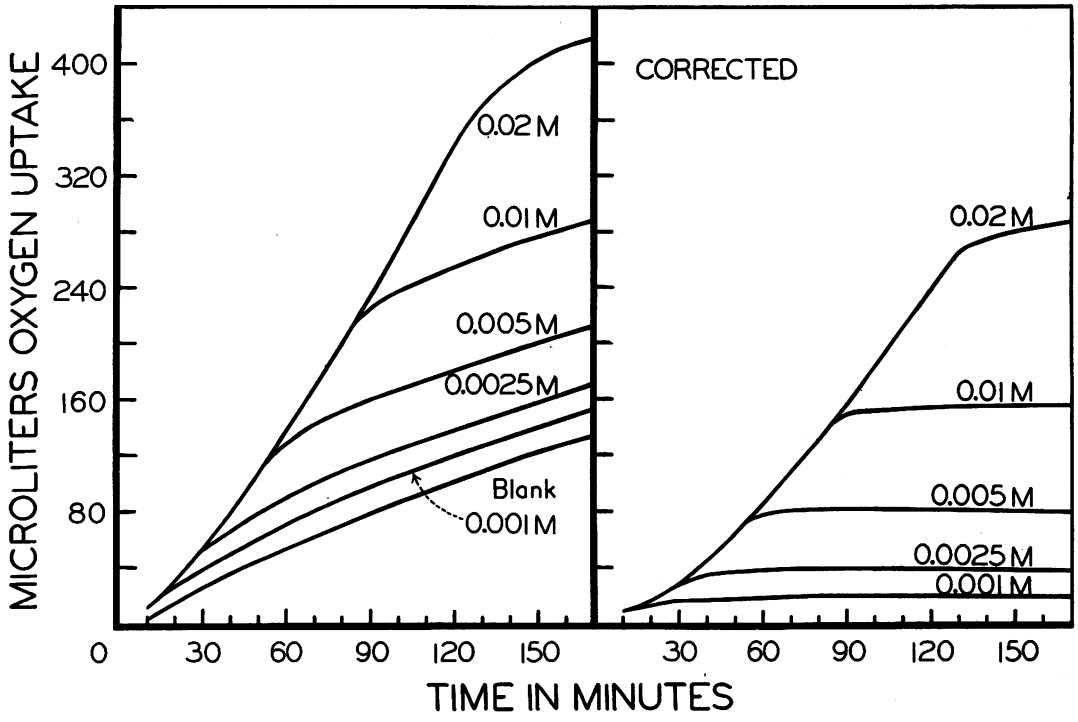

Figure 1. Oxygen consumption by washed suspensions of *Bacillus subtilis* in different concentrations of glucose as observed and following correction for endogenous respiration.

consumption proceeds at a fairly constant rate, and on reaching a level characteristic for the concentration of substrate employed, the rate decreases to a level near that of the endogenous control. This change in rate or "break" occurs at a time when all the substrate has been depleted from the medium. The total oxygen consumption up to the break points for the five concentrations of glucose employed varied from 71 to 97 per cent of the values for the amounts of oxygen required for complete combustion. The oxygen consumption values were corrected by subtracting the endogenous respiration observed in the control suspension and regraphed (figure 1). The break points are somewhat sharper in the graph of the corrected values and fall within the range of 50 to 60 per cent of the amounts required for complete combustion with an average value of 56 per cent. A similar behavior was noted with succinate, fumarate, or pyruvate as the substrate. Lactate or acetate was not oxidized at an appreciable rate by this strain of *B. subtilis*. With succinate the percentages of oxygen consumed up to the break varied with different concentrations from 100 to 192 per cent of the theoretical values for complete combustion. Subtracting the values for endogenous oxygen uptake before making the calculations resulted in values varying between 64 and 67 per cent, with one value of 51 per cent for the lowest concentration, 0.0025 M. The uncorrected values with fumarate ranged from 64 to 296 per cent of the theoretical. On correction for endogenous respiration the ranges were from 46 to 50 per cent for fumarate and 41 to 46 per cent for pyruvate. The relative constancy of the corrected values indicates that endogenous respiration of *B. subtilis* continues in the presence of an exogenous substrate, a point that will be considered in the discussion.

It is of interest in passing to record that the rate of oxygen consumption with glucose or succinate as the substrate was relatively independent of the initial concentration of the substrate while a progressive increase in rate was noted with increasing concentration of fumarate or pyruvate. Whether this behavior is due to permeability factors or to concentrations required to saturate the enzymes was not determined.

As an additional check on the manometric results a number of experiments were carried out

with the cooperation of Mr. Arthur Mercuri on heat production during oxidative assimilation. The amounts of heat produced from different concentrations of glucose, on correction for endogenous respiration, averaged around 52 per cent of the theoretical for complete combustion. This value agrees within the limits of experimental error with values calculated from the manometric results.

Determinations were made of both oxygen uptake and carbon dioxide production in the presence of 0.2 ml of 0.01 M glucose or 0.02 M succinate, fumarate, or pyruvate. The quantities of oxygen consumed and of carbon dioxide produced are recorded in table 1 together with values for the endogenous respiration. Calculated values for respiratory quotients and for moles of oxygen consumed per mole of substrate utilized are included in table 1. These data serve for the purpose of deriving equations to represent the overall assimilatory relationship noted with a given substrate. The equations thus developed are as follows:

Glucose:

RQ = 1.00; observed value = 0.96
Moles O_2 = 3.00; observed value = 3.07

$$C_6H_{12}O_6 + 3\ O_2 \rightarrow 3\ CO_2 + 3\ H_2O + 3(CH_2O)$$

Succinate:

RQ = 1.25; observed value = 1.17
Moles O_2 = 2.00; observed value = 2.12

$$2\ C_4H_6O_4 + 4\ O_2 \rightarrow 5\ CO_2 + 3\ H_2O + 3(HC_2O)$$

Fumarate:

RQ = 1.75; observed value = 1.69
Moles O_2 = 1.33; observed value = 1.34

$$3\ C_4H_4O_4 + 4\ O_2 \rightarrow 7\ CO_2 + H_2O + 5(CH_2O)$$

Pyruvate:

RQ = 1.50; observed value = 1.50
Moles O_2 = 1.00; observed value = 1.18

$$2\ C_3H_4O_3 + 2\ O_2 \rightarrow 3\ CO_2 + H_2O + 3(CH_2O)$$

Inspection of these equations that are based on the respiratory data indicates an assimilation of 50 per cent of the substrate in the case of glucose, 38 per cent for succinate, 43 per cent for fumarate, and 51 per cent for pyruvate, corresponding figures based on average corrected oxygen uptake being 44, 37, 52, and 57 per cent, respectively.

In order to determine the validity of the assumption that the portions of the substrates not accounted for in the manometric data are actually assimilated, determinations were made of the actual increase in cell carbon of washed cells respiring in a buffered glucose solution. As indicated under Methods, the amount of CO_2-carbon corrected for endogenous respiration is a measure of the amount of glucose oxidized by the cells while the increase in cellular carbon is a measure of the quantity of substrate assimilated. Together the two values represent the total amount of glucose carbon consumed by the cells. All determinations of increase in cell carbon were made with cells that were respiring in an excess of substrate and harvested before the break since in these larger scale experiments it was impossible to follow the rate of respiration and to determine the break point. The carbon exchange in all experiments showed the same general relationship. The results from two separate experiments with which the least technical difficulties were encountered are recorded in table 2. The equation derived from the manometric data indicated an assimilation of glucose amounting to 50 per cent of the glucose carbon disappearing from the medium which is in close agreement with the 48 per cent assimilation noted in the direct experiments summarized in table 2.

Oxidative assimilation by cultures of *B. subtilis* in the basal salt-glucose medium was determined at 2 hour intervals over a 6 hour period following inoculation of the medium. Calculations of the ratios of substrate carbon assimilated to that consumed by the cells indicated an assimilation of 33 per cent of the glucose carbon utilized during the first 2 hours, 55 during the second pe-

TABLE 1

Oxygen consumption and carbon dioxide production by Bacillus subtilis in the presence of various substrates

	GLUCOSE	SUCCINATE	FUMARATE	PYRUVATE
mm³ CO_2	263	311	352	238
mm³ CO_2, endogenous	105	79	137	81
mm³ O_2	261	282	257	179
mm³ O_2, endogenous	97	84	130	74
Observed RQ	1.01	1.10	1.37	1.33
Corrected RQ	0.96	1.17	1.69	1.50
Theoretical RQ	1.00	1.14	1.33	1.20
Corrected moles O_2 per mole substrate	3.07	2.12	1.34	1.18
Theoretical moles O_2 per mole substrate	6.00	3.50	3.00	2.50

TABLE 2

Carbon balance for assimilation of glucose by Bacillus subtilis*

	EXPERIMENT 1	EXPERIMENT 2
Initial cell carbon	7.68	8.84
Final cell carbon	8.08	9.28
Increase in cell carbon	0.40	0.44
CO_2-C	1.99	2.02
Endogenous CO_2-C	0.74	0.74
Corrected† increase in cell carbon	1.14	1.18
Corrected CO_2	1.25	1.28
Total substrate-carbon consumed	2.39	2.46
Per cent of utilized carbon assimilated	48	48

* All values are in mg/50 ml of cell suspension with the exception of the percentages.

† Increase in cell carbon plus cell carbon lost as indicated by endogenous CO_2-carbon production.

riod, and 28 per cent in the third 2 hour period or an over-all assimilation near 37 per cent. No correction can be made for any endogenous respiration of the cells in the cultures. If endogenous respiration does occur, correction for it would tend to increase the calculated values for percentage assimilation. Considering the technical and other difficulties involved in these studies on actively proliferating cells, the results are in fair agreement with those predicted from studies on washed cells. It is possible, however, that the extent of assimilation may vary with age of the culture, but this has not been definitely established.

DISCUSSION

The problem of an endogenous correction factor has plagued students of oxidative assimilation since the first study of this phenomenon by Barker (1936). The general tendency has been to assume that endogenous respiration is partially or completely suppressed in the presence of a substrate. A number of workers have reported that this assumption is not always applicable. Cochrane and Gibbs (1951), for example, concluded that concomitant oxidation of glucose or pyruvate had no suppressive effect on endogenous respiration of *Streptomyces coelicolor*. Wiame and Doudoroff (1951), on the other hand, reported that endogenous respiration of *Pseudomonas saccharophila* is inhibited during the oxidation of assimilable substrates.

In this study it was noted that oxygen consumptions of well over 100 per cent occurred in the presence of a number of substrates before the time at which respiration approached the level observed before the addition of the substrate. It is impossible to derive equations for oxidative assimilation from such data. On correction of the respiratory exchange figures by subtracting observed endogenous values from those observed in the presence of a substrate, quite constant values were obtained for the percentages of oxygen consumed and carbon dioxide produced. Equations, reported under Results, for oxidative assimilation could be derived from the corrected results, and these show the typical behavior noted with other cells where endogenous respiration is not as high in comparison with the exogenous rate as is noted with *B. subtilis*. Values for the amounts of carbon assimilated and for heat production with glucose as the substrate, when corrected for endogenous respiration, agree closely with those based on manometric data. These observations indicate that the endogenous respiration of *B. subtilis* is not suppressed in the presence of the substrates tested.

Comparisons of the free energy available on oxidation of the different substrates would indicate that chemical structure as well as the free energy change influences the extent of assimilation. For example, similar extents of assimilation were noted from one mole of glucose or two moles of pyruvate (6 carbon atoms) although more energy is theoretically available from the oxidation of glucose.

SUMMARY

Oxidative assimilation by suspensions of *Bacillus subtilis* oxidizing glucose, succinate, fumatate, or pyruvate follows the same general pattern as observed with other microorganisms. Manometric observations lead to the conclusion that endogenous respiration of this organism is not suppressed during exogenous respiration and satisfactory equations for assimilation can be advanced only from the corrected data. The observed percentage of carbon assimilation from glucose agrees closely with that calculated from corrected manometric data and proposed by the assimilatory equation for glucose and by implication for the other substrates tested.

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