Relation between Energy Production and Growth of *Aerobacter aerogenes*

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**SUMMARY**

Molar growth yields were measured for *Aerobacter aerogenes* growing with a number of substrates as sole carbon and energy source in a minimal medium. Under anaerobic conditions the molar growth yield for glucose was 26.1 g. This amount of dry weight is produced at the expense of 2.55 mole of ATP (1.71 mole from glycolysis and 0.84 mole from acetate produced from pyruvate by the thioclastic reaction). The yield per mole ATP is thus 10.2 g., which value is very close to the one found for other micro-organisms. Under aerobic conditions the molar growth yield for glucose is 72.7 g. During growth 1.14 mole of O₂ are taken up. The yield per atom oxygen is thus 31.9 g. By dividing the yield per atom oxygen by the yield per mole ATP we find the number of ATP mole formed per atom O. The values found are very close to 8, indicating that the efficiency of oxidative phosphorylation in this organism is the same as that in mitochondria. During the experiments it was observed that growth was maximal before the maximal O₂ uptake was reached. The explanation is that during aerobic growth acetate accumulates, which is oxidized after maximal growth. Acetate oxidation after glucose consumption does not contribute to the dry weight.

**INTRODUCTION**

It has been shown that the dry weight of various micro-organisms is proportional to the amount of energy source added (Monod, 1942) when the energy source in an otherwise complete medium is the growth limiting factor. The experiments were performed with *Bacillus subtilis, Escherichia coli* and *Salmonella typhimurium*, growing aerobically in a mineral medium. Under these circumstances, a considerable part of the energy source is used for the synthesis of cell material. De Moss, Bard & Gunsalus (1951) repeated this type of experiment with *Streptococcus faecalis* and *Leuconostoc mesenteroides* under anaerobic conditions. Both organisms have very complex nutritional requirements and almost the total amount of glucose is therefore used as energy source. They found that with *S. faecalis* the dry weight of organisms per mole glucose fermented was significantly greater than with *L. mesenteroides*. De Moss et al. (1951) concluded from these experiments that *S. faecalis* obtained more energy from the fermented glucose than did *L. mesenteroides*, a conclusion confirmed by later work. (For a review on the fermentation patterns in these organisms see Wood (1961).) Bauchop & Elsdon (1960) studied the growth of *S. faecalis, Saccharomyces cerevisiae, Pseudomonas lindneri* and *Propionibacterium*
pentosaceum growing anaerobically in complex media with limiting amounts of glucose or other compounds as energy sources. These organisms ferment glucose by different pathways, yielding different amounts of ATP per mole glucose fermented. From experiments with various energy sources these authors concluded that the four organisms formed about the same dry weight of organisms per mole ATP produced during the fermentation of the energy source ($Y_{ATP} = 10.5$). Recently Twarog & Wolfe (1963) found the same value for $Y_{ATP}$ with Clostridium tetanomorphum. The amount of growth per mole ATP found by these authors may be regarded as a constant which may be used to calculate the ATP production from the growth yields obtained with other micro-organisms. In a recent review on the energetics of bacterial growth Gunsalus & Shuster (1961) used this constant to calculate the ATP production from previous aerobic growth experiments with E. coli (Monod, 1942). The constant was also used to calculate the ATP yield from growth experiments with Desulfovibrio desulfuricans (Senez, 1962) and with Gluconobacter liquefaciens ($=Acetobacter aceti$) (Stouthamer, 1962). In the latter case a good agreement was found between the ATP production calculated from growth experiments and from direct measurements of P/O ratios in oxidative phosphorylation by cell-free extracts. By dividing the difference in the molar growth yields ($=Y$) of ethanol and acetate by $2 \times$ the P/O ratio for the conversion of ethanol to acetate it was found that $Y_{ATP}$ for G. liquefaciens is also about the same as that found by Bauchop & Elsdon (1960). In most cases the P/O ratios found in cell-free extracts of micro-organisms are small and it is not clear whether the energy of oxidation is really used inefficiently in micro-organisms or that it is used efficiently but that the P/O ratios are low because the phosphorylative system is damaged during the preparations of the cell-free extract. In G. liquefaciens there are indications (low molar growth yields and extensive heat production during growth) that the energy of oxidation is used inefficiently indeed.

To have an indication of the energy production in other bacteria these experiments have now been repeated with Aerobacter aerogenes, since this organism is known to give much higher growth yields than Gluconobacter liquefaciens (Pichinoty, 1960). Because this organism can be grown under anaerobic conditions it is possible to obtain an independent estimation of $Y_{ATP}$, which avoids the use of $Y_{ATP}$ obtained from other micro-organisms to calculate the aerobic ATP yield for A. aerogenes. As pointed out before, a large fraction of the carbon source is converted into cell material in aerobic experiments with bacteria growing in minimal medium. All kinds of carbon compounds are synthesized during growth, some compounds with a net yield of ATP and others with a consumption of ATP. We therefore decided to determine the oxygen consumption during growth as a measure of that part of the glucose which is completely oxidized, and to relate this oxygen uptake to the molar growth yield, as proposed by Whitaker & Elsdon (1963). The oxygen uptake (if all the oxygen participates in oxidative phosphorylation) and $Y$ was used to measure the efficiency of oxidative phosphorylation in this organism.

**METHODS**

*Aerobacter aerogenes* was kept on yeast extract agar slants at $4^\circ$. For the measurement of the molar growth yield the organism was grown in minimal medium. The salt solution of the minimal medium ($4 \times$ concentrated) contained $K_2HPO_4$. 


Energy production and growth of Aerobacter aerogenes

3.0% KH₂PO₄, 1.8% NH₄Cl, 0.8% MgSO₄·7H₂O, 0.02% and FeSO₄·0.002%; adjusted to pH 6.8. The solutions of carbon and energy sources were sterilized and stored at -20° or freshly prepared. Extinctometer tubes (diam. 2.5 cm.; length 8.5 cm.) containing concentrated salt solution, carbon source and distilled water to give a final volume of 10 ml., were inoculated with 0.1 ml. of an overnight culture in minimal medium, with the same substrate as carbon source. For every substrate 5 tubes were used with 5 different concentrations ranging from 0.25 to 6 μmole/ml., depending on the nature of the substrate. The tubes were incubated in a shaking machine at 37°.

To obtain anaerobic conditions tubes closed with rubber stoppers with inlet- and outlet-tubes, were gassed for 30 min. with nitrogen and then closed. In a number of experiments a small tube containing 1 ml. of a mixture of equal volumes of 15% (w/v) pyrogallol and of 10% (w/v) Na₂CO₃ was inserted in the extinctometer tubes. This did not influence the results however and therefore these precautions to ascertain absolute anaerobic conditions were not taken in later experiments. In the anaerobic experiments 2–10 μmole substrate/ml. were used and the tubes were inoculated from a pre-culture grown under anaerobic conditions on the same substrate.

The turbidity of the cultures in the extinctometer tubes was measured at regular intervals in a Moll extinctometer (built in this laboratory; see Buyze, 1949) until the turbidity became constant. Then the extinction of the culture was measured in a Unicam SP 600 spectrophotometer at 660 μm. Dry weight of bacteria was determined directly by centrifugation and washing the pellet three times with distilled water. The bacteria were then dried at 105° to constant weight.

Oxygen consumption of growing cultures was measured in sterile Warburg flasks, using conventional Warburg apparatus and methods. In a number of experiments the O₂ uptake was measured in the presence of CO₂ by the diethanolamine method of Pardee (1949), with 0.3% (v/v) CO₂ in the gas-phase.

Glucose and maltose were determined by the method of Dische, Shettles & Osnos (1949); acetate was determined as described by Rose, Grunberg-Manago, Korey & Ochoa (1954).

In experiments with radioactive glucose the radioactivity of the bacteria, the supernatant fluid and the CO₂ was measured after conversion to BaCO₃. The bacteria and the supernatant fluid were therefore oxidized with the van Slyke reagent in the apparatus for static wet combustion as described by Aronoff (1960) and the CO₂ collected as BaCO₃. Carbon dioxide liberated from the growing cultures in Warburg vessels was collected in KOH in the centre well and converted to BaCO₃. The BaCO₃ was handled as described by Aronoff and the radioactivity determined on a layer of infinite thickness, by using an end-window GM tube.

The following abbreviations will be used: ATP, adenosine triphosphate; CoA, coenzyme A and NADH, reduced nicotinamide-adenine dinucleotide.

RESULTS

Determination of the constant relating extinction to dry weight

The extinction and the number of viable cells of a culture grown aerobically with a limiting amount of maltose is shown in Fig. 1. It may be seen from Fig. 1 that the extinction of the culture decreases after a maximum. The same result was found in
similar experiments with *Bacillus subtilis* (Monod, 1942). In the case of *B. subtilis* this effect was found to be due to lysis of part of the bacteria (Hadjipetrou & Stouthamer, 1963). This is not the explanation for the decrease in extinction after the maximum with *Aerobacter aerogenes*, however, because the number of viable organisms remains constant after the maximum (Fig. 1). Moreover the dry weight bacteria in this and other experiments decreased only slightly on prolonged incubation (Table 1). It is thus evident, that the decrease in extinction after the maximum is mostly due to a difference in the light-scattering properties of the bacteria. For bacteria taken after the maximum the constant, which relates extinction to dry weight of bacteria, is higher than for bacteria at the maximum (Table 1). Therefore the constant which relates extinction to dry weight was determined with bacteria taken at the maximum extinction. From a number of experiments we found the relation: dry weight (\(\mu g./mL.\)) = 380 \times \text{extinction at 660 m\(\mu\)}. This relation was found to be valid for extinctions smaller than 0.9. The same relation was found when the bacteria were grown with glucose, maltose, citrate, galactose or ribose as growth-limiting carbon source. Thus the nature of the carbon source had no influence on the light-scattering properties of the bacteria. In all experiments for the determination of \(Y\) the extinction was followed until a maximum was reached. From the maximum extinction the dry weight was calculated with the constant given above.

### Table 1. Relation between optical density and dry weight in cultures of *Aerobacter aerogenes*

The conditions in this experiment are the same as those in the experiment of Fig. 1. At the time indicated the bacteria from 11. culture were harvested, washed and dried as described under Methods.

<table>
<thead>
<tr>
<th>Time of incubation (hr)</th>
<th>Extinction (660 m(\mu))</th>
<th>Bacterial dry weight (mg./l.)</th>
<th>Dry weight extinction</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.284</td>
<td>108.6</td>
<td>382</td>
</tr>
<tr>
<td>7</td>
<td>0.256</td>
<td>105.4</td>
<td>412</td>
</tr>
</tbody>
</table>

*Molar growth yields under anaerobic conditions*

It is known that at neutral pH value the fermentation of glucose by *Aerobacter aerogenes* closely resembles that of *Escherichia coli* (Mickelson & Werkman, 1938). The main fermentation products are then acetate, ethanol, formate and lactate. In this type of fermentation, pyruvate is decomposed by a thioclastic reaction to acetyl-CoA and formate. Acetyl-CoA is converted to acetate by means of phosphate acetyltransferase and acetatekinase. Thus above the net gain of 2 mole of ATP from the conversion of 1 mole of glucose to 2 mole of pyruvate, an additional mole of ATP is generated for each mole of acetate formed. Ethanol which is also formed in this fermentation is produced by reduction of acetyl-CoA (Dawes & Foster, 1956) and the energy-rich bond present in acetyl-CoA is lost during its conversion to ethanol. Therefore the net gain of ATP can only be calculated when the amount of acetate produced during the fermentation is known. The amount of acetate formed during the fermentation of the substrate was determined in the supernatant when the maximal optical density was reached.
Energy production and growth of Aerobacter aerogenes

The molar growth yield and the amount of acetate formed for glucose and fructose are given in Table 2. From the results of Table 2 we can calculate $Y_{\text{ATP}}$ (see Discussion). The value obtained in this calculation is 10.2.

Table 2. Molar growth yield and acetate production for Aerobacter aerogenes growing under anaerobic conditions in minimal medium with glucose or fructose as carbon source

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Molar growth yield (g./mole)</th>
<th>Acetate production (mole/mole substrate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>$26.1 \pm 0.5$ (17)*</td>
<td>$0.84 \pm 0.04$ (15)</td>
</tr>
<tr>
<td>Fructose</td>
<td>$26.7 \pm 0.4$ (7)</td>
<td>0.8 (3)</td>
</tr>
</tbody>
</table>

* The number of determinations is given in parentheses.

Molar growth yields under aerobic conditions

A linear relationship was found between the glucose content in the medium and the dry weight of bacteria produced, with glucose concentrations between 0 and 2.5 $\mu$ mole/ml. (Fig. 2). At higher concentrations a lower $Y$ was found. Similar results were found with other substrates. Therefore the molar growth yields were always determined at low substrate concentrations. The results are given in Table 3. To facilitate the comparison of the molar growth yields with different substrates the number of mole $O_2$ needed for complete oxidation is included in Table 3 for all substrates. It is evident from Table 3 that in most cases the molar growth yields for a group of compounds which need the same amount of $O_2$ for complete oxidation are very similar. For instance, mannitol and sorbitol form one group; the results for glucose, galactose, mannose and fructose are also similar. The results for the compounds which need 5.5 mole $O_2$ for complete oxidation are near each other and approach 10.2.

Fig. 1. Extinction and viable count of a culture of Aerobacter aerogenes growing aerobically with 0.5 $\mu$ mole maltose/ml. in a minimal medium: ○——○, optical density; ●——●, viable count.

Fig. 2. Growth yields with Aerobacter aerogenes growing aerobically with the indicated substrates as sole carbon source in a minimal medium: ○——○, sorbitol; ●——●, glucose; x——x, succinate.
the same applies (with the exception of L-arabinose) to the compounds which need 5 mole of O₂. For a number of substrates (rhamnose, inositol, DL-lactate, DL-malate, L-glutamate) the molar growth yields are low in comparison with substrates which need the same amount of O₂ for complete oxidation. On the other hand the growth yields with L-arabinose and citrate are unexpectedly high. Most of these irregularities are explained by the later observation that the O₂ uptakes during growth on these substrates are different from those obtained with corresponding compounds which need the same theoretical amount of O₂. Thus in most cases the Y° values (Y° = yield per atom oxygen taken up during growth) are normal.

The most important fact, which emerges from the results of Table 3 is that Ymannitol and Ysorbitol are much larger than Yfructose. This indicates that the O-atom

Table 3. Molar growth yields and O₂ consumption during aerobic growth for Aerobacter aerogenes with the indicated substrates as sole carbon source in a mineral medium

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Mole O₂ for complete oxidation</th>
<th>Y</th>
<th>O₂ consumption (mole/mole)</th>
<th>Y°</th>
<th>Y°/Y&lt;sub&gt;ATP&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltose*</td>
<td>12</td>
<td>149±2 ± 1-7 (8)</td>
<td>2-55</td>
<td>29-2</td>
<td>2-9</td>
</tr>
<tr>
<td>Sucrose*</td>
<td>12</td>
<td>172±2 ± 1-8 (12)</td>
<td>2-68</td>
<td>32-2</td>
<td>3-2</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>6-5</td>
<td>95-5 ± 1-1 (15)</td>
<td>2-31</td>
<td>19-0</td>
<td>1-9</td>
</tr>
<tr>
<td>D-Sorbitol*</td>
<td>6-5</td>
<td>97-4 ± 2-3 (5)</td>
<td>1-80</td>
<td>27-1</td>
<td>2-7</td>
</tr>
<tr>
<td>L-Rhamnose*</td>
<td>6-5</td>
<td>49-0±1 ± 1-1 (8)</td>
<td>1-24</td>
<td>19-7</td>
<td>1-9</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>6-0</td>
<td>72-7 ± 1-0 (21)</td>
<td>2-05 ± 0-05 (14)</td>
<td>17-7</td>
<td>1-7</td>
</tr>
<tr>
<td>D-Glucose*</td>
<td>6-0</td>
<td>72-7 ± 1-0 (21)</td>
<td>1-14 ± 0-05 (11)</td>
<td>31-9</td>
<td>3-1</td>
</tr>
<tr>
<td>D-Fructose*</td>
<td>6-0</td>
<td>76-1 ± 1-3 (15)</td>
<td>1-84</td>
<td>28-4</td>
<td>2-8</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>6-0</td>
<td>73-5 ± 1-9 (11)</td>
<td>1-93</td>
<td>19-0</td>
<td>1-9</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>6-0</td>
<td>69-4 ± 0-8 (18)</td>
<td>1-77</td>
<td>19-6</td>
<td>1-9</td>
</tr>
<tr>
<td>Myo-inositol*</td>
<td>6-0</td>
<td>52-2 ± 1-4 (9)</td>
<td>1-15</td>
<td>22-7</td>
<td>2-2</td>
</tr>
<tr>
<td>D-Gluconate*</td>
<td>5-5</td>
<td>62-2 ± 1-3 (21)</td>
<td>1-13</td>
<td>27-5</td>
<td>2-7</td>
</tr>
<tr>
<td>D-Galactonate*</td>
<td>5-5</td>
<td>66-3 ± 1-8 (6)</td>
<td>1-29</td>
<td>25-7</td>
<td>2-5</td>
</tr>
<tr>
<td>Ribitol</td>
<td>5-5</td>
<td>61-8 ± 1-5 (6)</td>
<td>1-97</td>
<td>15-7</td>
<td>1-5</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>5-0</td>
<td>53-2 ± 0-6 (13)</td>
<td>1-71</td>
<td>15-6</td>
<td>1-5</td>
</tr>
<tr>
<td>L-Arabinose*</td>
<td>5-0</td>
<td>65-2 ± 0-9 (17)</td>
<td>1-40</td>
<td>23-3</td>
<td>2-3</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>5-0</td>
<td>52-2 ± 0-9 (9)</td>
<td>1-86</td>
<td>14-0</td>
<td>1-4</td>
</tr>
<tr>
<td>D-Glucuronate*</td>
<td>5-0</td>
<td>55-3 ± 1-5 (6)</td>
<td>1-25</td>
<td>22-1</td>
<td>2-2</td>
</tr>
<tr>
<td>D-Galacturonate*</td>
<td>5-0</td>
<td>55-6 ± 1-5 (5)</td>
<td>1-11</td>
<td>25-3</td>
<td>2-5</td>
</tr>
<tr>
<td>Citrate</td>
<td>4-5</td>
<td>61-8 ± 0-7 (12)</td>
<td>1-73</td>
<td>18-0</td>
<td>1-8</td>
</tr>
<tr>
<td>L-Glutamate</td>
<td>4-5</td>
<td>27-1 ± 0-8 (17)</td>
<td>1-86</td>
<td>7-3</td>
<td>0-7</td>
</tr>
<tr>
<td>Succinate</td>
<td>3-5</td>
<td>29-7 ± 0-9 (10)</td>
<td>1-48</td>
<td>10-0</td>
<td>1-0</td>
</tr>
<tr>
<td>Glyeerol</td>
<td>3-5</td>
<td>41-8 ± 0-6 (14)</td>
<td>1-35</td>
<td>15-5</td>
<td>1-5</td>
</tr>
<tr>
<td>Dihydroxyacetone</td>
<td>3-0</td>
<td>31-9 ± 0-9 (12)</td>
<td>0-90</td>
<td>17-7</td>
<td>1-7</td>
</tr>
<tr>
<td>Fumarate</td>
<td>3-0</td>
<td>26-3 ± 0-7 (15)</td>
<td>1-24</td>
<td>10-6</td>
<td>1-0</td>
</tr>
<tr>
<td>L-Aspartate</td>
<td>3-0</td>
<td>28-3 ± 1-4 (8)</td>
<td>1-45</td>
<td>9-8</td>
<td>1-0</td>
</tr>
<tr>
<td>DL-Lactate*</td>
<td>3-0</td>
<td>16-6 ± 0-4 (14)</td>
<td>1-41</td>
<td>5-9</td>
<td>0-6</td>
</tr>
<tr>
<td>DL-Malate</td>
<td>3-0</td>
<td>14-8 ± 0-4 (12)</td>
<td>1-16</td>
<td>6-4</td>
<td>0-6</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>2-5</td>
<td>17-9 ± 0-4 (16)</td>
<td>1-18</td>
<td>7-6</td>
<td>0-7</td>
</tr>
<tr>
<td>Acetate</td>
<td>2-0</td>
<td>10-5 ± 0-3 (8)</td>
<td>1-06</td>
<td>5-0</td>
<td>0-5</td>
</tr>
</tbody>
</table>
needed for the conversion of the hexitols to fructose supplies energy for the assimilation of a larger part of the substrate. Similar conclusions can be reached by comparison of the results with ribitol/ribose and glycerol/dihydroxyacetone.

**Oxygen consumption during growth**

As described in the introduction we followed the method of Whitaker & Elsden (1963) to relate the oxygen uptake during growth to the \( Y \). In the first experiments the molar growth yields in Warburg flasks were much lower than those found in extinctometer tubes. Originally we supposed that the absence of \( CO_2 \) due to the presence of KOH in the centre well of the Warburg vessel was the cause of the low yields. In some experiments the \( O_2 \) uptake and the molar growth yield were measured in the presence of \( CO_2 \) by the method of Pardee (1949). The presence or absence of \( CO_2 \) had no influence on \( Y \). Finally, we found that the low yields in Warburg flasks were caused by a difference in the efficiency of shaking of the extinctometer tubes and the Warburg vessels. By varying the rate of shaking we found that \( Y \) for glucose under these circumstances varied from 55 with inefficient shaking (25 complete 2.5 cm. strokes/min.) to 71 with good shaking (125 complete 5 cm. strokes/min.); although the \( O_2 \) uptake during growth was always the same. A further increase of the shaking rate did not influence the results. A similar influence of the oxygen supply on the growth yield of *Aerobacter cloacae* was found by Pirt (1957). From this result we may conclude that \( O_2 \) consumption and molar growth yield may vary independently. Even with efficient shaking \( Y \) found in the Warburg experiments was somewhat smaller than the value found in the extinctometer tubes.

After we had found the influence of the shaking rate on \( Y \) we measured the \( O_2 \) uptake with the same (efficient) shaking for a number of substrates. In these experiments we noticed that maximal \( O_2 \) uptake in the Warburg flasks was reached at a later time than maximal growth in extinctometer tubes. This suggested that maximal growth in Warburg flasks was obtained before the \( O_2 \) uptake was maximal. This possibility was tested by growing the bacteria in several Warburg flasks with a growth limiting concentration of substrate. At intervals the content of a Warburg flask was taken for the measurement of the extinction at 660 mp. In this experiment the rate of oxygen uptake increases in a logarithmic fashion until growth stops and thereafter the rate becomes linear (Fig. 3). The results indicate that indeed maximal growth is obtained much earlier than maximal \( O_2 \) uptake. We must assume that during growth a compound is formed, which is oxidized during the second stage of the curve. The oxidation of this compound does not seem to be accompanied by an increase in the dry weight of bacteria. Dagley, Dawes & Morrison (1951) found that pyruvate accumulated during glucose oxidation by *Aerobacter aerogenes* and that aeration had a strong influence on this accumulation. We found, however, that in our case pyruvate accumulation was very unimportant. Instead of pyruvate significant amounts of acetate were found in the supernatant fluid. The kinetics of acetate accumulation during glucose oxidation are shown in Fig. 4. Acetate accumulation during aerobic growth was also found by Pirt (1957) for *A. cloacae* when the oxygen supply was insufficient. It is evident from Fig. 4 that during the second stage of the \( O_2 \) consumption curve acetate was oxidized. We must thus conclude that acetate oxidation after glucose disappearance does not
L. P. Hadjipetrou and others

lead to an increase in the dry weight of bacteria. The curves for the \(O_2\) uptakes during growth do not show a bend for a number of substrates. It is not known whether there is acetate accumulation in these cases or whether acetate is immediately oxidized. In this aspect it is important to note that acetate oxidation in the presence of glucose seems to contribute to the dry weight of bacteria (Table 4). Under these circumstances the energy of oxidation of acetate seems to be utilized for the assimilation of part of the glucose. The same may happen with acetate oxidation during growth with the substrates that do not show the bend in the curve for the \(O_2\) uptake during growth.

![Fig. 3](image)

**Fig. 3.** Extinction, \(O_2\) consumption and maltose concentration in a culture of *Aerobacter aerogenes* growing in minimal medium: \(\bigcirc--\bigcirc\), extinction; \(\bullet--\bullet\), \(O_2\) consumption; \(\times--\times\), maltose concentration.

![Fig. 4](image)

**Fig. 4.** Extinction, glucose concentration and acetate accumulation in a culture of *Aerobacter aerogenes* growing with 1 \(\mu\)mole glucose/ml.: \(\bullet--\bullet\), extinction; \(\bigcirc--\bigcirc\), acetate concentration; \(\times--\times\), glucose concentration.

<table>
<thead>
<tr>
<th>Glucose (pmole/ml.)</th>
<th>Acetate (pmole/ml.)</th>
<th>Growth yield per mole glucose (mg. dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>0</td>
<td>72.7 ± 1.0 (21)</td>
</tr>
<tr>
<td>1.5</td>
<td>0.5 or 1.0</td>
<td>78.0 ± 0.5 (6)</td>
</tr>
</tbody>
</table>

The results are given in Table 3. For the substrates, which are oxidized with a sharp bend in the curve for the \(O_2\) uptake (as in Fig. 3), the \(O_2\) uptake at the moment of the bend is given (substrates marked with an asterisk). For the substrates which are oxidized without a bend in the curve the final \(O_2\) consumption is given. For comparison both values for glucose are given. When we take the maximal \(O_2\) uptake for glucose, we find a similar value for \(Y^o\) as for galactose and mannose, which do not give a bend in the curve for the \(O_2\) uptake. It is very likely that acetate is
Energy production and growth of Aerobacter aerogenes

oxidized during growth on these substrates, but that the oxidation of acetate does not lead to an increase or only a very small increase in the dry weight of bacteria. In this respect we note that the increase in dry weight in the experiments with glucose and acetate is very low (Table 4). It is evident that \( Y^o \) for the substrates, which do not show the bend in the \( O_2 \) consumption curves, is too low and that it should be regarded as a minimal value. For the substrates which show the bend in the curve for the \( O_2 \) uptake, the \( Y^o \) values are much larger. When we divide \( Y^o \) by \( Y^{ATP} \) we find the number of ATP molecules per atom O taken up. The results with these substrates are sometimes in the neighbourhood of 3.

From the results of Table 3 it is evident that the \( Y^o \) values for the carbohydrates, or the compounds which are easily converted into carbohydrates, are much higher than for the substrates which are more difficult to convert into carbohydrates. When we take only the maximal \( O_2 \) uptakes into account, \( Y^o \) varies from 14-0 (D-xylose) to 19-6 (D-mannose) for the first class of compounds and from 5-0 (acetate) to 10-6 (fumarate) for the second class. The only exception is citrate, which gives an \( Y^o \) of 18-0. No explanation can be given for this result.

Table 5. Assimilation of glucose by growing Aerobacter aerogenes

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Radioactivity (counts/min.)</th>
<th>% (^{14}C) added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>12-870</td>
<td>100</td>
</tr>
<tr>
<td>Maximal growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>4-885</td>
<td>39-5</td>
</tr>
<tr>
<td>Supernatant</td>
<td>1-444</td>
<td>11-7</td>
</tr>
<tr>
<td>( CO_2 )</td>
<td>3-817</td>
<td>30-8</td>
</tr>
<tr>
<td>Recovery</td>
<td>10-146</td>
<td>82-0</td>
</tr>
<tr>
<td>Maximal ( O_2 ) uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>4-544</td>
<td>36-7</td>
</tr>
<tr>
<td>Supernatant</td>
<td>1-409</td>
<td>11-9</td>
</tr>
<tr>
<td>( CO_2 )</td>
<td>5-365</td>
<td>43-4</td>
</tr>
<tr>
<td>Recovery</td>
<td>11-378</td>
<td>92-0</td>
</tr>
</tbody>
</table>

Assimilation of glucose

The assimilation of glucose was studied by measuring the incorporation of \(^{14}C\) from uniformly labelled glucose into cell material. In these experiments the radioactivity was measured in the bacteria, in the \( CO_2 \) and in the supernatant fluid at the moment of maximal growth and maximal \( O_2 \) uptake. The results are shown in Table 5. It is evident that the recovery of the radioactivity at the point of maximal growth is low. This is due to the fact that acetate is not converted to \( CO_2 \) by the combustion method used. At the point of maximal \( O_2 \) uptake the recovery is much better and the radioactivity in the \( CO_2 \) is much larger. This confirms our earlier observation that during this stage acetate is oxidized to \( CO_2 \). The radioactivity in the bacteria decreased slightly during acetate oxidation which confirms our earlier observation (Table 1) that the dry weight decreases somewhat on prolonged incubation. The nature of the compound (or compounds) in which the radioactivity in the supernatant fluid is present is unknown. It is not glucose remaining in the medium,
because chemical estimation showed that no glucose was present after maximal growth (Fig. 4).

From the result of Table 5 (39.5% of $^{14}$C in the bacteria and 70.5 g. dry weight) we find that the C-content of the bacteria is 40.0%.

DISCUSSION

From the anaerobic experiments (Table 2) we can calculate $Y_{\text{ATP}}$. The molar growth yield for glucose is 26.1. The result of the radioactive experiment shows that the carbon content of the bacteria is 40.0%. The C-content of the bacteria is the same as that of glucose. This means that in the anaerobic experiment 26.1 g. glucose have been converted to cell material and $180 - 26.1 = 153.9$ g. have been fermented. As the net gain of ATP per mole glucose transformed into pyruvate is 2 mole and as one extra ATP is formed per mole of acetate produced, the amount of ATP formed from 153.9 g. glucose is $2 \times (153.9/180) + 0.84 = 2.55$ mole of ATP. From these results we can calculate $Y_{\text{ATP}}$ as 10.2. From the molar growth yield and the acetate production with fructose we find a value of 10.7 for $Y_{\text{ATP}}$. It is thus evident, that we find a similar value for $Y_{\text{ATP}}$ as Bauchop & Elsden found (1960) for Streptococcus faecalis, Saccharomyces cerevisiae and Pseudomonas lindneri, as Stouthamer (1962) found for Gluconobacter liquefaciens and as Twarog & Wolfe (1963) found for Clostridium tetanomorphum. Because $Y_{\text{ATP}}$ is the same for so many organisms it is very likely that it may be used to calculate the ATP yield from growth experiments with all micro-organisms.

In the aerobic experiments we found with some minor exceptions that $Y$ was about the same for each class of substrate (disaccharides, hexitols, hexoses, hexonates, pentoses, etc.). $Y$ for mannitol and sorbitol was much higher than $Y$ for fructose indicating that the oxygen atom needed for the conversion of mannitol to fructose allowed the assimilation of a larger part of the substrate. Some of the irregularities in the values for $Y$ (Table 3) are eliminated when we take the $Y^o$ values. For instance the low $Y$ values for rhamnose, inositol and glutamate lead to normal $Y^o$ values when divided by the $O_2$ uptake. In contrast to the results of Whitaker & Elsden (1968) we found that $Y^o$ for a given substrate is not a constant. By varying the shaking rate of the Warburg apparatus we found that $Y^o$ for glucose varied from 81.9 with efficient shaking to 25.7 with inefficient shaking. It should be pointed out, however, that under identical conditions $Y^o$ is a constant. These results cannot be ascribed to a difference in acetate accumulation because similar amounts of acetate were found at the moment of maximal growth under both conditions. With inefficient shaking both the growth rate and $Y^o$ are low. This may be explained by supposing that there exists some mechanism either by passing ATP generation or permitting the dissipation of ATP in the cell.

At high glucose concentrations the molar growth yield is lower. Under these circumstances we must also assume that less ATP is formed per mole of $O_2$ taken up or that the normal amount of ATP is formed, but that part of the ATP is wasted.

Acetate accumulation under the conditions of our experiments is not caused by an insufficient rate of oxygenation. In none of these experiments (with small or high glucose concentrations) was the formation of any of the fermentation products (formic acid, butanediol or ethanol) from glucose observed (Hadjipetrou &
Energy production and growth of *Aerobacter aerogenes* Stouthamer, unpublished experiments). Even with very vigorous aeration acetate accumulation was obtained. We must suppose, therefore, that the ability of *Aerobacter aerogenes* to oxidize acetate is insufficient to prevent its accumulation during glucose oxidation.

Acetate oxidation in the absence of glucose does not lead to an increase in dry weight of bacteria. This can be explained by the fact that during growth on acetate two adaptive enzymes, which constitute the glyoxylate by-pass, must be induced (for reviews see Kornberg, 1959; Kornberg & Elsden, 1961). Before the induction in the culture has taken place the acetate may already have been oxidized by the bacteria. How the energy of oxidation during acetate oxidation is used is not known.

The $Y^o$ values obtained by dividing the $Y$ values by the maximal $O_2$ uptakes fall in two classes. The compounds which are intermediates of the citric acid cycle or which are easily fed into the citric acid cycle give (with the exception of citrate) low $Y^o$ values. This is very clear when we compare the results with glycerol and succinate, which need both 3-5 mole $O_2$ per mole of substrate for complete oxidation. For glycerol we found $Y = 41.8$ and for succinate $Y = 29.7$; the $O_2$ uptakes during growth for these substrates were 1-85 mole and 1-48 mole per mole substrate respectively. The $Y^o$ for glycerol is 15-5 and that for succinate is 10-0. This may be explained by the hypothesis that the assimilation of succinate to cell material needs more energy than assimilation of glycerol. The conversion of each mole of succinate to glyceraldehyde-3-phosphate, which is necessary for the synthesis of all carbohydrate components of the cell needs two ATP. Besides NADH is used for this conversion, which limits the amount of NADH available for oxidation and ATP production.

The $Y^o/Y_{ATP}$ values of Table 3 are the same as the phosphorus/oxygen (P/O) ratios. Some of the values of Table 3 are minimal values because we found that maximal $O_2$ uptake was reached much later than maximal growth (Fig. 3). This was found to be due to acetate oxidation. For a number of substrates the $Y^o$ values could be corrected by dividing $Y$ by the $O_2$ uptake at the moment the curve for the $O_2$ uptake showed a sharp bend. The $Y^o/Y_{ATP}$ values obtained with these $O_2$ uptakes are very close to 3. This indicates that in the living bacterial cell oxidative phosphorylation is as efficient as in mitochondria. This is in contrast with the low P/O ratios found in cell-free extracts of *Aerobacter aerogenes* (Nossal, Keech & Morton, 1956; Hadjipetrou & Stouthamer, unpublished results). Thus the low P/O ratios in cell-free extracts are most probably caused by the damage in the phosphorylative system, which occurs during the preparation of the cell-free extract.

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