Yields of *Hydrogenomonas eutropha* from Growth on Succinate and Fumarate

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Molar growth yields were determined from chemostat cultures of *Hydrogenomonas eutropha* on succinate and on fumarate. The yields from culture on succinate were about 12 g higher than on fumarate. Assuming this difference to be equivalent to 1 molecule of adenosine triphosphate, it is concluded that the oxidation by oxygen of the *Hydrogenomonas* cytochrome *b* yields 1 molecule of adenosine triphosphate.

The ratio of H₂ oxidation (gram-atoms of oxygen utilized in oxidation of H₂) to CO₂ fixation (gram-atoms of carbon converted into cellular matter), defined as the O/C value, was found to vary appreciably in autotrophically grown *Hydrogenomonas eutropha*. The observed variation appeared dependent on the availability of inorganic nutrients and the supply rate of H₂, O₂, and CO₂ (3). Since the amount of adenosine triphosphate (ATP), or equivalent, made available per consumed atom of oxygen was not precisely known, the extent of coupling between energy supply and energy utilization could not be evaluated.

Our earlier observations (2) from experiments with cell-free preparations substantiated the occurrence of oxidative phosphorylation. Measurements of ratios of phosphorylative activity (gram-atoms of phosphorus converted to ATP) to oxidative activity (gram-atoms of oxygen utilized in oxidation of substrate) suggested a respiratory chain with a single coupling site between H₂ and the *Hydrogenomonas* cytochrome *b* (P/O = 1). On the other hand, our (3) efficient growth yields (O/C ~ 2.5) are difficult to explain unless one assumes the operation of an additional coupling site between cytochrome *b* and O₂ (P/O ≥ 2). To test the presence of such an additional coupling site in the respiratory chain, we used a procedure suggested by Günsalanus and Shuster (4). The procedure is based on measuring the difference in cell yield between succinate and fumarate and assuming that some 10 to 12 g of cells are synthesized per mole of ATP (1).

*H. eutropha* was cultivated in a chemostat (Pyrex, 1,000 ml liquid volume) under aerobic conditions with either succinate or fumarate as the sole energy and carbon source. The mineral medium used for these experiments was described previously (2). It contained 1.5 × 10⁻² m NH₄Cl as a nitrogen source. The culture chamber and mineral medium were autoclaved. The organic substrates were filter-sterilized (Nalgene, 0.20-μm membrane filter) and added to the mineral medium prior to use. Substrate utilization was determined from the difference in organic carbon content of the diluent medium and the supernatant effluent liquid. Cells were rapidly removed by filtration through a membrane filter (Millipore Corp., Bedford, Mass., HA 0.45 μm type).

The content of organic carbon was determined by a method described by Van Hall et al. (10), and cell concentration was measured as described previously (2). The molar growth yields (grams of cells per gram-mole of utilized substrate) obtained with these two substrates are recorded in Table 1.

The yield values reported in Table 1 were obtained during steady-state growth. The dilution rates of 0.40/hr indicate doubling times of approximately 1.75 hr. Since dilution rates of 0.48 to 0.50/hr resulted in culture wash-out, the reported values approach the maximum rate for *H. eutropha* on both substrates. Consequently, we may assume that the energy requirement for such functions as cell maintenance (8) was relatively low and about equal in both cases.

Since the concentration of succinate in the medium was virtually equal to that of fumarate, and since the cultures were diluted at equal rates, on a molar basis, the rate of substrate supply was the same for both conditions. Hence, the observed difference in steady-state cell concentration, which amounted to 12 g of cells per mole of substrate (see Table 1), reflects the difference in energy content between these two substrates.
Table 1. Molar growth yields on succinate and fumarate with Hydrogenomonas eutropha

<table>
<thead>
<tr>
<th>Expt no</th>
<th>Substrate</th>
<th>Dilation rate (per hr)</th>
<th>Cell conc. (mg/liter)</th>
<th>Yield (g/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Succinate</td>
<td>0.40–0.43</td>
<td>740 ± 19</td>
<td>51 ± 1</td>
</tr>
<tr>
<td>9</td>
<td>Fumarate</td>
<td>0.40–0.42</td>
<td>603 ± 25</td>
<td>39 ± 2</td>
</tr>
</tbody>
</table>

*H. eutropha* (ATCC 17697) was cultivated at 33°C in a chemostat. The culture was aerated (four times the culture volume per minute) with sterilized air, introduced through a fritted disc of medium porosity. A succinate concentration of 13.1 mM and a fumarate concentration of 15.8 mM were used for all experiments. Conditions were arranged to approach maximal growth rate and to allow utilization of ≥90% of added substrate. The amount of carbon source utilized was calculated from direct measurements of the organic carbon content of the growth medium before and after cell growth.

All enzymes of the tricarboxylic acid cycle are present in both autotrophically and heterotrophically grown hydrogen bacteria (6, 9). The metabolism of succinate and fumarate and the generation of the requisite reducing equivalents through this cycle thus seem likely. Although it is not known whether reducing equivalents are generated at all possible sites, one would expect equal amounts to be generated with both substrates, with the exception of an extra pair of hydrogen atoms obtained per mole of succinate. These hydrogens are transported through the succinate dehydrogenase system to an oxygen atom. Previous observations with cell-free preparations (2) showed the succinate-fumarate couple to be linked to cytochrome b, and we can assume a similar pathway for the intact cell. The succinate-fumarate difference of 12 g seen between succinate and fumarate (Table 1) thus should reflect the additional energy obtained from succinate oxidation via the Hydrogenomonas cytochrome b. On the basis of estimations concerning ATP requirement for cell synthesis (1, 5, 7), it appears that this difference is equivalent to 1 ATP. Consequently, we may conclude that the oxidation of cytochrome b yields H. eutropha at least at the equivalent of 1 mole of ATP.

If one assumes a similar electron transport chain for autotrophic and heterotrophic metabolism, it follows that the P/O ratio for hydrogen oxidation by the intact cell is 2. The O/C ratio of 2.5, as observed under optimal growth conditions (3), then suggests a requirement of 5 molecules of ATP for the conversion of 1 mole of CO₂ into cellular constituents. If we assume that, in the classic Bauchop and Elsden (1) experiments, energy conversion was perfect (~2 molecules of ATP per mole of C for polymerization), and that the operation of the pentose phosphate cycle requires 3 molecules of ATP per molecule of CO₂, it follows that little or no energy is uselessly dissipated when conditions for cell synthesis are optimized.

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Literature Cited