

Methanol Oxidation and Growth Yields in Methylo-trophic Bacteria: A Review

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Summary

In all bacteria growing on methane or methanol the methanol is oxidised by way of methanol dehydrogenase. This unusual quinoprotein interacts with the electron transport chain at the level of cytochrome *c*. All methylotrophs having methanol dehydrogenase contain 2 different types of soluble cytochrome *c*. One of these, but not the other, reacts with methanol dehydrogenase; no intermediate electron carrier is required for this reaction. The P/O ratio for methanol oxidation is 1 although the midpoint redox potential of the methanol/formaldehyde couple suggests that a higher ATP yield might be theoretically possible. Curves are presented that allow the prediction of cell yields, and the effects of altering the system for methanol oxidation, on these cell yields.

Zusammenfassung

Methanol wird in allen auf Methan oder Methanol wachsenden Bakterien durch die Methanoldehydrogenase oxidiert. Dieses NAD-unabhängige Chinoprotein steht mit der Atmungskette auf der Stufe des Cytochrom *c* in engem Kontakt. Alle Methanoldehydrogenase besitzenden methylo-trophen Mikroorganismen enthalten zwei unterschiedliche Typen von löslichem Cytochrom *c*. Nur einer davon reagiert direkt ohne zwischengeschalteten Elektronencarrier mit der Methanoldehydrogenase. Der P/O-Quotient für die Methanoloxidation beträgt 1, obwohl das Normal-Redoxpotential für die Umwandlung von Methanol zu Formaldehyd theoretisch eine höhere ATP-Ausbeute ermöglichen würde. Es werden Kurven vorgelegt, die eine Vorhersage der Zellausbeuten und der Effekte bei Veränderung des Methanoloxidationssystems auf diese Zellausbeuten erlauben.

All bacteria growing on methane or methanol oxidise methanol by way of methanol dehydrogenase which is an unusual, NAD-independent dehydrogenase having pyrrolo-quinoline quinone (PQQ) as its prosthetic group [1, 2]. Because cell carbon is assimilated at the level of formaldehyde, every molecule of methanol metabolised by the bacteria must be oxidised by this dehydrogenase. The midpoint redox potential of the HCHO/CH₃OH couple is -0.182 V; it might therefore be expected that methanol dehydrogenase would interact with the electron transport chain at or before the level of cytochrome *b*, and that the P/O ratio would be 2 for the oxidation of methanol to formaldehyde. However, all the available evidence indicates that methanol dehydrogenase interacts at the level of cytochrome *c*, thus by-passing cytochrome *b* and the earlier energy-transducing parts of the electron transport chain [3, 2]. This is consistent with

the fact that the P/O ratio for oxidation of methanol to formaldehyde is only 1, and not 2 as might have been expected.

This raises a number of questions with respect to electron transport and energy transduction during methanol oxidation, which this review will briefly attempt to answer.

1. How many cytochromes *c* are present in methylotrophs?
2. Is the reaction between methanol dehydrogenase and cytochrome *c* direct, or is there an intermediate electron carrier?
3. Is there a special cytochrome *c* involved in methanol oxidation?
4. How are methanol dehydrogenase and cytochrome *c* arranged to generate ATP?
5. Does the unusual system for methanol oxidation have any special effect on growth yields of methylotrophs, and could a genetically-engineered change in this system for methanol oxidation lead to greater cell yields?

1. How many cytochromes *c* are present in methylotrophs?

All methylotrophs studied in any detail have at least 2 soluble cytochromes *c*, and some membrane-bound cytochrome *c* which may or may not be the same as the soluble cytochromes. Some of these are listed in Table 1. We have also confirmed the presence of 2 soluble cytochromes *c* in the methane-utilising bacteria, in the facultative autotroph and methylotroph *Paracoccus denitrificans*, and in *Hyphomicrobia*. The two types of cytochrome *c* differ in their midpoint redox potentials, their molecular weights and their isoelectric points. They are completely different proteins; one type is not a dimer or degradation product of the other [7].

Table 1. Properties of the two types of soluble cytochromes *c* of methylotrophs
The data for *Pseudomonas* AM1 are from O'KEEFE and ANTHONY [4]; for *Methylophilus methylotrophus* from CROSS and ANTHONY [5]; and for *Methylomonas* J from OHTA and TOBARI [6].

	Isoelectric point	Molecular weight	Midpoint redox potential
<i>Pseudomonas</i> AM 1			
Cytochrome <i>c</i> _H	8.8	11,000	+294 mV
Cytochrome <i>c</i> _L	4.2	20,900	+256 mV
<i>Methylophilus methylotrophus</i>			
Cytochrome <i>c</i> _H	8.9	8,500	+373 mV
Cytochrome <i>c</i> _L	4.0–4.4	21,000	+310 mV
<i>Methylomonas</i> J			
Cytochrome <i>c</i> ₅₅₁ (I)	5.3	16,000	+290 mV
Cytochrome <i>c</i> ₅₅₁ (II)	4.3	12,500	+240 mV

2. Is the reaction between methanol dehydrogenase and cytochrome *c* direct, or is there an intermediate electron carrier?

The answer to this question has been difficult to determine unequivocally with purified proteins. This is because addition of methanol dehydrogenase to cytochrome *c* leads to reduction of the cytochrome even in the absence of methanol [8]. A less direct approach has now solved this problem. This has been achieved by using small amounts of the cytochrome *c* from methylotrophs to mediate between methanol dehydrogenase and large quantities of mammalian cytochrome *c* — used as a terminal electron acceptor [9].

Methanol-dependent reduction of the cytochrome *c* was recorded with concomitant production of stoichiometric amounts of formaldehyde. Thus, using this system, it has now been conclusively shown that no intermediate electron carrier is required between methanol dehydrogenase and pure cytochrome *c* from methylotrophs. It should be noted that the rate measured for the methanol: cytochrome *c* oxidoreductase activity of methanol dehydrogenase was too low to account for the rate of respiration observed in whole cells; this may be related to the change in environment occurring on release of the cytochrome *c* and methanol dehydrogenase from the membrane surface of the bacteria.

3. Is there a special cytochrome *c* involved in methanol oxidation?

The fact that methylotrophs have two very distinct types of cytochrome *c* suggests that one of them may have a special role in methanol oxidation, and the results in Table 2 appear to confirm that this is the case. The first point to note from Table 2 is that neither horse heart cytochrome *c* nor the cytochrome from the non-methylotroph, *Pseudomonas aeruginosa*, is able to react with any of the 3 dehydrogenases. The second point is that only one of the two cytochromes from each methylotroph is able to react with methanol dehydrogenase. The third point is that the cytochromes *c* from *Methylophilus methylotrophus* and from *Paracoccus denitrificans* are only able to react with their own dehydrogenase, whereas the cytochrome *c* from *Pseudomonas* AM 1 is less specific and is able to react with all three dehydrogenases.

Table 2. The specificity of reaction between methanol dehydrogenases and cytochromes *c*. This Table is taken from BEARDMORE-GRAY et al. [9].

Cytochrome <i>c</i>	Reduction of cytochromes by methanol dehydrogenase from the following bacteria		
	<i>M. methylotrophus</i>	<i>Pa. denitrificans</i>	<i>Pseudomonas</i> AM 1
Horse heart cytochrome <i>c</i> (pI, 10.4)	—	—	—
<i>Pseudomonas aeruginosa</i> cytochrome <i>c</i> ₅₅₁ (pI, 4.7)	—	—	—
<i>M. methylotrophus</i> cytochrome <i>c</i> _H (pI, 8.85)	—	—	—
cytochrome <i>c</i> _L (pI, 4.35)	+	—	—
<i>Pa. denitrificans</i> cytochrome <i>c</i> (1), (pI, acidic)	—	—	—
cytochrome <i>c</i> (2) (pI, more acidic)	—	+	—
<i>Pseudomonas</i> AM 1 cytochrome <i>c</i> _H (pI, 8.8)	—	—	—
cytochrome <i>c</i> _L (pI, 4.2)	+	+	+

4. How are methanol dehydrogenase and cytochrome *c* arranged to generate ATP?

The two main options for arrangement of the 'methanol oxidase' system are shown in Fig. 1. The protonmotive redox 'loop' (Fig. 1a) is unlikely because the dehydrogenase and its prosthetic group are very hydrophilic and unlikely to span a lipoprotein mem-

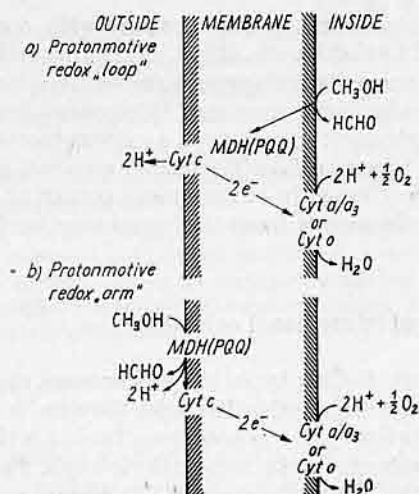


Fig. 1. Possible arrangements of methanol dehydrogenase and cytochrome *c* in the bacterial membrane. In both schemes 2H^+ are effectively moved from the inside to the outside for every molecule of methanol oxidised to formaldehyde. In (a) the MDH is transmembranous and in (b) it is on the outer surface. [2]

brane. All the evidence at present supports the protonmotive redox 'arm' is shown in Fig. 1b [10, 11, 12, 13, 14]. This arrangement of the 'methanol oxidase' system leads to translocation of one pair of protons across the bacterial membrane and thus to a P/O ratio of 1, and this value has been confirmed by more direct measurements [15]. It is of interest that if the methanol dehydrogenase is on the outer surface, as indicated here, the formaldehyde produced must be able to cross the membrane for its further oxidation and assimilation into cell material. It will thus be of interest to determine how it is transported, and whether formaldehyde transport is involved in the regulation of oxidation and assimilation of this critical 'branch point' intermediate.

5. Does the unusual system for methanol oxidation have any special effects on cell yields of methylotrophs, and could a genetically-engineered change in this system lead to greater cell yields?

The summary above shows that there are two unusual features of the system for methanol oxidation in bacteria. The first is that no NADH is produced during the first step in the oxidation of methanol; and the second is that the P/O ratio for this step is only 1, whereas the value of 2 is theoretically possible. Table 3 illustrates one effect of these features on methylotrophic metabolism; that is, during growth on methane or methanol, usually less than 30% of electron transport is by way of NADH dehydrogenase. In methylotrophs growing on methane or methanol most electron transport is by way of the methanol dehydrogenase whose activity is associated with a P/O ratio of only 1. This raises the question of whether or not cell yields might be increased if the methanol dehydrogenase were to be changed to an NADH-yielding enzyme, or if the P/O ratio for methanol oxidation could be increased to 2.

For some methylotrophs the answer is immediately obvious; in those methylotrophs that are NADH-limited rather than ATP-limited (this includes most methanotrophs), increasing the P/O ratio for methanol oxidation would have a negligible effect on cell yields [16, 17, 2]. In these bacteria, however, if the methanol dehydrogenase were to become NADH-yielding, then the cell yields would be much higher even if the P/O ratio were to remain low.

In those methanol-utilizers having the RuMP assimilation pathway, higher yields would be obtained if the P/O ratio were to be increased or if the methanol dehydrogenase were

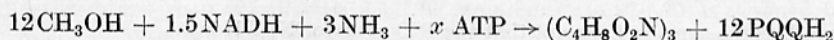
Table 3. Electron flow from each dehydrogenase expressed as a proportion of total electron transport

Growth substrate	Percentage of electron flow from each dehydrogenase			
	Methanol	Formaldehyde	Flavoprotein	NADH
Methane				
Serine pathway bacteria (e.g. <i>Methylosinus trichosporium</i>)	78–88	(NAD ⁺ -linked)	9–12	0–13
RuMP pathway bacteria (e.g. <i>Methylococcus capsulatus</i>)	84–92	(NAD ⁺ -linked)	0	8–16
Methanol				
Serine pathway bacteria				
(a) HCHO oxidation yields 1 NADH (e.g. <i>Pseudomonas</i> AM 1)	53–56	35	10	0–3
(b) HCHO oxidation yields 2 NADH	53–64	(NAD ⁺ -linked)	9–15	21–40
RuMP pathway bacteria (e.g. <i>Methylophilus methylotrophus</i>)	57–70	(NAD ⁺ -linked)	0	30–43
RuBP pathway bacteria (e.g. <i>Paracoccus denitrificans</i>)	52–62	(NAD ⁺ -linked)	0	38–48
Conventional substrates (including formate)	0	0	0–26	74–100

The range of values predicted is for a range of assumed P/O ratios; the lowest is assumed to be one for each oxidation step and the highest P/O ratios are assumed to be 1, 2 or 3 for methanol dehydrogenase, flavoprotein and NADH, respectively. Higher P/O ratios lead to a lower proportion of electron transport from NADH dehydrogenase and a higher proportion from other dehydrogenases. During growth on methane only 25–40% of the total oxygen consumed is by way of electron transport and oxidases, the remainder being used in the initial hydroxylation reaction (assumed here to be NADH-linked). In serine pathway bacteria the oxidation of acetyl-CoA to glyoxylate is assumed to involve a flavoprotein.

This Table is taken from ANTHONY [2].

to be NADH-yielding. The extent of the expected increases can be estimated from the Fig. 2 and 3. These data have been calculated using methods previously described [16, 17, 2]. The formula for calculation of yields is:



To use this formula for predicting yields a value for the ATP requirement for biosynthesis (x) must be chosen and values for the P/O ratios assumed for oxidation of NADH and PQQH₂ (reduced methanol dehydrogenase). The value for x can be calculated, or the measured yield (Carbon Conversion Efficiency) can be used for estimating a value for x for a given organism from curve A in Figs. 2 and 3. Using this same value for x the effect of changing the nature of the system for oxidation of methanol can be calculated by using the alternative curves in Figs. 2 and 3.

Curve A is the normal situation; the methanol dehydrogenase (MDH) is a PQQ-containing enzyme coupling to the electron transport chain at the level of cytochrome *c*. The P/O ratio is 1 for PQQH₂ and 3 for NADH oxidation.

Curve B is the same as A except that the P/O ratio for NADH is 2.

Curve C is the result of coupling the MDH so that the P/O ratio for PQQH₂ is 2 instead of 1 (P/O ratio for NADH is 3).

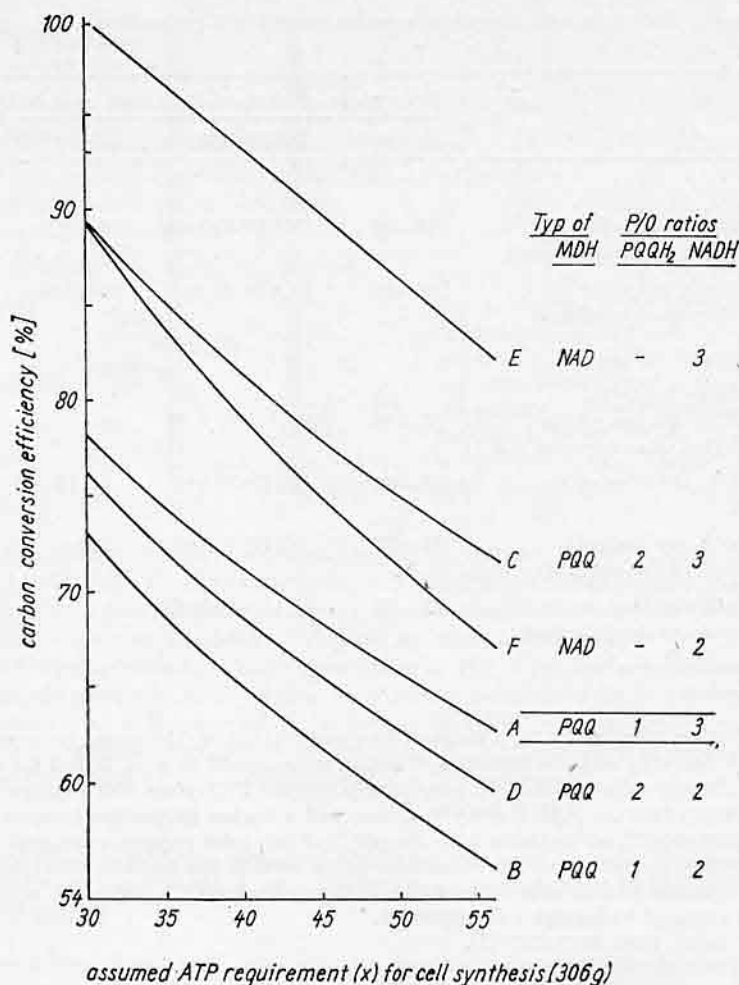


Fig. 2. The variation of carbon conversion efficiency with ATP requirement

Curve D is the same as C except that the P/O ratio for NADH is 2.

Curve E is the result of changing the methanol dehydrogenase to an NAD-linked dehydrogenase (P/O ratio for NADH is 3).

Curve F is the same as E except that the P/O ratio for NADH is 2.

Table 4 presents predictions derived from the use of Figs. 2 and 3 for an obligate methanol-utilizer growing by way of the RuMP carbon assimilation pathway. It can be seen that the greatest potential increase in yield would be achieved by changing the methanol dehydrogenase to an NAD-linked dehydrogenase. It should be borne in mind that the very high yield that is predicted in this case might not be obtainable in practice on thermodynamic grounds. Such a modified organism might only be able to exist if it also 'adapted itself' by lowering the P/O ratio for NADH oxidation from 3 to 2. Even if this did occur then, as shown in Table 4, the predicted yield would still be higher than in the organism having the normal methanol dehydrogenase. In summery, bacteria growing on methanol by way of the RuMP pathway are some of the most efficient

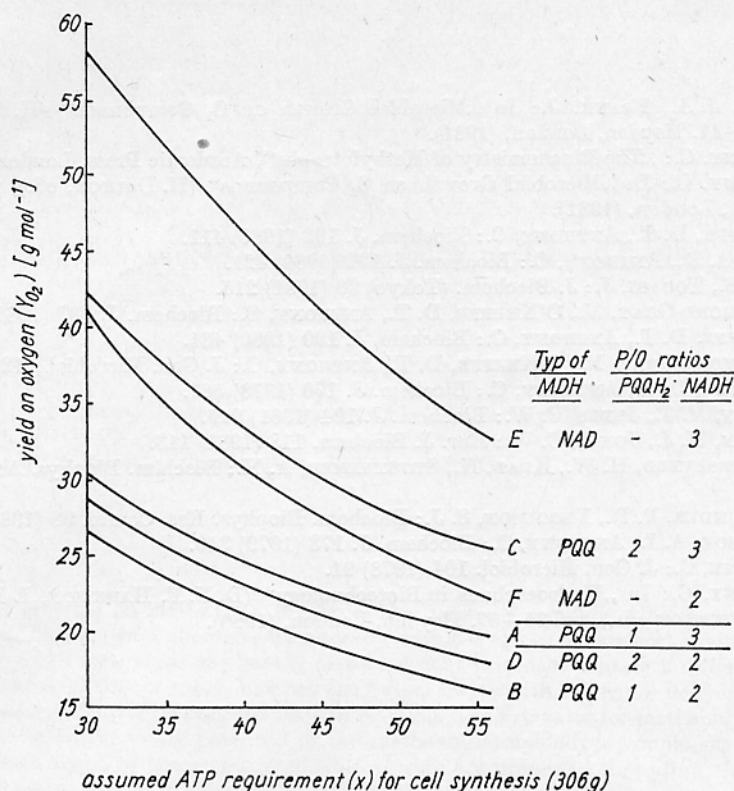


Fig. 3. The variation of Y_{O_2} with ATP requirement for growth

organisms known (in terms of carbon conversion efficiency), and the 'altered' organisms discussed here would be even more efficient. The free energy change during such efficient growth would tend to be much smaller than normal, and it must be concluded that rapid growth of such altered organisms might not be possible on thermodynamic grounds.

Table 4. Examples of changes in yields predicted from Figs. 2 and 3

These yields are for an obligate methanol-utilizer growing by way of the RuMP pathway of carbon assimilation. An ATP requirement of 37 ATP is assumed as the requirement for synthesis of 306 g of bacteria. The initial carbon conversion efficiency for such an organism would be 73% and the yield with respect to oxygen (Y_{O_2}) would be 26 g mol⁻¹.

Change in the organism proposed	Increases in yields	
	CCE (%)	Y_{O_2} (g mol ⁻¹)
1. Increase in P/O ratio for methanol oxidation from the usual 1 to a value of 2		
if the P/O ratio for NADH is 3	73 to 83	26 to 36
if the P/O ratio for NADH is 2	67 to 73	23 to 25
2. Methanol dehydrogenase changes to NAD-linked enzyme		
if the P/O ratio for NADH is 3	73 to 95	26 to 49
if the P/O ratio for NADH is 2	67 to 82	23 to 33

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