CHAPTER 1

Energy metabolism and phylogenetic diversity of sulphate-reducing bacteria

Rudolf K. Thauer, Erko Stackebrandt and W. Allan Hamilton

1.1 INTRODUCTION

Sulphate-reducing bacteria (SRB) are those prokaryotic microorganisms, both bacteria and archaea, that can use sulphate as the terminal electron acceptor in their energy metabolism, i.e. that are capable of dissipimulatory sulphate reduction. Most of the SRB described to date belong to one of the four following phylogenetic lineages (with some examples of genera): (i) the mesophilic δ-proteobacteria with the genera Desulfovibrio, Desulfobacterium, Desulfobacter, and Desulfobulbus; (ii) the thermophilic Gram-negative bacteria with the genus Thermodesulfovibrio; (iii) the Gram-positive bacteria with the genus Desulfotomaculum; and (iv) the Euryarchaeota with the genus Archaeoglobus (Castro et al., 2000). A fifth lineage, the Thermodesulfobiaceae, has been described recently (Mori et al., 2003).

Many SRB are versatile in that they can use electron acceptors other than sulphate for anaerobic respiration. These include elemental sulphur (Bottcher et al., 2005; Finster et al., 1998), fumarate (Tomei et al., 1995), nitrate (Krekeler and Cypionka, 1995), dimethylsulfoxide (Jonkers et al., 1996), Mn(IV) (Myers and Nealson, 1988) and Fe(III) (Lovley et al., 1993; 2004). Some SRB are even capable of aerobic respiration (Dannenberg et al., 1992; Lemos et al., 2001) although this process appears not to sustain growth, and probably provides these organisms only with energy for maintenance. Since dissimilatory sulphate reduction is inhibited under oxic conditions, SRB can grow at the expense of sulphate reduction only in the complete absence of molecular oxygen. SRB are thus considered to be strictly anaerobic microorganisms and are mainly found in sulphate-rich anoxic habitats (Cypionka, 2000; Fareleira et al., 2003; Sass et al., 1992). These conditions apply in marine sediments since ocean water is rich in sulphate,
its concentration being as high as 30 mM. SRB are also present, however, in freshwater sediments, where the sulphate concentration is generally well below 1 mM but is continuously maintained at this level by the re-oxidation of the H$_2$S to sulphate at the oxic/anoxic interface due to the action of chemolithotrophic and photolithotrophic bacteria (Holmer and Storkholm, 2001). Since most SRB may use electron acceptors other than sulphate, they can also be found in anoxic habitats depleted in sulphate such as the human intestinal tract (Chapter 18). SRB are, however, most abundant in habitats where the availability of sulphate is not limiting.

1.1.2 Thermodynamics

The discussion of the energetics of sulphate reduction is introduced with consideration of the thermodynamic parameters that determine the possible interactions between potential electron donors and sulphate as electron acceptor.

The redox potential, $E^\circ$, of the sulphate/HS$^-$ couple is $-217$ mV under standard conditions, which are 1M concentrations of sulphate and HS$^-$ at pH 7.0 and 25°C. Under physiological conditions, however, where the concentrations of sulphate are generally $<30$ mM and of HS$^-$ $<1$ mM, the redox potential, $E'$, is a little more positive and of the order of $-200$ mV. Thus at all expected concentrations of sulphate and HS$^-$, almost any organic compound generated by plants or animals, including carbohydrates, fatty acids, alkanes and aromatic compounds, should, in theory, be able to be completely oxidized to CO$_2$ since the redox potentials of each of these possible electron donors is significantly more negative than the $-200$ mV of the sulphate/HS$^-$ couple (Table 1.1). Indeed, each of these bio-organic materials has now been shown to be completely mineralized by individual SRB, either alone or in syntrophic association with other organisms. This is even true for methane with a redox potential $E^\circ$ of the CO$_2$/methane couple of $-244$ mV and therefore a redox potential difference $\Delta E' \left[ E'(SO_4^{2-}/HS^-) - E'(CO_2/CH_4) \right]$ of only $+44$ mV. Remarkably, it has recently been shown that a $\Delta E'$ of $+25$ mV, equivalent to a free energy change $\Delta G'$$=\approx$ $-20$ kJ/mol ($\Delta G' = -nF\Delta E'$; $n = 8$) is sufficient to sustain growth of SRB (Hoehler et al., 2001).

For the prediction of electron flow in natural environments, in most cases $E^\circ$ rather than $E'$ of the reductant can be used since the two generally differ by only around 20 mV. There are, however, important exceptions, e.g. the H$^+$/H$_2$ couple and the S$^0$/HS$^-$ couple. The redox potential at pH 7.0 (H$^+$ concentration constant at $10^{-7}$ M) of the H$^+$/H$_2$ couple increases
Table 1.1. Redox potentials $E^\circ$ of electron donors thermodynamically capable and not capable of dissimilatory sulphate, thiosulphate or bisulphite reduction

<table>
<thead>
<tr>
<th>Redox couple</th>
<th>$n$</th>
<th>$E^\circ$ (Mv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2\text{CO}_2 + 2\text{acetate} / \text{hexose}$</td>
<td>8</td>
<td>$-670$</td>
</tr>
<tr>
<td>$\text{CO}_2 + \text{acetate} / \text{pyruvate}$</td>
<td>2</td>
<td>$-660$</td>
</tr>
<tr>
<td>$\text{FeS}_2 /\text{FeS} + \text{H}_2\text{S}^d$</td>
<td>2</td>
<td>$-613$</td>
</tr>
<tr>
<td>$\text{SO}_4^{2-} /\text{HSO}_3^-$</td>
<td>2</td>
<td>$-516$</td>
</tr>
<tr>
<td>$\text{CO}_2 /\text{CO}$</td>
<td>2</td>
<td>$-520$</td>
</tr>
<tr>
<td>$\text{Fe}^{2+} /\text{Fe}^{0}$</td>
<td>2</td>
<td>$-447$</td>
</tr>
<tr>
<td>$\text{CO}_2 + \text{acetate} / \text{lactate}$</td>
<td>4</td>
<td>$-430$</td>
</tr>
<tr>
<td>$\text{CO}_2 / \text{formate}$</td>
<td>2</td>
<td>$-432$</td>
</tr>
<tr>
<td>$2\text{H}^+ /\text{H}_2$</td>
<td></td>
<td>$-414$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>($-270$ to $-300)^a$</td>
</tr>
<tr>
<td>$6\text{CO}_2 /\text{hexose}$</td>
<td>24</td>
<td>$-410$</td>
</tr>
<tr>
<td>$\text{S}_2\text{O}_5^{2-} /\text{HS}^- + \text{HSO}_3^-$</td>
<td>2</td>
<td>$-402$</td>
</tr>
<tr>
<td>$\text{CO}_2 + \text{acetate} + \text{NH}_3 /\text{alanine}$</td>
<td>4</td>
<td>$-400$</td>
</tr>
<tr>
<td>Acetate/ethanol</td>
<td>4</td>
<td>$-390$</td>
</tr>
<tr>
<td>$\text{CO}_2 / \text{methanol}$</td>
<td>6</td>
<td>$-370$</td>
</tr>
<tr>
<td>$4\text{CO}_2 / \text{succinate}$</td>
<td>12</td>
<td>$-312$</td>
</tr>
<tr>
<td>$7\text{CO}_2 / \text{benzoate}$</td>
<td>30</td>
<td>$-300$</td>
</tr>
<tr>
<td>$2\text{Acetate} / \text{butyrate}$</td>
<td>4</td>
<td>$-290$</td>
</tr>
<tr>
<td>$\text{CO}_2 + \text{acetate} / \text{glycerol}$</td>
<td>6</td>
<td>$-290$</td>
</tr>
<tr>
<td>$2\text{CO}_2 / \text{acetate}$</td>
<td>8</td>
<td>$-290$</td>
</tr>
<tr>
<td>$4\text{CO}_2 / \text{butyrate}$</td>
<td>20</td>
<td>$-280$</td>
</tr>
<tr>
<td>$3\text{CO}_2 / \text{propionate}$</td>
<td>7</td>
<td>$-280$</td>
</tr>
<tr>
<td>$\text{N}_2 /\text{NH}_3^f$</td>
<td>6</td>
<td>$-276$</td>
</tr>
<tr>
<td>$\text{SO}_2^+/\text{H}_2\text{S}^*$</td>
<td>2</td>
<td>$-270$ $(–120)^h$</td>
</tr>
<tr>
<td>$6\text{CO}_2 / \text{hexane}^e$</td>
<td>38</td>
<td>$-250$</td>
</tr>
<tr>
<td>$\text{CO}_2 / \text{CH}_4$</td>
<td>8</td>
<td>$-244$</td>
</tr>
<tr>
<td>$\text{SO}_4^{2-} /\text{HS}^-$</td>
<td>8</td>
<td>$-217$ $(–200)^i$</td>
</tr>
<tr>
<td>$\text{SO}_3\text{H}^- /\text{HS}^-$</td>
<td>6</td>
<td>$-116$</td>
</tr>
<tr>
<td>Glycine/acetate $+ \text{NH}_3$</td>
<td>2</td>
<td>$-10$</td>
</tr>
<tr>
<td>Fumarate/succinate</td>
<td>2</td>
<td>$+33$</td>
</tr>
<tr>
<td>Trimethylamine N-oxide/trimethylamine</td>
<td>2</td>
<td>$+130$</td>
</tr>
<tr>
<td>Dimethylsulfoxide/dimethylsulphide</td>
<td>2</td>
<td>$+160$</td>
</tr>
<tr>
<td>$\text{Fe(OH)}_3 + \text{HCO}_3^- / \text{FeCO}_3^h$</td>
<td>1</td>
<td>$+200$</td>
</tr>
<tr>
<td>$\text{NO}_2^- /\text{NH}_3$</td>
<td>6</td>
<td>$+330$</td>
</tr>
<tr>
<td>$\text{NO}_3^- /\text{NH}_3$</td>
<td>8</td>
<td>$+360$</td>
</tr>
</tbody>
</table>
from –414 mV at an H₂ partial pressure of 10⁵ Pa (standard condition) to values between –270 mV and –300 mV at H₂ partial pressures between 1 and 10 Pa, which is the concentration range of H₂ prevailing in sediments. Thus the oxidation of acetate to CO₂ (E⁰′ = –290 mV) with H⁺ as electron acceptor (E′ = –270 mV at 1 Pa: H₂) becomes thermodynamically feasible and there are organisms that appear to live at the expense of this equation (Galouchko and Rozanova, 1996; Lee and Zinder, 1988; Shigematsu et al., 2004).

\[
\text{CH}_3\text{COO}^- + H^+ + 2H_2O = 2\text{CO}_2 + 4H_2 \quad \Delta G^{\circ\prime\prime} = +95 \text{ kJ/mol} \quad (1.1)
\]

The redox potential of the S⁰/HS⁻ couple (S⁰ in the solid state and therefore constant) increases from –270 mV under standard conditions

<table>
<thead>
<tr>
<th>Reaction</th>
<th>(n)</th>
<th>(\Delta G^{\circ\prime\prime}) (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn⁴⁺/Mn²⁺</td>
<td>2</td>
<td>+407</td>
</tr>
<tr>
<td>NO₃⁻/NO₂⁻</td>
<td>2</td>
<td>+430</td>
</tr>
<tr>
<td>2NO⁻/N₂</td>
<td>10</td>
<td>+760</td>
</tr>
<tr>
<td>O₂/2H₂O</td>
<td>4</td>
<td>+818</td>
</tr>
<tr>
<td>2NO/N₂O</td>
<td>2</td>
<td>+1175</td>
</tr>
<tr>
<td>H₂O₂/2H₂O</td>
<td>2</td>
<td>+1350</td>
</tr>
<tr>
<td>N₂O/N₂</td>
<td>2</td>
<td>+1.360</td>
</tr>
</tbody>
</table>

\(a\) Calculated for a H₂ partial pressure of 1 Pa and 10 Pa, respectively.

\(b\) Calculated for a [HS⁻] = 0.1 mM.

\(c\) Calculated for [sulphate] = 30 mM and [HS⁻] = 0.1 mM.

\(d\) (Wächtershäuser, 1992)

\(e\) Calculated from the free energy of formation. \(\Delta G^{\circ\prime\prime}\) of benzoate was estimated from \(\Delta G^{\circ}\) for benzoic acid (crystalline solid state) (–245 kJ/mol), from the solubility of benzoic acid at 25 °C (27.8 mM) and from the pK of benzoic acid (4.2) to be –212.3 kJ/mol (Thauer and Morris, 1984).

\(f\) \(N₂\) cannot be used as electron acceptor for energy conservation because of the too high energy of activation required for its reduction.

\(g\) (Zengler et al., 1999)

\(h\) (Ehrenreich and Widdel, 1994)

Notes: E⁰ at pH 7.0 are given for H₂, CO₂, CO, CH₄ and O₂ in the gaseous state at 10⁵ Pa, for S⁻ in the solid state and for all other compounds in aqueous solution at 1M concentration. The values in brackets are E⁰ values calculated for physiological substrate and product concentrations. E⁰ values were calculated from \(\Delta G^{\circ}\values were taken from (Thauer et al., 1977).
to $-120$ mV under physiological conditions where the HS$^-$ concentrations can be 0.1 mM or even lower. As a consequence, under in situ conditions the reduction of sulphate with H$_2$S to S$^0$ is endergonic. In agreement with this prediction, SRB have been found that can grow at the expense of S$^0$ disproportionation to sulphate and HS$^-$ (Finster et al., 1998):

$$4S^0 + 4H_2O = SO_4^{2-} + 3HS^- + 5H^+ \quad \Delta G^{\circ'} = +41 \text{ kJ/mol} \quad (1.2)$$

### 1.1.3 Energy coupling

Growth of SRB with dissimilatory sulphate reduction indicates that substrate oxidation is coupled with adenosine triphosphate (ATP) synthesis from adenosine diphosphate (ADP) and inorganic phosphate. It leaves open, however, whether coupling is by substrate level phosphorylation and/or electron transport-linked phosphorylation (Thauer et al., 1977). In substrate level phosphorylation an “energy-rich” intermediate is formed from organic substrates during exergonic oxidation reactions. The “energy-rich” intermediate is generally an acyl phosphate or an acyl thioester, which have group transfer potentials equivalent to that of ATP, and are in an enzyme-catalyzed equilibrium with the ADP/ATP system. In electron transport-linked phosphorylation the redox potential difference between electron carriers and the terminal electron acceptor is conserved in a transmembrane electrochemical proton or sodium ion gradient, which drives the phosphorylation of ADP via a membrane-bound ATP synthase.

For many years it was thought that it was only possible to grow SRB on organic substrates as electron donors for dissimilatory sulphate reduction, and this led to the belief that in these organisms energy is conserved mainly or exclusively via substrate level phosphorylation. However, in 1978 it was unambiguously shown that *Desulfovibrio vulgaris* can grow with H$_2$ and sulphate as the sole energy source (Badziong and Thauer, 1978).

$$4H_2 + SO_4^{2-} + H^+ = HS^- + 4H_2O \quad \Delta G^{\circ'} = -151.8 \text{ kJ/mol} \quad (1.3)$$

During growth on H$_2$ and sulphate, energy must be conserved by electron transport-linked phosphorylation since substrate level phosphorylation is only possible when the substrate oxidized is organic. There is, however, an exception to this general rule. The oxidation of bisulphite to sulphate can be coupled by substrate level phosphorylation via an energy-rich adenosine phosphosulphate (APS) intermediate. Using this reaction some SRB can grow at the expense of bisulphite disproportionation to sulphate and
hydrogen sulphide (Bak and Cypionka, 1987; Frederiksen and Finster, 2003; Kramer and Cypionka, 1989).

\[ 4\text{HSO}_3^- + 4\text{H}^+ = 3\text{SO}_4^{2-} + \text{HS}^- \quad \Delta G^{\circ} = -235.6 \text{kJ/mol.} \] (1.4)

This chapter first summarizes what is presently known about the biochemistry of dissimilatory sulphate reduction with \( \text{H}_2 \). Thermodynamic problems associated with the sulphate-dependent oxidations of substrates other than \( \text{H}_2 \) are then outlined. Subsequently, also under energetic aspects, the trophic interactions of SRB with other microorganisms in their habitats are described. Finally, the phylogenetic diversity of SRB is discussed.

Some of the arguments that will be put forward are based on genome sequence information. Until now, only the genome sequences of three SRB, of \textit{Archaeoglobus fulgidus} (Klenk et al., 1997), of \textit{Desulfovibrio vulgaris} (Hildenborough) (Heidelberg et al., 2004; Hemme and Wall, 2004) and \textit{Desulfotalea psychrophila} (Rabus et al., 2004), have been published. The genomes of many other SRB with different metabolic capacities and from different phylogenetic origins are presently being sequenced. Only when we have these data will we have a complete picture of the energy metabolism of SRB (Chapter 3).

For a previous review on the bioenergetic strategies of sulphate-reducing bacteria see Peck (1993).

### 1.2 DISSIMILATORY SULPHATE REDUCTION WITH \( \text{H}_2 \)

The equations and proteins involved in dissimilatory sulphate reduction with \( \text{H}_2 \) will be described for \textit{Desulfovibrio vulgaris} (Hansen, 1994; Matias et al., 2005). The biochemistry of this \( \delta \)-proteobacterium and of closely related species has been studied in detail and the genome sequence of the Hildenborough strain has recently been published (Heidelberg et al., 2004). Most of the results can probably be generalized to other SRB capable of growth on \( \text{H}_2 \) as sole energy source such as \textit{D. desulfuricans}, \textit{Thermodesulphobacterium commune}, \textit{Desulfobacterium autotrophicus}, \textit{Desulfotomaculum orientis} and \textit{Archaeoglobus profundus}.

#### 1.2.1 Sulphate activation

Dissimilatory sulphate reduction with \( \text{H}_2 \) to \( \text{H}_2\text{S} \) in \textit{D. vulgaris} proceeds via \( \text{HSO}_3^- \) as intermediate. The redox potential, \( E^{\circ} \), of the \( \text{SO}_4^{2-}/\text{HSO}_3^- \) couple is \(-516 \text{mV}\) and thus more than 100 mV more negative that of
the $\text{H}^+/\text{H}_2$ couple (Table 1.1). Reduction of sulphate to bisulphite with $\text{H}_2$ can therefore only proceed after some input of energy. It has been shown that sulphate is first activated with $\text{ATP}$ to adenosine phosphosulphate ($\equiv \text{adenylylsulphate}$) (APS), and that the redox potential of the resulting APS/HSO$_3^-$ couple is $-60 \text{ mV}$. The reaction is catalyzed by ATP sulphurylase (Sperling et al., 1998; Taguchi et al., 2004) and consumes the energy of up to two energy-rich bonds.

$$\text{SO}_4^{2-} + \text{ATP} + 2\text{H}^+ = \text{APS} + \text{PPi} \quad \Delta G^\circ = -46 \text{kJ/mol}$$

$$\text{PPi} + \text{H}_2\text{O} = 2\text{Pi} \quad \Delta G^\circ = -21.9 \text{kJ/mol}.$$  

(1.5)  

(1.6)

*Desulfovibrio* contain an active cytoplasmic inorganic pyrophosphatase that probably catalyzes the hydrolysis of most of the inorganic pyrophosphate generated in reaction 1.5 (Kobayashi et al., 1975; Liu and Legall, 1990; Ware and Postgate, 1971; see also Weinberg et al., 2004). Based on the genome sequence of *D. vulgaris*, there is no evidence of a membrane-associated inorganic pyrophosphatase which would allow conservation of part of the energy released during pyrophosphate hydrolysis in the form of a trans-membrane electrochemical proton potential, thus reducing somewhat the energy cost of sulphate activation.

### 1.2.2 Cytoplasmic APS reduction

The sulphate activation to APS increases the redox potential of the first step in dissimilatory sulphate reduction from $-516 \text{ mV}$ to $-60 \text{ mV}$. This is well above the redox potential of the $\text{H}^+/\text{H}_2$ couple and allows the reduction with $\text{H}_2$ to proceed even at low $\text{H}_2$ concentrations. *Desulfovibrio* contain a cytoplasmic APS reductase ($\equiv \text{adenylylsulphate reductase}$) whose direct electron donor is not yet known (Fritz et al., 2002; Kremer and Hansen, 1988; Lopez-Cortes et al., 2005; Yagi and Ogata, 1996).

$$\text{APS} + 2\text{e}^- + 2\text{H}^+ = \text{HSO}_3^- + \text{AMP} \quad E^\circ = -60 \text{ mV}$$

(1.7)

### 1.2.3 Cytoplasmic bisulphite reduction

The reduction of APS to HSO$_3^-$ is followed by the reduction of HSO$_3^-$ to $\text{HS}^-$, a reaction catalyzed by a cytoplasmic bisulphite reductase (Crane et al., 1997; Friedrich, 2002; Kremer and Hansen, 1988; Larsen et al., 1999; Steger et al., 2002; Zverlov et al., 2005), whose direct electron donor has
also still to be identified. For assay of enzyme activity reduced violagen dyes are generally used.

\[
\text{HSO}_3^- + 6e^- + 6H^+ = HS^- + 3H_2O \quad E^{\text{ox}} = -116 \text{ mV} \quad (1.8a)
\]

With respect to the mechanism of this equation, there has been considerable controversy over the last 30 years, which is not yet completely resolved. One of the reasons for this is that bisulphite reductase also catalyzes reactions 1.8b and 1.8c when the \( \text{HSO}_3^- \) concentration is high and the reductant concentration is limiting (Akagi, 1995). SRB contain a thiosulphate reductase which catalyzes equation 1.9.

\[
3\text{HSO}_3^- + 2e^- + 3H^+ = S_3\text{O}_6^{2-} + 3H_2O \quad E^{\text{ox}} = -173 \text{ mV} \quad (1.8b)
\]

\[
S_3\text{O}_6^{2-} + 2e^- + H^+ = S_2\text{O}_3^{2-} + \text{HSO}_3^- \quad E^{\text{ox}} = +225 \text{ mV} \quad (1.8c)
\]

\[
S_2\text{O}_3^{2-} + 2e^- + H^+ = HS^- + \text{HSO}_3^+ \quad E^{\text{ox}} = -402 \text{ mV} \quad (1.9)
\]

Thus bisulphite reduction could proceed in three two-electron steps rather than in one six-electron step, especially when SRB are grown with bisulphite or thiosulphate rather than sulphate as terminal electron acceptor (Fitz and Cypionka, 1990; Sass et al., 1992). However, recently it has been shown that when \( D. vulgaris \) is genetically impaired in thiosulphate reduction, this does not affect its ability to grow on sulphate and \( H_2 \) (Broco et al., 2005). This is interpreted as indicating that, at least under these growth conditions, bisulphite is reduced in a single step. In the following, it is therefore assumed that APS and \( \text{HSO}_3^- \) are the only intermediary electron acceptors involved in dissimilatory sulphate reduction with \( H_2 \).

### 1.2.4 Periplasmic \( H_2 \) oxidation

\( D. vulgaris \) contains four periplasmic hydrogenases, three [NiFe]-hydrogenases and one [FeFe]-hydrogenase: of these, three hydrogenases couple with the major periplasmic poly-heme cytochrome \( c \) (TpI-c3), and one, the [NiFe]-hydrogenases 2, most probably with a second poly-heme cytochrome \( c \) (TpII-c3) (Heidelberg et al., 2004; Matias et al., 2005). When the organism is grown on \( H_2 \) and sulphate in medium depleted in nickel, only the [FeFe]-hydrogenase is synthesized without this having a noticeable effect on the growth rate (R. K. Thauer, unpublished results). Growth of \( D. vulgaris \) is also not impaired when the genes for the [FeFe]-hydrogenase (Haveman et al., 2003; Pohorelic et al., 2002) or one of the NiFe hydrogenases (Goenka et al., 2005) are deleted. These findings indicate that the four hydrogenases
can fully functionally replace each other, at least under the growth conditions employed in the laboratory where the H₂ concentration in the fermenters is kept high.

### 1.2.5 Transmembrane electron transport

Coupling of periplasmic hydrogen oxidation with cytoplasmic APS and HSO₃⁻ reduction must involve electron transport through the cytoplasmic membrane. The electron transport is most probably catalyzed by the Hmc complex, which is associated on the periplasmic side with a poly-heme cytochrome c, and on the cytoplasmic side with an iron-sulphur protein with sequence similarity to heterodisulphide reductase (Keon and Voordouw, 1996; Matias et al., 2005; Rossi et al., 1993). *D. vulgaris* deleted in the hmc genes grew normally on lactate and sulphate, but growth on H₂ and sulphate was hampered (Dolla et al., 2000; Haveman et al., 2003; Keon and Voordouw, 1996). The genome of *D. vulgaris* harbours two other polycistronic transcription units predicted to encode for transmembrane protein complexes (TpII-c₃ and Hme), associated on the periplasmic side with a cytochrome c and on the cytoplasmic side with an iron-sulphur protein, again with sequence similarity to heterodisulphide reductase (Heidelberg et al., 2004; Matias et al., 2005). The Hme genes are part of a locus that includes the genes for bisulphite reductase. The three transmembrane complexes Hmc, Hme and TpII-c₃ could have overlapping functions, in a like manner to the four periplasmic hydrogenases. In the genome, a further gene cluster for a transmembrane protein complex is found which lacks the periplasmic cytochrome c (Qmo complex) (Pires et al., 2003). There is indirect evidence that the Qmo complex is involved in APS reduction (Matias et al., 2005).

Heterodisulphide reductase from methanogens catalyzes the reduction of the heterodisulphide CoM-S-S-CoB to coenzyme M (HS-CoM) and coenzyme B (HS-CoB) (Hedderich et al., 1998). Both coenzymes are absent from SRB, and cell extracts of SRB neither catalyze the reduction of CoM-S-S-CoB nor the oxidation of CoM-SH plus CoB-SH (Mander et al., 2002; 2004). The iron-sulphur proteins in SRB with sequence similarity to heterodisulphide reductase must therefore have a different substrate specificity and/or a different function. However, since in methanogenic archaea heterodisulphide reduction links H₂ oxidation with methyl-coenzyme M reduction to methane, it is tempting to speculate that in SRB a disulphide/-SH couple might also be involved in the electron transport from H₂ to HSO₃⁻.
1.2.6 ATP synthesis and sulphate transport

As indicated above, up to two ATP equivalents are required to activate sulphate before it can be reduced to $\text{HSO}_3^-$. The reduction of $\text{HSO}_3^-$ to $\text{HS}^-$ must therefore be coupled with the phosphorylation of at least 2 mol ADP in order that the SRB can grow on $\text{H}_2$ and sulphate. Growth yield data, extrapolated to infinite growth rates, for *D. vulgaris* on $\text{H}_2$ and sulphate were 12.2 g/mol sulphate, and on $\text{H}_2$ and thiosulphate 33.5 g/mol, indicating that $\text{HSO}_3^-$ reduction with $\text{H}_2$ to $\text{HS}^-$ is coupled with the net synthesis of approximately three ATP (Badziong and Thauer, 1978). In the interpretation of these growth yield data it has to be considered that sulphate must be transported into the cells before it can be reduced and that this transport also requires energy. Available evidence indicates that sulphate is symported with three protons (Cypionka, 1987; Kreke and Cypionka, 1992), or three sodium ions (Kreke and Cypionka, 1994) which is probably equivalent to the consumption of one third or one fourth of an ATP, as discussed below.

1.2.7 Proton stoichiometries

*D. vulgaris* contains a $F_0F_1$-type proton-translocating ATPase/ATP synthase (Heidelberg et al., 2004; Hemme and Wall, 2004) of as yet unknown $H^+$ to ATP stoichiometry. Recent structural analyses of $F_0F_1$ ATPases from different organisms indicates that the $H^+$ to ATP stoichiometry may differ from organism to organism, and may be as high as five or as low as three in some organisms (Dimroth and Cook, 2004; Meier et al., 2005; Mueller, 2004; Murata et al., 2005). If the enzyme in *D. vulgaris* has a stoichiometry of three protons per ATP, then at least nine electrogenic protons are required for the synthesis of the three ATP predicted from growth yields to be formed during bisulphite reduction to $\text{HS}^-$. If the stoichiometry is five protons per ATP, then fifteen protons are required. Of these, six are generated from $\text{H}_2$ in the periplasm in a scalar reaction catalyzed by the periplasmic hydrogenases. The other protons required for ATP synthesis must therefore be generated during bisulphite reduction with $\text{H}_2$ by electrogenic proton translocation from the cytoplasm to the periplasm. Although menaquinone is the major quinone found in the cytoplasmic membrane of all SRB, its involvement in this proton translocation is unlikely since the redox potential, $E^{\circ\text{iso}}$, of the menaquinone ox/red couple is $-74 \text{ mV}$ and thus more positive than that of the $\text{HSO}_3^-/\text{HS}^-$ couple ($-116 \text{ mV}$).