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# Growth by Aerobic Oxidation of Molecular Hydrogen in *Archaea* – a Metabolic Property so far Unknown for this Domain

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## Summary

Members of the genera *Sulfolobus, Acidianus* and *Metallosphaera* were found to be able to grow chemolithoautotrophically on $H_2/O_2$. Under these conditions, the strains grew between about 0.2 and 10% $O_2$ per vol. (opt: ≈1% $O_2$). The oxidation of $H_2$ by $O_2$ was confirmed by the addition of $D_2$ as a tracer. To our knowledge, this is the first demonstration of $H_2$ oxidation by $O_2$ among the *Archaea*.

Key words: Hydrogen oxidation – *Sulfolobaceae* – *Archaea* – Thermophilic – Leaching

Chemolithoautotrophic growth by hydrogen oxidation (Knallgas reaction) occurs among many different phylogenetic groups of the bacterial domain of life (*Kaserer,* 1906; *Schlegel,* 1989; *Woese* et al., 1990). These hydrogen oxidizers have been shown to include facultative organotrophs (*Schlegel,* 1989) and the ore-leaching acidophiles *Thiobacillus ferrooxidans* and *Thiobacillus plumbophilus* (*Drobner* et al., 1990; *Drobner* et al., 1992). *Aquifex pyrophilus* is a hyperthermophilic oxidizer of molecular hydrogen and sulfur and it represents the deepest phylogenetic branch within the domain *Bacteria* (*Huber* et al., 1992).

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Within the *Archaea,* energy conservation by aerobic oxidation of molecular hydrogen has not previously been described. Members of the extremely thermoacidophilic *Sulfolobales* are able to grow by oxidation of reduced sulfur compounds (*Brock* et al., 1972; *Stetter* and *Zillig,* 1985; *Segerer* et al., 1986). In this respect they resemble members of the genus *Thiobacillus.* In order to identify possibly existing archaeal $H_2$-oxidizers we examined different members of the *Sulfolobales* for this metabolic property (Table 1). Type strains and new isolates within the genera *Acidianus, Metallosphaera* and *Sulfolobus* were precultured on suitable substrates (Table 1) under shaking (100 rev/min) in 100 ml Erlenmeyer flasks containing 30 ml of Allen's medium, adjusted to pH 2.5 (*Allen,* 1959).

Table 1. Sources and culture conditions of the strains

| Strains and sources | Temp. (°C) | Substrates | Reference |
|---|---|---|---|
| *Acidianus brierleyi* DSM 1651 | 65 | S° 2%; y.e. 0.1% | *Brierley* and *Brierley* (1973) |
| *Acidianus infernus* DSM 3191 | 75 | S° 2%; y.e. 0.1% | *Segerer* et al. (1986) |
| *Metallosphaera sedula* DSM 5348 | 65 | ore mixture "G6" | *Huber* et al. (1989) |
| *Metallosphaera sedula* strains TH4, SP3a | 65 | ore mixture "G6" | *Huber* et al. (1989) |
| *Sulfolobus acidocaldarius* DSM 639 | 65 | yeast extract 0.1% | *Brock* et al. (1972) |
| *Sulfolobus metallicus* DSM 6482 | 65 | ore mixture "G6" | *Huber* et al. (1991) |
| *Sulfolobus shibatae* DSM 5389 | 65 | yeast extract 0.1% | *Grogan* et al. (1990) |
| *Sulfolobus solfataricus* DSM 1616 | 65 | yeast extract 0.1% | *Zillig* et al. (1980) |
| *Sulfolobus* isolates Hv5, VE2, VE6, Ron12 | 65 | ore mixture "G6" | *Huber, G.* (1987) |

y.e. = yeast extract

Growth was determined directly by a Thoma counting chamber (depth: 0.02 mm). Thightly stoppered 120 ml serum bottles containing 20 ml of Allen's medium (gas phase $H_2/CO_2/air = 80:20:10$; 250 kPa) were inoculated by the precultures (3% inoculum). After 2 to 4 days incubation at 65 or 78 °C (Table 1), turbidity of the culture media resulting from cell growth was observed in the cultures of *Acidianus brierleyi, Acidianus infernus, Metallosphaera sedula* (final cell concentration: each $\approx 10^8$/ml) and of the *Sulfolobus* isolates Hv5, VE2, VE6 and Ron12 (final cell conc.: each $\approx 5 \times 10^7$/ml). *Sulfolobus acidocaldarius, Sulfolobus solfataricus* and *Sulfolobus shibatae* grew poorly (final cell conc.: $\approx 8 \times 10^6$/ml). *Sulfolobus metallicus* failed to grow under these conditions. Attempts to adapt cells by subcultivation on $H_2$ and air in the presence of decreasing amounts of S° were unsuccessful. With the exception of *Sulfolobus metallicus*, all strains were successfully transferred more than 10 times in sequence into fresh mineral medium with $H_2/CO_2/O_2$ as gas phase always yielding approximately the same final cell concentrations (not shown). This indicated that the cultures were able to grow on $H_2/O_2$ as energy source. During growth, $H_2$ was determined by gas chromatography (Hewlett-Packard 5890). Consumption of $H_2$ correlated with growth, as shown for *Metallosphaera sedula* DSM 5348 (Fig. 1). In Allen's medium, in the absence of $O_2$, all strains failed to grow (not shown). In order to prove the new metabolic property of the organisms, $H_2$ in the gas phase was replaced by $D_2$ (99.9% pure; Linde, Höllriegelskreuth, Germany). After 4 days incubation, the cultures were centrifuged and the concentration of HDO in the supernatants were determined by NMR spectroscopy (reference: $D_2O$). The results are shown in Table 2. Significant amounts of HDO had been formed (for example: 200 µmol/ml HDO in the case of *Metallosphaera sedula;* cell concentration $2 \times 10^8$ cells/ml), indicating that the organisms were $H_2$ oxidizers. The strains grew in the presence of $O_2$ concentrations from about 0.2% to 10% $O_2$ with an optimum around 0.5% (not shown). Therefore, they can be considered as microaerophilic. Since members of the deepest bacterial phylogenetic branch *(Aquifex pyrophilus)* exhibit the same type of metabolism, hydrogen-oxidation may be a rather ancient property of microbial life on Earth.

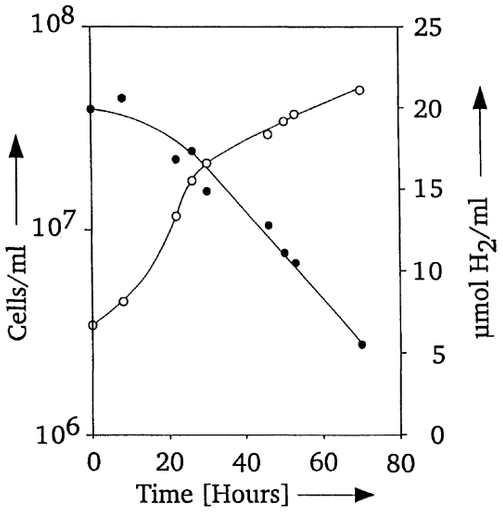


Fig. 1. Growth of *Metallosphaera sedula* by hydrogen oxidation. Symbols: ○ = cell concentration; ● = hydrogen concentration/ml cell suspension (100 ml gas phase; 250 kPa).

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Table 2. Formation of HDO/ml cell suspension in cultures grown with $D_2$ in the gas phase

| Strains | Cell densities (cells/ml) | HDO conc. (µmol/ml) |
|---|---|---|
| *Acidianus brierleyi* | $5 \times 10^7$ cells/ml | 83 |
| *Acidianus infernus* | $4 \times 10^7$ cells/ml | 75 |
| *Metallosphaera sedula* strain TH2 | $2 \times 10^8$ cells/ml | 200 |
| *Metallosphaera sedula* strains TH4 | $4 \times 10^7$ cells/ml | 73 |
| *Sulfolobus acidocaldarius* | $3 \times 10^6$ cells/ml | 2 |
| *Sulfolobus metallicus* | no growth | 0 |
| *Sulfolobus solfataricus* | $4 \times 10^6$ cells/ml | 2 |
| *Sulfolobus* isolates (VE6) | $5 \times 10^7$ cells/ml | 86 |

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