Ecophysiological aspects of the electron donor metabolism of sulfate-reducing bacteria
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SUMMARY

Sulfate reducers form an ecophysiological group of bacteria which grow at the expense of the anaerobic oxidation of organic compounds or hydrogen in processes linked to the reduction of sulfate to sulfide. This thesis mainly deals with the electron donor metabolism of these types of bacteria. The introduction of this thesis (Chapter 1) gives a survey of the properties of sulfate reducers and of their role in the anaerobic mineralization of organic matter in natural sediments. Chapters 2 and 3 deal with the lactate metabolism of Desulfovibrio HL21, a bacterium which utilizes lactate, pyruvate, ethanol, formate and hydrogen as growth substrates. At the start of the experimental work described in this thesis hardly anything was known about the biochemistry of the oxidation of lactate to pyruvate in sulfate-reducing bacteria. In Desulfovibrio HL21 an extremely oxygen-sensitive, NAD(P)-independent, membrane-bound L(+) lactate dehydrogenase was demonstrated. The synthesis of the enzyme is regulated as appears from the fact that the activities in pyruvate and hydrogen-grown cells are lower than in lactate-grown cells. The oxidation of lactate to pyruvate ($E^\prime\prime = -190 \text{ mV}$) is thermodynamically unfavourable compared with the oxidation of pyruvate to acetylCoA ($E^\prime\prime = -496 \text{ mV}$) or of $H_2$ to $H^+$ ($E^\prime\prime = -414 \text{ mV}$). Yet Desulfovibrio HL21 does not have a special preference for one of the substrates; L-lactate, pyruvate and hydrogen are utilized simultaneously. Hydrogen inhibited lactate utilization by lactate-grown cells for about 50%. This finding makes the hydrogen cycle for growth on lactate as postulated by Odom and Peck very unlikely.

Desulfovibrio propionicus is a recently isolated new type of sulfate-reducing bacterium. Besides the substrates that were mentioned for Desulfovibrio HL21, this species can also oxidize propionate to acetate and $CO_2$. In the absence of sulfate it ferments lactate, pyruvate and ethanol ($+ CO_2$) to propionate and acetate. The pathway that Desulfobulbus follows for the formation of propionate was elucidated and is described in chapter 4. Experiments with labeled ethanol as well as measurements of enzyme levels showed that the so-called succinate pathway is present in Desulfobulbus. This route shows close similarities with that of Propionibacterium. Most probably the same pathway is used for the oxidation of propionate to acetate.

The formation of poly-β-hydroxybutyrate (PHB) as a storage compound had been described for Desulfovibrio gigas and some of
the new types of sulfate reducers. In chapter 5 it is shown that also polyglucose can occur as a storage compound in certain strains of sulfate-reducing bacteria. The reserve material of *D. gigas* turned out to be polyglucose and not PHB. Some strains form polyglucose only in media with limiting amounts of phosphate, nitrogen or iron. The polyglucose of *Desulfovibrio HL21* can serve as a reserve carbon source and most likely also as a reserve energy source. The formation of polyglucose by sulfate reducers is remarkable, especially since the strains were unable to grow with glucose.

The chapters 6 to 9 refer directly or indirectly to the utilization of amino acids by sulfate-reducing bacteria. At the start of the research project only very few reports had appeared which mentioned the utilization of amino acids as energy substrates for sulfate-reducing bacteria. On the other hand, it was reported that the degradation of amino acids in freshwater sediments was inhibited for the greater part by molybdate, a specific inhibitor of sulfate reduction. The results of the experiments with molybdate can be interpreted in two ways: amino acids are either directly oxidized by sulfate reducers or are degraded by syntrophic associations of amino acid-fermenting and sulfate-reducing bacteria. It was shown in this thesis that both possibilities may occur. Chapter 6 describes the isolation and characterization of a glutamate-fermenting bacterium, which in pure cultures degrades glutamate slowly and incompletely to acetate, NH$_4^+$, hydrogen, CO$_2$ and formate. In the presence of sulfate-reducing or methanogenic bacteria (organisms which cannot grow on glutamate) a fast and complete oxidation was observed. In these cultures propionate was an additional important fermentation product. Further work showed that several amino acids, among which alanine, valine, leucine, threonine and lysine were only degraded in coculture with sulfate-reducing or methanogenic bacteria. The formation of propionate as well as the syntrophic degradation of amino acids are due to a decrease of the hydrogen partial pressure by sulfate-reducing or methanogenic bacteria.

The utilization of amino acids as energy substrates by sulfate reducers occurs, but is not a common property of these types of bacteria. In chapter 7 two marine *Desulfovibrio* strains are described which grow with alanine, serine, glycine, aspartate and some other amino acids as energy substrates. *Desulfovibrio HL21*, however, is a representative of the sulfate reducers that are not able to grow with amino acids as energy sources. In chapter 8 it is shown that this bacterium can use alanine, serine and aspartate as a source of carbon and nitro-
gen in the presence of H₂ as the energy substrate. The alanine metabolism was investigated. As it was shown that alanine is metabolized via an NAD(P) dependent alanine dehydrogenase, which leads to the formation of pyruvate, it was difficult to conceive why the strain does not grow with alanine as an energy substrate. Unfavourable kinetic properties of the alanine dehydrogenase and a substantial repression of its synthesis at physiological NH₄⁺ concentrations were identified as factors of major importance in this respect. Furthermore, growth in the presence of alanine led to an increase of the intracellular NADH/NAD ratio, from which it was concluded that a poor coupling between NADH oxidation and the process of sulfate reduction might be an additional factor contributing to the inability of the strain to grow with alanine as an energy substrate.

Finally in chapter 9 the alanine metabolism of the above mentioned marine Desulfovibrio strains and of Desulfotomaculum ruminis, a spore-forming, sulfate-reducing bacteria which is also able to grow with alanine, was investigated. In all strains alanine most probably is degraded via an NAD-dependent alanine dehydrogenase. The activities of this enzyme, however, differed strongly among the three strains. With respect to the oxidation of NADH, it appeared that in the two Desulfovibrio strains, in contrast with Desulfovibrio HL21, membrane-bound NADH dehydrogenases were present, while in D. ruminis very high activities of a cytoplasmic NADH dehydrogenase were demonstrated.