Sulfate-Reducing Bacteria and Their Activities in Oil Production

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Summary. This paper presents an overview of the microbiology of sulfate-reducing bacteria (SRB) and their detrimental effects in oil technology and summarizes a study on SRB in an oil field.

SRB are a group of specialized microorganisms that occur in aqueous environments in the absence of oxygen. The main nutrients for SRB are simple organic acids and molecular hydrogen (H_2) from decomposing natural organic matter. The nutrients are oxidized, with sulfate being reduced to sulfide (hydrogen sulfide, H_2S). The formed H_2S is the principal agent in the disastrous effects caused by SRB. It contaminates gas and stored oil, precipitates ferrous sulfide that plugs injection wells, and promotes corrosion of iron and steel in the absence of oxygen (anaerobic corrosion). Another principal mechanism by which SRB are involved in corrosion is their ability to depolarize iron surfaces by consumption of cathodically formed hydrogen. The postulated mechanisms in anaerobic corrosion are briefly explained. As an example for a microbiological study of SRB in oil technology, examination of an oil treater in a field in northern Germany is presented. On the basis of measured growth characteristics of the SRB, possibilities for controlling their activity are discussed.

Introduction

Biological sulfate reduction by SRB is the only known process by which, in aquatic environments of moderate temperatures (0 to 75°C [32 to 167°F]), H_2S is formed from sulfate. In sediments of ponds, lakes, and marine environments, SRB are usually part of the indigenous community of microorganisms and are rather inconspicuous in nonpolluted waters. In oilfield water systems, however, SRB cause serious problems: (1) corrosion of iron in the absence of air (anaerobic corrosion), (2) precipitation of amorphous ferrous sulfide that, by plugging, diminishes the injectivity of water injection wells, (3) contamination of fuel gas with H_2S, and (4) contamination of stored fuel oil with H_2S. Furthermore, H_2S is extremely toxic if inhaled; it easily escapes from contaminated waters and may accumulate under poorly ventilated conditions. It is usually recognized by its distinctive, unpleasant odor, but high concentrations anesthetize the sense of smell.

The objective of this paper is to present an overview of the biological features of SRB and of their activities in oil technology with emphasis on anaerobic corrosion. We also include results from our studies on SRB in an oil field in northern Germany.

Microbiology of SRB

SRB are an assemblage of specialized bacteria that thrive in the absence of oxygen and obtain energy for growth by oxidation of organic nutrients, with sulfate being reduced to H_2S.[1,2] The biological significance of this form of life is best understood within the overall natural decomposition process carried out by living organisms.

Processes in Biological Decomposition. The natural decomposition of organic material in our biosphere through a food chain of oxygen-breathing (respiring) organisms—namely, animals, fungi, and bacteria—is a well-known process. Biochemically, respiration is a transport of reducing power (hydrogen, "electrons") from the organic nutrients (organic substrates, electron donors) being oxidized to oxygen (electron acceptor) being reduced (Fig. 1a). Respiration liberates the energy that has been originally conserved in the organic matter during photosynthesis by green plants and cyanobacteria (blue-green algae). In the oxygen-breathing organisms, the liberated energy is used for maintenance of their living structures and for growth—i.e., a net synthesis of their own cell material from the nutrients. Thus every organic substrate of a respiring organism is partly decomposed for obtaining energy and partly converted into new cell material. These functionally distinctive reactions in living organisms are designated catabolism or dissimilation (energy metabolism) and anabolism or assimilation (cell synthesis), respectively. An amount of biomass initially synthesized by photosynthesis is diminished more and more by passing through the food chain because of respiratory losses. The final result is a reoxidation (mineralization) of the chemically complex biomass to CO_2, H_2O, and other minerals (Fig. 1a). These inorganic end products are used by green plants and cyanobacteria for photosynthesis of new organic substances (the natural cycle of matter).

The total reoxidation of biomass is possible only if the conditions are aerobic—i.e., if sufficient oxygen is present. If biomass gets into stagnant or rather closed water systems where the gas exchange with the atmosphere is limited, dissolved oxygen may be completely consumed. Despite the absence of oxygen in such waters, the organ-

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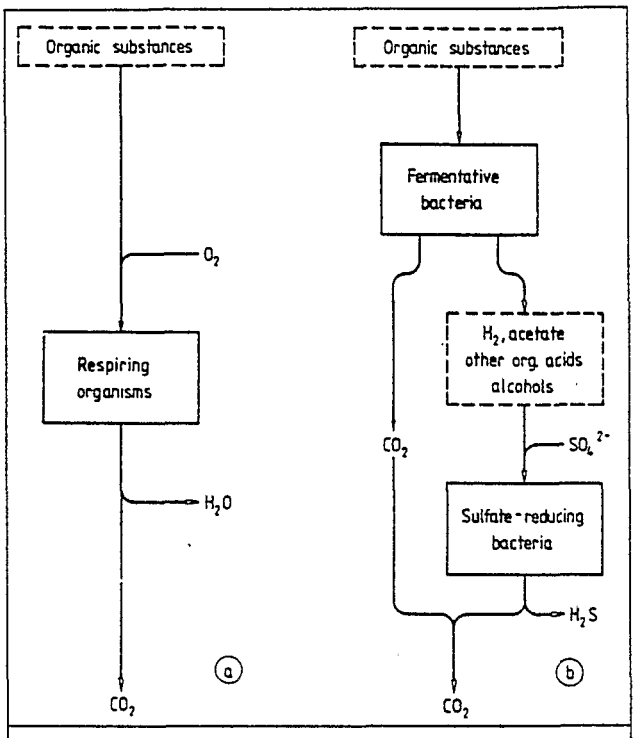

Fig. 1—Simplified scheme of (a) aerobic and (b) anaerobic biological decomposition.

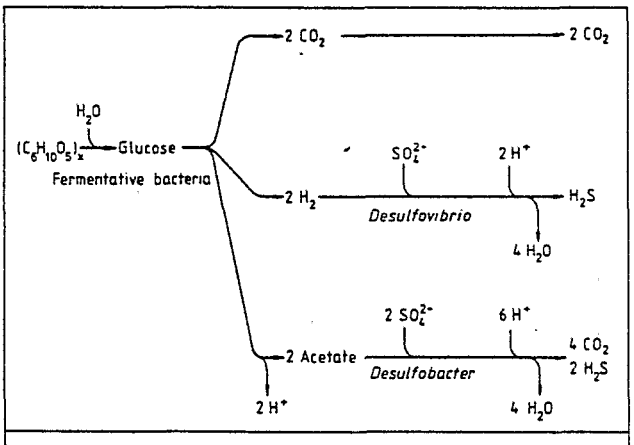

Fig. 2—Possible reactions in anaerobic cellulose decomposition.

ic matter undergoes further biological decomposition by a complex population of so-called fermentative bacteria. The conditions in the absence of oxygen and the bacteria living under these conditions are generally designated as anaerobic. Most fermentative bacteria are even obligately anaerobic and become inactive in air. Because no external electron acceptor (oxidant), such as oxygen, is used by fermentative bacteria, the overall oxidation state of the degraded matter cannot change. The degradation reactions by which most fermentative bacteria gain energy are disproportionations of the organic matter; a part of this is converted to CO_2; another part is necessarily converted to reduced products, such as fatty acids, H_2, and alcohols (Fig. 1b). In many natural anaerobic environments, the quantitatively most important fermentation products formed with CO_2 are H_2, acetate, propionate, and butyrate.[3]

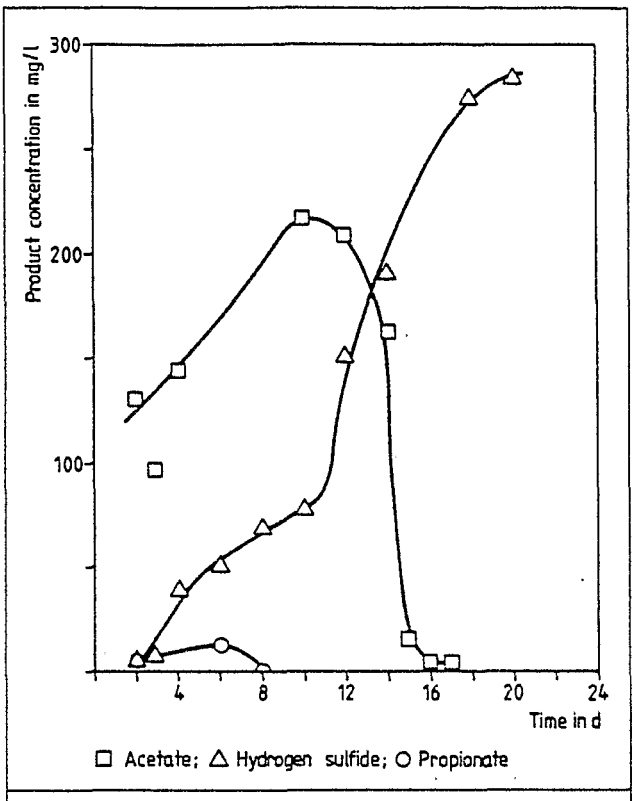

Fig. 3—Product formation during anaerobic xanthan (0.5 g/L) decomposition.

If sulfate is present under anaerobic conditions, the fermentation products are used further by SRB (Fig. 1b). Because these bacteria use an inorganic oxidized compound—sulfate—as electron acceptor in their energy metabolism, a net oxidation of the organic substrates is effected without free oxygen; the reducing power from the decomposed organic matter appears as H_2S. SRB are obligate anaerobes and become inactive in air, like most fermentative bacteria. With respect to the organic compounds used, SRB are more restricted than fermentative bacteria. Several fermentative bacteria decompose chemically complex compounds, e.g., such polymers as cellulose or proteins. SRB were never observed to use a polymer directly. Typical nutrients for SRB are simple compounds of low molecular weight, such as the indicated fermentation products. Therefore, SRB in nature depend on fermentative bacteria that cleave and ferment the complex organic matter (cellulose, starch, and other biopolymers) to low-molecular-weight compounds (Fig. 1b).

Fig. 2 is an example for detailed bacterial processes by which cellulose may be degraded completely under anaerobic conditions. Through fermentative bacteria and SRB (*Desulfovibrio* or *Desulfobacter*), each molecular cellulose unit (glucose) enables the production of three molecules of H_2S; thus 1,000 g cellulose would yield 630 g H_2S, provided sulfate is not limiting. If sulfate is limiting or absent under anaerobic conditions, the fermentation products are used by methane-forming bacteria that cooperate with some other, special anaerobic bacteria, and the degradable biomass is finally converted to methane (CH_4 or "biogas") and CO_2.[4]

An anaerobic degradation experiment with a biotechnologically produced polymer, xanthan, is shown in Fig.

Fig. 4—*Desulfovibrio vulgaris* (viable cells, phase-contrast micrograph, bar = 10 μm).

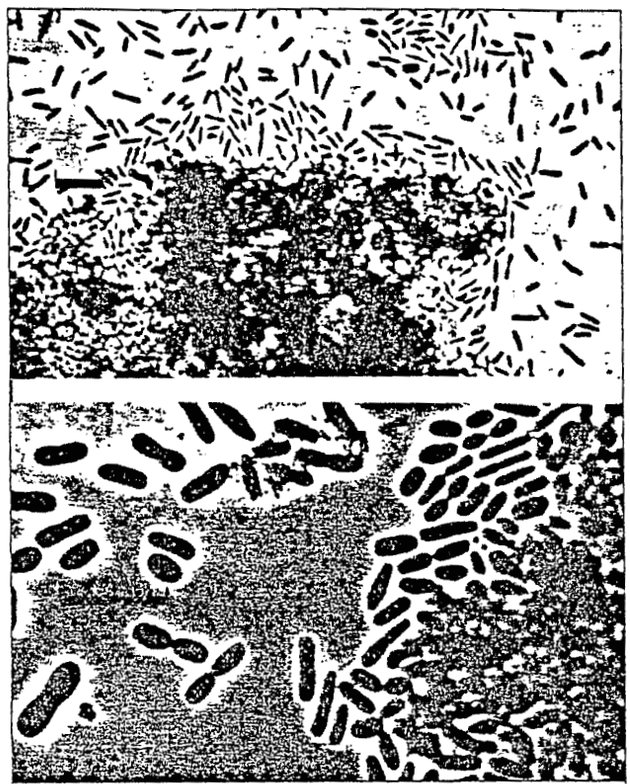

Fig. 5—A *Desulfobacter* species (viable cells at different magnifications, phase-contrast micrographs, bar = 10 μm).

3. Deaerated seawater (containing 2.7 g SO_4^{2-}/L) with 0.50 g xanthan (dry weight)/L was inoculated with some marine sediment as a source of anaerobic bacteria. By consecutive transfer into new oxygen-free seawater with the polymer, a xanthan-degrading mixed culture of fermentative bacteria and SRB was selected. The concentration of formaldehyde (0.05%) present as a biocide in the used xanthan was insufficient to inhibit these bacteria. After inoculation, fermentation of xanthan to acetate and propionate started first. Somewhat later, these products were used by SRB that formed H_2S. The viscosity caused by xanthan disappeared. In controls without xanthan, neither fatty acids nor H_2S was formed.

Apart from the sulfate reduction by SRB, the normal decay of biomass yields H_2S by liberation from proteins (desulfuration). The content of bound sulfur in living and dead organisms, however, is only about 1% (weight per dry weight). Therefore, decomposition of 1,000 g (dry weight) biomass in the absence of sulfate would yield not more than about 10 g H_2S from the proteins.

Thermodynamically, fermentation or sulfate reduction yields less energy than respiration with oxygen. This can be demonstrated, e.g., with acetate as electron donor, by calculation of the free-energy change ($\Delta G^{\circ\prime} = \Delta G^{\circ}$ at pH 7) that is a measure of the available energy.

Sulfate reduction:

$$CH_3COO^- + SO_4^{2-} + 3H^+ \rightarrow 2CO_2 + H_2S + 2H_2O; \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$\Delta G^{\circ\prime} = -41$ kJ/mol acetate.

Respiration:

$$CH_3COO^- + 2O_2 + H^+ \rightarrow 2CO_2 + 2H_2O; \quad \ldots\ldots (2)$$

$\Delta G^{\circ\prime} = -837$ kJ/mol acetate.

The different free-energy changes are approximately reflected by the cell mass synthesized per amount of a utilized substrate. Aerobic bacteria can convert 50% or more of the total substrate to cell material because they obtain much energy from respiration (Eq. 2). Fermentative bacteria or SRB convert only about 10% of the total substrate to cell material; the bulk of the substrate has to be decomposed for providing energy (in SRB, e.g., according to Eq. 1). In short, anaerobic bacteria, including SRB, make themselves conspicuous by the degradation products rather than by formed cell mass.

A consequence of the relatively low free-energy change of anaerobic reactions is that fermentation products and H_2S still carry a significant part of the energy that was conserved in the original biomass. If the bacterial products from the anaerobic environment contact air, they are energetically exploited by specialized aerobic bacteria. Among these, the H_2S-oxidizing bacteria, mainly *Thiobacillus* species, are ecologically and economically important.[5] They reoxidize H_2S to sulfate, and the environment may become acidic.

Sulfide oxidation:

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+; \quad \ldots\ldots\ldots\ldots\ldots (3)$$

$\Delta G^{\circ\prime} = -796$ kJ/mol sulfide.

TABLE 1—CHARACTERISTICS OF REPRESENTATIVE SRB

	Cell Form	Approximate Optimum Temperature for Growth (°C)	Compounds Oxidized				
			H_2	Acetate	Fatty Acids	Lactate	Others
Incomplete oxidation							
Desulfovibrio							
desulfuricans	Curved	30	+	–	–	+	Ethanol
vulgaris	Curved	30	+	–	–	+	Ethanol
gigas	Curved	30	+	–	–	+	Ethanol
salexigens	Curved	30	+	–	–	+	Ethanol
sapovorans	Curved	30	–	–	C_4 through C_{16}	+	
thermophilus	Rod-shaped	70	+	–	–	+	
Desulfotomaculum							
orientis	Rod-shaped	30 to 35	+	–	–	+	Methanol
ruminis	Rod-shaped	37	+	–	–	+	
nigrificans	Rod-shaped	55	+	–	–	+	
Desulfobulbus							
propionicus	Oval	30 to 38	+	–	C_3	+	Ethanol
Complete oxidation							
Desulfobacter							
postgatei	Oval	30	–	+	–	–	
Desulfovibrio							
baarsii	Curved	30 to 38	–	(+)	C_3 through C_{18}	–	
Desulfotomaculum							
acetoxidans	Rod-shaped	35	–	+	C_4, C_5	–	Ethanol
Desulfococcus							
multivorans	Spherical	35	–	(+)	C_3 through C_{14}	+	Ethanol, benzoate
niacini	Spherical	30	+	(+)	C_3 through C_{14}	–	Ethanol, nicotinate, glutarate
Desulfosarcina							
variabilis	Cell packets	30	+	(+)	C_3 through C_{14}	+	Ethanol, benzoate
Desulfobacterium							
phenolicum	Oval	30	–	(+)	C_4	–	Phenol, *p*-cresol, benzoate, glutarate
Desulfonema							
limicola	Filamentous	30	+	(+)	C_3 through C_{12}	+	Succinate

Symbols: + = utilized; (+) = slowly utilized; – = not utilized.

Types of SRB and Their Substrates. SRB are not homogeneous.[1,2] Properties of representatives that have been studied in detail are listed in Table 1. It is very likely that many more types of SRB occur in nature.

The cell forms of SRB most commonly found by light microscopy are curved and oval to rod-shaped; their diameters usually range from 0.5 to 2 μm, their lengths from 1 to 5 μm. Many SRB are actively motile by flagella. Other forms are spheres and long multicellular filaments. Several types of SRB tend to grow in clumps or cell aggregates and stick to surfaces.

Nutritionally, SRB may be divided into two major groups. Species of the first group carry out an incomplete oxidation of organic substrates with acetate as an end product. Species of the second group oxidize organic substrates, including acetate, completely to CO_2.

Most incompletely oxidizing SRB may grow rather fast under optimum conditions and reach doubling times of about 3 hours. The best-studied representatives are *Desulfovibrio* species (Fig. 4) that can be easily isolated from nearly every aquatic sediment.[1] For most, lactate is an excellent substrate that is oxidized to acetate and CO_2. Many *Desulfovibrio* species also grow well with H_2 as electron donor; the equation is obvious from Fig. 2.[6] If *Desulfovibrio* species grow with H_2 and sulfate as energy source, they require acetate and CO_2 as carbon sources for cell synthesis.[6] *Desulfovibrio sapovorans* and some similar, as yet unnamed SRB oxidize long-chain fatty acids to acetate.[7] *Desulfobulbus* species oxidize propionate to acetate. Most of the known spore-forming *Desulfotomaculum* species resemble nutritionally the commonly found *Desulfovibrio* species.

The completely oxidizing SRB grow relatively slowly, with optimum doubling times seldom shorter than 15 hours. The nutritionally specialized *Desulfobacter* species (Fig. 5) prefer acetate as substrate (Eq. 1), the quantitatively most important organic fermentation product; higher fatty acids are not used. Other completely oxidizing SRB (e.g., *Desulfococcus* species and *Desulfosarcina variabilis*) are nutritionally more versatile; they may oxidize propionate, higher fatty acids, dicarboxylic acids, lactate, alcohols, and even aromatic organic acids. Some of the completely oxidizing SRB can use H_2 as electron donor and synthesize cell material from CO_2 as the sole carbon source.

In nature, the completely oxidizing SRB, especially *Desulfobacter* species, may cooperate with incompletely oxidizing types by using the acetate excreted by the latter.

SRB Distribution in Nature and Their Growth Conditions. Development of SRB in nature can be expected whenever decomposable organic matter gets into sulfate-containing waters where O_2 is limited. Typical habitats are aquatic sediments where settled organic particles accumulate. Significant activities of SRB are measured in salt-marsh or marine sediments because of the high sul-

TABLE 2—PROPOSED REACTIONS IN IRON DEPOLARIZATION BY SRB	
Anodic reaction	$4Fe \rightarrow 4Fe^{2+} + 8e^-$
Water dissociation	$8H_2O \rightarrow 8H^+ + 8OH^-$
Cathodic reaction	$8H^\bullet + 8e^- \rightarrow 8H \rightarrow 4H_2$
Hydrogen oxidation	$SO_4^{2-} + 4H_2 \rightarrow H_2S + 2H_2O + 2OH^-$
Precipitation	$Fe^{2+} + H_2S \rightarrow FeS + 2H^+$
Total reaction	$4\,Fe + SO_4^{2-} + 4H_2O \rightarrow FeS + 3Fe(OH)_2 + 2OH^-$

fate concentration of seawater (28 mmol=2.7 g SO_4^{2-}/L).[8] H_2S production is often visible by blackening of the sediment, which is a result of the formation of ferrous sulfide (FeS) from iron minerals.

Despite the inhibitory effect of oxygen on SRB, these bacteria are sometimes active in aerobic aquatic sediments, where SRB thrive in anaerobic microniches. Formation and maintenance of such microniches is explained by two factors. First, the respiration of aerobic bacteria scavenges oxygen and favors growth conditions for SRB. Second, H_2S produced by SRB is a reductant that reacts with oxygen at normal temperature; thus, if once established, colonies of SRB can protect themselves against oxygen.[9] If organic matter increases, the anaerobic microniches can soon expand in a self-stimulating process.

In a homogeneously aerated environment, SRB become inactive. Nevertheless, they can survive many hours or days in aerated water.[9,10] If under anaerobic conditions again, such SRB recover their activity. SRB of the genus *Desulfotomaculum* form spores like the anaerobic fermentative *Clostridium* species. The spores are resistant not only to oxygen but also to heat (80°C [176°F]) or desiccation and are therefore present even in dry soils. The spores germinate under favorable growth conditions.

SRB prefer neutral pH for growth. In the laboratory, activity is observed within a pH range of about 5.5 to 8.5. Nevertheless, SRB have been observed in more acidic waters.[11] In environments with an unfavorable pH, SRB probably occur in microniches of more neutral conditions. The metabolic products of SRB represent buffers—namely, the HS^-/H_2S and the HCO_3^-/CO_2 systems—that may protect against extreme pH values.

The optimum temperature for most known SRB is about 20 to 40°C [68 to 104°F]. In natural aquatic sediments, low activities of sulfate reduction can still be measured close to 0°C [32°F]. Relatively few types of SRB have been described so far that prefer high temperatures (thermophilic SRB). *Desulfotomaculum nigrificans* may grow at temperatures up to 65 to 70°C [149 to 158°F], *Desulfovibrio thermophilus* and the similar *Thermodesulfobacterium commune* up to 80 to 85°C [176 to 185°F].[1] High pressures may increase or diminish the temperature tolerance of SRB.[12,13]

SRB show various reactions with respect to salt concentrations.[1,2,12] Freshwater species may be inhibited by more than 20 to 30 g NaCl/L. In contrast, many marine species are moderately halophilic; i.e., they do not develop in freshwater environments but require 10 to 30 g NaCl/L, and sometimes magnesium salts for optimum growth.[2] Halotolerant SRB grow in both freshwater and seawater environments. Activity of most SRB declines drastically if the NaCl concentration exceeds 50 to 100 g/L.[1,12] In natural saline habitats (salt lakes and brines), activity of SRB is sometimes found near salt saturation.[12] However, there are no reports on extremely halophilic SRB that really require such high salt concentrations for optimum growth. Apparently, a few SRB can tolerate rather high salt concentrations and live, though with diminished activity, near salt saturation far beyond the optimum.

Economic Activities of SRB

Considering the growth conditions of SRB, it is not surprising that these bacteria may find a suitable environment in oilfield water systems if degradable organic compounds and sulfate are present. Of course, activity can be expected only if the temperature is not significantly higher than 80°C [176°F] and if the water is not too acidic. The manifold problems caused by SRB have been reviewed in Ref. 1.

Principles of Metal Corrosion by SRB. Several mechanisms have been proposed by which different microorganisms corrode metals, and the subject has been reviewed in detail.[1,14-17] Two major biologically mediated processes by which metals corrode may be visualized. First, microorganisms may favor or initiate anodic, oxidative processes on a metal surface by direct contact. Second, excreted metabolic products may be chemically aggressive and may dissolve the metal. Both types of processes play a role in corrosion by SRB.

A principal mechanism by which SRB corrode iron is proposed by the cathodic depolarization theory first postulated in 1934 by von Wolzogen Kühr and van der Vlugt.[18] The detailed reactions are listed in Table 2. Metal becomes polarized in water by loss of positive metal ions (anodic reaction). The electrons left in the metal reduce protons from dissociation of water to atomic hydrogen (cathodic reaction). Atomic or molecular hydrogen remains on the metal surface where a dynamic equilibrium is established. SRB are supposed to remove H_2 permanently from the metal surface by oxidation with sulfate as electron acceptor (cathodic depolarization). The result is a net oxidation of the metal. Some of the metal ions react with sulfide to form FeS; others form ferrous hydroxide.

All species of SRB that are able to use H_2 (Table 1) are potentially corrosive by acting as depolarizers. *Desulfovibrio vulgaris* and SRB in marine environments were shown to have a high affinity to H_2, which can be removed down to an extremely low concentration, around 10^{-9} mol/L.[19,20] The uptake of H_2 by the cells of the SRB is always mediated by the enzyme hydrogenase.

The ability of SRB to use hydrogen from steel surfaces has been demonstrated experimentally. By application of an electromotive force to a steel and a platinum electrode in a mineral solution, Hardy[21] demonstrated an increase of the current density and a production of sulfide from

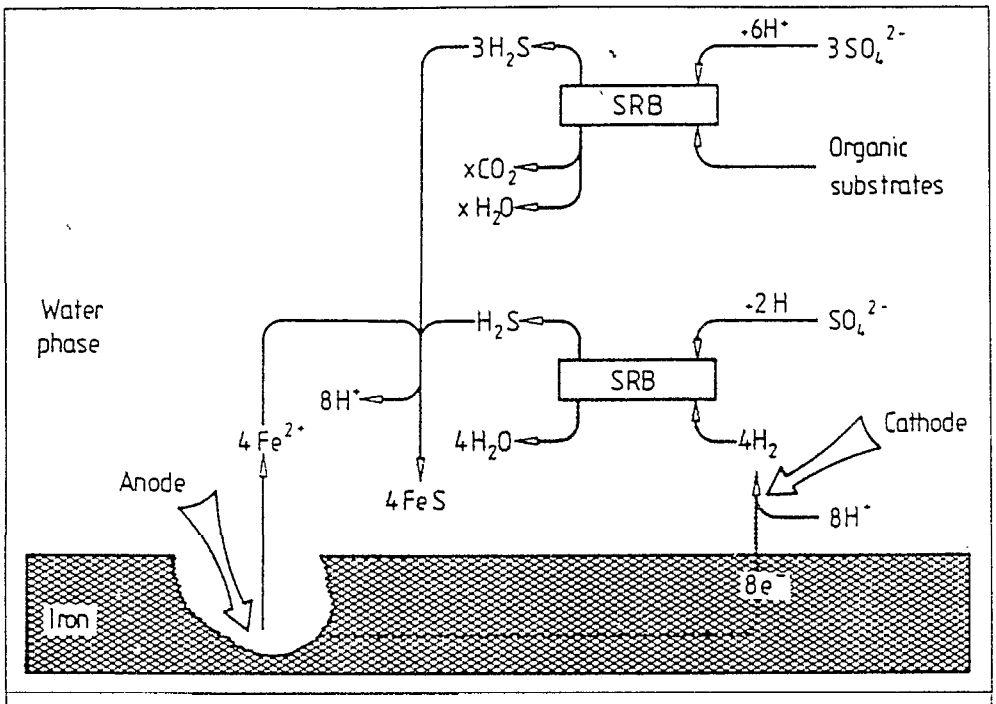

Fig. 6—Proposed main reactions in anaerobic corrosion.

sulfate when cells of hydrogenase-positive SRB (i.e., SRB able to utilize hydrogen) were added. In corrosion experiments in our own laboratory, we incubated steel wool in media of different types of SRB.[22] The quantification of formed sulfide revealed that in the presence of hydrogenase-positive *Desulfovibrio* species, the steel wool provided H_2 for sulfate reduction, indicating that corrosion occurred. In the 2-week short-term experiments, however, the utilization of hydrogen from the steel surface was observed only when organic compounds (e.g., lactate) were also present as additional substrates for sulfate reduction. In cultures of the hydrogenase-negative *Desulfovibrio sapovorans* (Table 1), the steel wool was not corroded. These results not only confirm the cathodic depolarization theory, but also demonstrate the significance of organic substrates for SRB in anaerobic corrosion. The stimulation of the corrosion by the additional organic substrates may be explained by two probably cumulative effects. First, the organic substrates stimulate the activity of the SRB and, as a consequence, also their hydrogen uptake. Second, as Postgate[1] mentioned, the additionally formed sulfide reacts with the remaining ferrous ions or hydroxide from the anodic process (Table 2) and leads to FeS as the entire corrosion product. Thermodynamically, FeS precipitation is an effective removal of ferrous iron from the anodic reaction and therefore should promote the oxidative destruction of the metal. Because the additional sulfide may be formed not only by the hydrogenase-positive, depolarizing SRB but also by hydrogenase-negative SRB, the latter are expected to contribute to corrosion.

The detailed mechanisms with their spatial separations in anaerobic corrosion are not yet fully understood. A depolarization reaction occurring merely on the iron surface was regarded as insufficient to account for the striking corrosion phenomena under anaerobic conditions. Instead, the precipitated FeS was assumed to be the main site of depolarization by acting as a cathode, like a noble metal in contact with iron.[23] Hence, the SRB remove the cathodically formed H_2 from the FeS attached to the metal. This view and our laboratory observations led to the corrosion model proposed in Fig. 6.

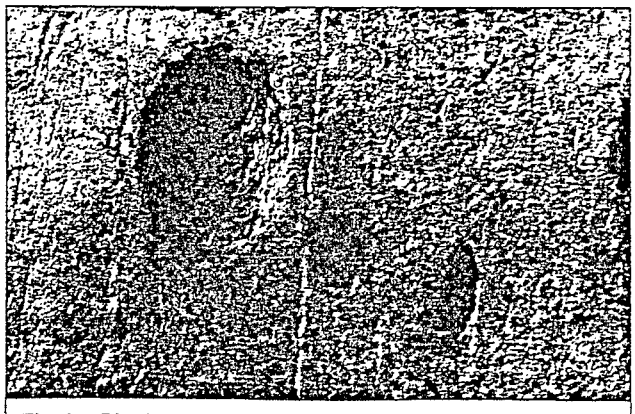

Fig. 7—Pits in anaerobically corroded iron (scanning electron micrograph, bar = 100 μm).

Fig. 8—Anaerobically corroded iron surface (scanning electron micrograph, bar = 10 μm).

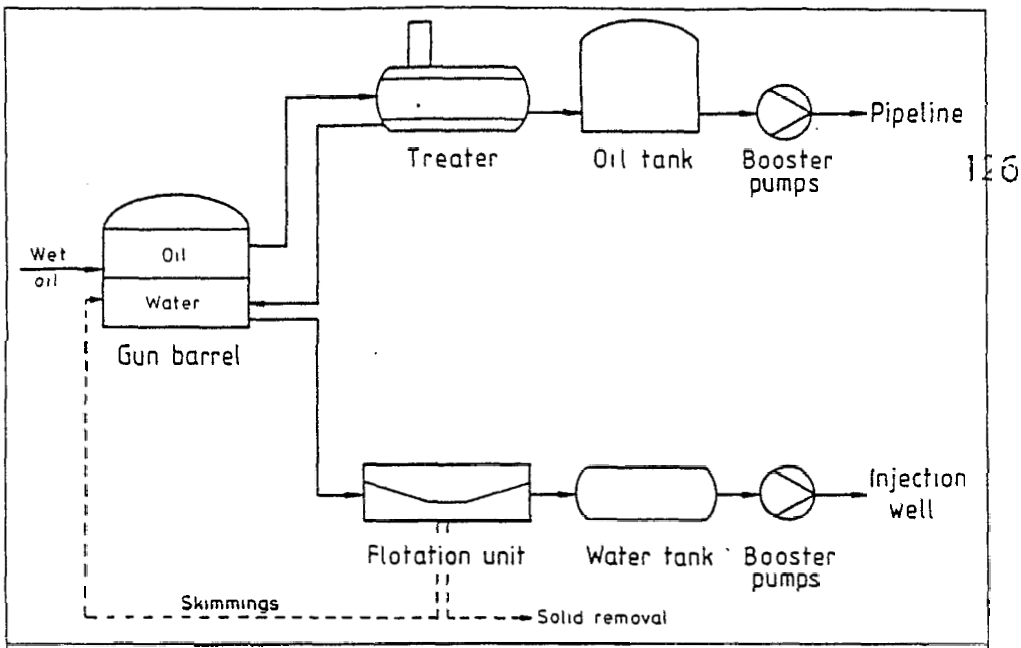

Fig. 9—Oil and water flow in the examined oil field.

In a quite different corrosion theory, the depolarization resulting from H_2 removal by SRB was questioned.[24] Instead, the depolarization was ascribed to the cathodic activity of H_2S itself, which receives electrons from the metal and yields H_2. According to this assumption, SRB act only indirectly on iron by their reactive end product, sulfide. Other studies on anaerobic corrosion furnished evidence for the involvement of a phosphorus compound as an additional reactive agent.[25]

A corrosion mechanism with indirect involvement of SRB may also occur in environments with intermittent anaerobic/aerobic conditions. Produced sulfide reacts with O_2 and yields elemental sulfur, which, with excess sulfide (especially at pH > 7), forms polysulfides. Both sulfur and polysulfides are highly corrosive.[26]

The different corrosion mechanisms proposed indicate the complexity of the whole process. On the basis of our observations, we prefer the explanation by the classic depolarization mechanism and the reaction of sulfide with ferrous ion as the two fundamental chemical processes in anaerobic corrosion. Nevertheless, other reactions may contribute to different extents to the destruction of iron, depending on the environmental conditions. Actually, the corrosion process usually takes place in a chemically and physically very inhomogeneous microenvironment. As Hamilton[17] explained, SRB and many other types of bacteria often occur in a polymeric layer (biofilm) that forms on the metal surface. Within such layers, various gradients of substrates, products, or even O_2 from the air are established. The inhomogeneity of the corrosive environment is also indicated by the pitting, rather than an even corrosion, of the iron or steel. The anodic destruction tends to continue in the same area where the process has started. The establishment of the corroding areas may be initiated by irregularities in the metal surface structure and by special sites in the aqueous surroundings that favor an anodic process. Fig. 7 shows a distinctively marked hole in steel that has been incubated for 10 months in a *Desulfovibrio* culture. The higher magnification (Fig. 8) exhibits a fissured structure of the corroded area.

Refs. 1 and 14 summarize the possibilities for controlling anaerobic corrosion. If practical, use of inert, noncorrosive material appears to be the best solution. Cathodic protection of iron by sacrificial anodes or application of an electromotive force, first used for inhibiting nonbiological corrosion, has also been successful against corrosion by SRB.[14] Although it protects the iron, this method does not inhibit SRB activity. On the contrary, the increased negative potential imposed on the iron provides further H_2 as electron donor for SRB. Generally, soil and water that are poor in biologically degradable organic matter are expected to be less corrosive than organic-rich surroundings of the metal.

Examination of SRB from an Oil Field in Northern Germany. The details of a microbiological study on SRB in an oil field in northern Germany near Hamburg have been reported elsewhere.[27] In this oil field, an increase of H_2S was observed during several years of operation. Serious corrosion effects have not been observed so far; however, formation of H_2S in the water led to plugging of the injection wells by FeS flocs. The whole flow diagram is shown in Fig. 9. Analyses of samples taken from the aqueous phase at different points revealed that the crude oil processing unit (treater) was the main source of sulfide. The operating temperature of the treater measured in the oil phase was 60 to 70°C [140 to 158°F]. The temperature in the water phase was between 30 and 45°C [86 and 113°F], depending on the crude oil throughput and on the distance from the inlet.

Water samples for studying SRB were taken near the bottom of the treater. The samples contained a sediment consisting of FeS, other precipitates, and oil particals. Microscopic examination revealed the presence of mainly oval bacterial cells, most of which were attached to the particles.

For counting cells of different SRB in the treater, a fresh sample with flocs was homogenized under an atmosphere of N_2 and diluted stepwise in test tubes with anaerobic agar media containing bicarbonate buffer, mineral salts,

TABLE 3—COUNTING AND DETERMINATION OF SRB FROM AN OIL TREATER

Substrate for Counting	Cells of SRB per mL	Cell Morphology	Substrates Oxidized: H_2	Acetate	Butyrate, Caproate	Lactate	Ethanol	Benzoate	Type of SRB
Acetate	6.3×10^6	Oval	–	+	–	–	(+)	–	*Desulfobacter* species
Butyrate + caproate	270	Oval or curved	–	–	+	–	–	–	Similar to *Desulfovibrio sapovorans*
Lactate	1.4×10^5	Curved	+	–	–	+	+	–	*Desulfovibrio* species
Benzoate	1.1×10^6	Rod-shaped	(+)	(+)	(+)	(+)	(+)	+	Unknown

Symbols: + = utilized; (+) = slowly utilized; – = not utilized.

trace elements, vitamins, sulfate, and an organic substrate; the organic substrates were acetate, lactate, benzoate, or a fatty acid mixture of butyrate and caproate.[2] After 2 to 4 weeks of incubation at 30°C [86°F], the SRB in the solidified agar had grown into colonies that were counted; for further studies and identification, the SRB colonies were isolated in pure culture in liquid media. The results are shown in Table 3. Acetate-oxidizing SRB of the *Desulfobacter* type were dominant, followed by yet unknown benzoate-utilizing rod-shaped SRB and lactate-utilizing *Desulfovibrio* species. SRB oxidizing butyrate and higher fatty acids occurred at relatively low numbers. Fig. 5 shows *Desulfobacter* cells, many of which are sticking to each other or to FeS particles added to the growth medium.

The pure cultures of SRB obtained at a temperature of 30°C [86°F] exhibited optimum growth at 30 to 36°C [86 to 97°F]; at temperatures higher than 44°C [111°F], these SRB died. If, however, the initial incubation temperature was elevated to 45°C [113°F], additional types of SRB developed and were isolated with H_2, acetate, or lactate; these SRB were active at 40 to 50°C [104 to 122°F]. One H_2-utilizing strain was thermophilic and grew between 45 and 65°C [113 and 149°F].

Growth of SRB isolated from the treater water was tested at different salt concentrations. Although the salt concentration of the treater water was about 100 g/L (mainly NaCl), the pure cultures of the SRB exhibited optimum growth with 10 to 50 g NaCl/L. With one exception, the obtained SRB were retarded at higher NaCl concentrations and inhibited above 150 g NaCl/L. The only exception was a rather slowly growing, rod-shaped type of SRB with an optimum temperature of 45 to 52°C [113 to 126°F]. This type was not retarded by NaCl concentrations up to 200 g/L and still developed slowly with 270 g/L. Lactate, fatty acids, and ethanol were used, but not H_2.

Examination of the treater shows that oilfield waters may harbor various types of SRB. We can conclude from the determined numbers of SRB cells and their nutritional capacities (Table 3) that acetate and benzoate were nutrients of potential significance in the treater. The source of these compounds is still unknown. One possibility is that acetate and benzoate are compounds of the crude oil and are enriched in the polar organic precipitates (asphaltic and resin-like crude oil components); these accumulated especially in the treater at the oil/water interface and on the bottom. Oilfield chemicals could not have contributed significantly to degradable substances; only 50 ppm of a nonionic surfactant was applied as an oil-soluble demulsifier to favor the separation of oil/water emulsion.

The acetate-oxidizing SRB that were dominant in the oil treater did not grow with lactate. Therefore, the counting techniques with lactate that are routinely applied in oil fields may significantly underestimate the real number of SRB. Further underestimation of cell numbers results from the tendency of many SRB to grow in dense clumps and to attach to particles and other surfaces (Figs. 4 and 5).

Experiments with different salt concentrations showed that no extremely halophilic SRB existed in the oil field. The detected types of SRB preferred moderate salt concentrations. In the water of the examined oil field, these SRB have to grow beyond their salt optimum at relatively high salt concentrations that apparently diminish the activity.

Some General Comments on SRB Control in Oilfield Waters

It appears unlikely that ancient SRB in the oil-bearing strata have endured geological periods. We must therefore assume that SRB are imported with surface waters or ground waters. The gradual increase of sulfide production after the beginning of operations in oil fields may reflect the multiplication and spreading of the SRB.

Sterile operation in oil fields to avoid contamination ("infection") with SRB is nearly impossible. Several biocides are known to inhibit SRB.[1,13] Application of biocides in large waterflooding projects presents problems because of the costs. Also, the environmental problems have to be considered. Inhibitory concentrations of biocides determined in laboratory studies may be insufficient in the field where strains other than those detected in the laboratory are present and where several SRB occur in protected niches. It is also possible, however, that high pressures or temperatures in oil wells increase the effectiveness of biocides.[13] Biocides may also become inactive—e.g., by adsorption to minerals or by reaction of aldehyde groups with sulfide.

The possibilities for controlling SRB in other ways than by application of biocides must not be forgotten. A more causative limitation of sulfate reduction in oil fields is the control of the biological factors that govern SRB. Such factors are the availability of organic electron donors and sulfate or the salt concentrations.

Some substrates for SRB might be organic low-molecular-weight compounds from the oil (e.g., acids) that diffuse from the precipitated polar fraction into the water phase. Removal of precipitates that harbor SRB in their interstitial water would help diminish H_2S production. Reports on a utilization by SRB of saturated hydrocarbons, the main oil constituents, are controversial.[1] No recent microbiological studies clearly demonstrate a degradation of saturated hydrocarbons under exclusively anaerobic conditions. Under intermittent aerobic/anaerobic conditions, the possibility exists that aerobic hydrocarbon-oxidizing bacteria release some intermediates (such as fatty acids) that can be oxidized by SRB.[28] Such conditions are likely in fields where oil/water emulsions are not protected against air. However, further investigation is necessary to estimate the significance of an aerobic, incomplete hydrocarbon oxidation.

Sulfate reduction in oilfield waters may also be caused by added chemicals. Every type of biologically decomposable organic matter in anaerobic waters is a potential source of substrates for reduction of sulfate to H_2S. A commonly used solvent for oilfield chemicals is methanol. It is true that the majority of known SRB do not use methanol directly (Table 1); however, methanol plus CO_2 can be converted to acetate by specialized anaerobes[29]; acetate is a substrate for many SRB. Further examples of degradable substances applied in oil fields are citric acid used as a complexing agent and xanthan applied in EOR projects. Citric acid and xanthan are not used directly by SRB but are easily fermented to suitable substrates for sulfate reduction. Even chemically synthesized substances have to be considered as precursors for sulfate reduction. Polyethyleneglycol, the hydrophilic part in molecules of nonionic tensides (surfactants), was shown to be fermented by anaerobic bacteria to acetate plus ethanol[30]; both products are excellent substrates for SRB. Control of SRB would require keeping the addition of degradable organic substances as minute as possible.

A natural limitation of the activity of SRB is given if the primary oilfield waters contain high NaCl concentrations or are poor in sulfate. If possible, such waters should not be mixed with less-saline waters or with sulfate-rich waters, respectively.

Conclusions

To control bacterial sulfate reduction in the aforementioned oil field near Hamburg, the following measures were taken and observed by chemical analyses of the water quality and by counting of SRB.

1. Removal of precipitates from oil tanks and avoidance of a re-entrance of precipitates during recycling of the separated oil.
2. Limitation of added chemicals to an oil-soluble demulgator and corrosion inhibitor; no application of biocides.
3. Removal of particles from the injection water by a flotation unit.
4. Lowering of the pH to nearly 5.0 by addition of hydrochloric acid, which also favored oil/water separation and diminished FeS precipitation.
5. Avoidance as far as possible of a dilution of the high salt concentrations that diminish SRB growth; recycling of all separated water and limitation of added fresh water to the oil replacement volume.

In the examined oil field, these measures improved the water quality significantly. Since the beginning of the measures 3 years ago, no plugging by precipitated FeS or other problems resulting from SRB have been observed.

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SI Metric Conversion Factor

°F (°F−32)/1.8 = °C

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