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Abstract:

Acidic and metal-polluted drainage water from abandoned pyrite mines affect aquatic life in several resipient watercourses in Norway. A possible role of sulfate-reducing bacteria (SRB) in treatment of acidic mine water is discussed on the basis of literature studies and own experiments. SRB can generate alkalinity, remove sulfate and precipitate metal cations effectively when given appropriate growth conditions. Pure cultures of SRB were isolated from several mining sites and characterized physiologically and immunologically. pH-tolerance and copper sensitivity are comparable to type strains of SRB. Process studies will be conducted in the near future.

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1. sulfatreduksjon
2. gruveavrenning
3. tungmetaller
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For the Administration

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Biological fixation of metals in mine drainage and ore wastes

Progress report

Oslo, 1. oct. 1992.

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Rolf T. Arnesen
Eigil R. Iversen

PREFACE

This report summarizes the activities on the project "Biological fixation of metals in mine drainage and ore wastes" (O-90090) in the period sept. 1990 - sept. 1992. The project is part of Nordic Environmental Biotechnology Programme 1990-93, funded by Nordic Industrial Fund and industrial partners (Norwegian Miners Association Research Fund (BVLI) and Løkken Mines A/S). NIVA has also supported the project (E-89540). All fundings are gratefully acknowledged.

The idea of the project is to utilize sulfate-reducing bacteria in a treatment process for acidic drainage water from mines. These anaerobic bacteria can remove sulfate, generate alkalinity and precipitate metals from contaminated water if they are given appropriate growth conditions. Hopefully, this concept can be useful for *in situ* treatment in water-filled mines as well as treatment in artificial wetlands and bioreactors.

The background for the project and results generated in the period march 1990 - sept. 1990 has been discussed in NIVA-report No. 2542 (Laake, 1991), and the reader is referred to this for details. Results have also been presented at the "Second International Conference on the Abatement of Acidic Drainage", Montreal, Canada, 16-18. sept.1991 (Arnesen et al., 1991), and in progress reports to Nordic Industrial Fund (Christensen and Kallquist, 1991)(Christensen and Laake, 1992). The practical work was done at Institute for Microbiology and Plant Physiology, University of Bergen during the first year and at NIVA since august 1991.

Torsten Kallquist was engaged as project leader until 1.oct. 1991 when Morten Laake took over. Since 1.sept. 1992, Merete Johannessen has been project leader. The microbiological studies have been conducted as part of my dr.scient (PhD)-work, with Torleiv Lien, University of Bergen, and Morten Laake, NIVA, as supervisors. I wish to thank all these persons for their contributions to the project. I am also thankful to Rolf Tore Arnesen and Eigil Rune Iversen, NIVA, for valuable discussions concerning chemical aspects.

Oslo, 2. oct. 1992.

Bjørn Christensen

CONTENTS

1.Introduction

1.1 Treatment of acid mine drainage

Acid mine drainage (AMD) is probably the most serious environmental problem facing the pyrite and coal mining industry today. AMD is formed by chemical and biological oxidation processes when sulfidic ores are exposed to water and oxygen. Characteristic features for AMD are low pH-values, high levels of sulfate and high levels of heavy metals.
Sulfidic ores are widely distributed in Norway and have been exploited for more than 350 years. These activities caused substantial pollution problems in downstream watercources. Today most of the pyrite mines have been closed, but generation of AMD continue from waste rock piles, tailings and low grade ores.

Existing technology for treatment of AMD is based on neutralization with lime or other alkaline compounds. This technique is fairly effective in raising pH and removing metals by precipitation from the drainage water. The flotation process is often based on lime addition, and a high pH effluent from this process is often used to neutralize the acid mine drainage by mixing the two flows. However, additional lime treatment is rather expensive and many abandoned mining sites will probably continue to produce AMD for hundreds of years. In addition to this, large amounts of low density sludge is produced, and this sludge should be stored properly in order to prevent leakage of heavy metals into ground water and surface water. The costs for handling this sludge may exceed the lime costs by several folds. It was recently shown that recirculation of the sludge can give a more concentrated product , thus reducing costs associated with sludge handling (Kuyucak et al., 1991).

During the last years, considerable attention has been paid to the development of alternative strategies for AMD prevention and treatment, and several approaches to the problem have been proposed.
The most obvious way to prevent bacterial oxidation of ores, waste rocks and tailings is to minimize the flow of oxygen to the acid-producing sites. Waste rock piles can be covered by clay and till (dry cover) to prevent diffusion of air, or the waste rocks and tailings can be disposed under water. Also plstic cover to prevent flowthrough of water has been successfully used. Underwater disposal in lakes seems to be efficient under given conditions, although it might be controversial in the public.

Promising results have also been achieved by the use of biological treatment systems. In principal, these systems can be designed to favour either oxidative or reductive processes. More than 400 artificial wetlands have been constructed for treatment of acid coal mine drainage in the US during the last decade (Kleinmann et al., 1991). Most of these are constructed in a way which tend to favour bacterial oxidative processes, particulary oxidation of ferrous iron and precipitation of iron hydroxides (FeOOH etc.). Additional treatment of the effluent with lime or other chemicals is usually necessary to remove net acidity and reduce the content of other metals than iron to acceptable levels. However, mine operators often find that the costs of constructing wetlands are recovered within one year through savings in chemical usage and storage pond maintenance (Kleinmann et al., 1991).

During the last years the potential benefits of anaerobic operation of artificial wetlands have been emphasized by several authors, and bench scale/pilot scale experiments have been conducted. Several anaerobic microorganisms, including nitrate-, ferric iron-, manganese- and sulfate-reducing bacteria can generate alkalinity as part of their metabolism. Among

these, SRB are particulary interesting since they also consume sulfate and cause precipitation of metals by their production of hydrogen sulfide. The use of SRB for treatment of AMD will be further discussed in this progress report.
In order to optimize the design of the wetlands for anaerobic processes, various physical arrangements including upflow and downflow systems have been evaluated (Wildemann et al., 1990). Different carbon amendments have also been assessed and it has been found that spent mushroom compost is well suited as a source of organic material and solid matrix to support growth of SRB and retain precipitates.
The results obtained so far is promising. pH can be raised by 4-5 units to circumneutral values, metals are removed effectively and effluent water may contain net alkalinity. Bacterial sulfate-reduction have been identified as the most important process in these systems by several workers.

Treatment of AMD in anaerobic bioreactors is also an interesting concept. Use of bench scale packed bed reactors was reported by Maree and Strydom (1985, 1987). Using molasses and other carbon sources to support growth of a SRB-population, it was shown that significant amounts of sulfate and metals could be removed. Hammack and Edenborn (1992) have recently shown that nickel can be effectively removed from artificial mine water in bench scale experiments. Columns packed with mushroom compost were used as simple model reactors and lactate was provided as an additional carbon-source. Barrels and tanks filled with spent mushroom compost were used to treat AMD in pilot scale at two locations in Pennsylvania, US. Concentrations of Al, Cd, Fe, Mn, Ni and Zn were typically lowered by over 95 % in these systems, and effluent water contained net alkalinity (Dvorak et al., 1992).

As far as we know, publications on *in situ*-treatment of mine water ii water-filled mines has not been recognized. The idea is to stimulate bacterial sulfate-reduction, for example by adding organic material into water-filled open pits and underground mines.

In summary, three different concepts have been proposed for treatment of acidic, metal-contaminated water based on bacterial sulfate-reduction; in situ precipitation in water-filled mines and open pits, treatment in anoxic artificial wetlands and bioreactor treatment.

1.2.Physiology and ecology of sulfate-reducing bacteria

Sulfate-reducing bacteria gain energy by coupling oxidation of hydrogen or organic compounds to dissimilatory reduction of sulfate. The substrates can be oxidized completely to CO_2 or incompletely to acetate and CO_2.
In general, the dissimilatory sulfate-reduction process can be described as follows:

$$2CH_2O + SO_4^{2-} = 2HCO_3^- + H_2S$$

Autotrophic species able to grow on H_2 and CO_2 have been identified during the last years (Widdel, 1988). In the absence of sulfate, most species are able to grow fermentatively on pyruvate. Some species are able to obtain energy by disproportion of sulfite and thiosulfate. Twelve genera of SRB have been reported so far, including both eubacteria and archaebacteria. Growth of SRB have been shown to occur between 0 and 92°C

SRB have always been regarded as strict anaerobes. However, it has recently been shown that several SRB-species are capable of aerobic respiration under microaerophilic conditions (Dilling and Cypionka, 1990). A number of inorganic and organic compounds were utilized as electron donors (Danneberg et al., 1992). ATP was produced, but no growth could be found. It is believed that the oxygen consumption might be important for survival in oxygenated water and soil, and also for colonization of new habitates. Particle-associated SRB are more protected against oxygen than free-living ones (Fukui and Takii, 1990)

Pure cultures of acidophilic SRB have not been obtained so far (Guyre et al., 1990). However, it has been reported by several authors that dissimilatory sulfate reduction takes place in strongly acidified natural habitates (pH 2,0) and in mixed laboratory cultures at pH 3,0-4,0.
Sulfate-reducers can generate alkalinity according to the formula above and will therefore tend to increase pH in their growth habitate. This alkalinization is particulary evident if both CO_2 and H_2S escape from the water phase as gases. If metal ions are present in the water phase, H_2S may form insoluble precipitates, thus releasing protons. In such cases the net pH-effect depends on the balance between the bicarbonate alkalinity produced and the amount of protons released.

In anoxic habitates, the sulfate-reducers play a crucial role in the mineralization of organic material along with the methanogenic bacteria. These two processes are competitive, but dissimilatory sulfate-reduction tend to dominate in habitates where sulfate is available. This is mainly due to the fact that the sulfate-reducers have a greater affinity for common electron donors than methanogenic bacteria.

2.Sampling programme and chemical analyses

Løkken mine has been most extensively studied. Since this mine was shut down some years ago, one part (Wallenberg mine) has been gradually filled with water. Samples were taken as depth profiles in the Wallenberg shaft twice a year during this period and analysed for chemical parameters as part of a monitoring programme conducted by NIVA. The analyses showed that water quality improved significantly with time. It was difficult to explain the improvement in water quality by dilution-effects and chemical precipitations alone, and this lead to the hypothesis that sulfate-reducing bacteria were active in the mine and caused precipitation of metal-sulfides. Since 1990, samples were also collected for enrichment of bacteria and counting of bacterial numbers.

In order to isolate a representative choice of SRB-strains from Norwegian pyrite mines, samples have been collected at 14 mining sites geographically scattered over a large part of the country (Tab.1). Both watersamples and sediment samples from habitates receiving AMD were collected. Samples were brought to the laboratory in sterile, sealed bottles and sediment core samplers for enrichment of SRB. Samples were collected in separate bottles for chemical analyses.

The sampling programme is summarized in tab.1.

Location	Date	Samples collected
Løkken	06.03.90	Mine water
"	26.10.90	"
"	23.05.91	"
"	28.11.91	Mine water/sediments
"	18.03.92	"
Raubekken stream	28.11.91	Sediments
Stordø	25.02.91	Drainage/sediments
Vigsnes	26.02.91	Mine water
Lake Vigsnesvann	26.02.91	Lake water/sediments
Sulitjelma	18.05.91	Drainage/sediments
Bleikvassli	19.05.91	"
Joma	20.05.91	"
Skorovass	20.05.91	"
Kongen	21.05.91	Drainage
Storwartz	21.05.91	"
Orvdalen	21.05.91	Drainage/sediments
Sextus	21.05.91	"
Folldal	22.05.91	"
N. Geiteryggen	22.05.91	"

Tab.1: Sampling programme for enrichment of SRB and chemical analyses from norwegian pyrite mines.

Samples have been analysed for the following parameters: pH, conductivity, temperature, sulfate, calcium, magnesium, iron, copper, zink, total organic carbon (TOC), total nitrogen (TOT-N), ammonium (NH_4-N), total phosphorus (TOT-P) and phosphate (PO_4-P). Results are shown in tab.2-4.

During the last year Løkken Mine A/S has made efforts to reduce pollution from the mining area at Løkken. The concept is to utilize the water-filled part of the mine (6 mill. m^3) as a natural "treatment plant". A map of the mine is shown by Arnesen et. al., (1991). Acidic drainage water enriched with heavy metals is now collected in ditches from the entire area and pumped back into the mine through Fearnley shaft. The water level in the system is controlled by a pump in the Wallenberg shaft. The pumping of water out of the Wallenberg shaft started in april this year. Since the water quality in the upper part of Wallenberg shaft is fairly good, no further treatment of the outlet is planned at the moment. However, water quality in the outlet will be surveyed carefully, and treatment may be necessary in the future.

Wallenberg sjakt 1991

Dato	Prøve-nivå	Vann-stand	Temp gr.C	pH	Kond mS/m	SO4 mg/l	Ca mg/l	Mg mg/l	Al mg/l	Fe mg/l	Cu mg/l	Zn mg/l	Cd ug/l
07.01.91	178.7	178.7		6.25							0.25	1.5	
01.02.91	177.2	177.2								0.2	0.10	1.2	
01.03.91	176.1	176.1								0.7	0.13	1.3	
02.04.91	174.8	174.8								0.6	0.12	1.1	
03.05.91	173.2	173.2								0.4	0.13	1.1	
23.05.91	171.6	171.6	16.4	6.85	89.8	308	124	22.3	0.1	1.6	0.13	1.2	4
23.05.91	200.0		16.5	6.99	94.2	318	124	22.8	0.1	0.5	0.13	1.1	4
23.05.91	300.0		16.8	6.01	613.0	4420	484	730.0	0.8	242.0	0.49	32.8	35
23.05.91	340.0		16.2	5.72	652.0	5340	450	840.0	1.0	345.0	1.75	79.0	130
23.05.91	380.0		15.5	5.33	855.0	10700	413	1350.0	4.4	1880.0	0.78	294.0	70
23.05.91	430.0		16.2	4.52	2641.0	51800	465	2670.0	323.0	17700.0	0.35	2758.0	190
23.05.91	490.0		14.4	4.29	2856.0	56000	475	2520.0	708.0	19800.0	0.42	2994.0	150
01.07.91	169.5	169.5		6.11	81.7					2.4	0.71	3.8	
05.08.91	166.9	166.9									0.14	1.7	
02.09.91	165.6	165.6								0.4	0.15	1.7	6
01.10.91	163.4	163.4								0.9	0.16	1.8	
01.11.91	161.7	161.7								3.0	0.17	1.8	
28.11.91	165.0	158.8	13.6	6.61	89.1	333	155	23.2		19.0	1.36	1.8	
28.11.91	195.0		13.1	6.78	82.6	327	147	24.1		3.9	0.55	1.7	
28.11.91	200.0		16.0	5.49	401.0	3595	550	409.0		265.0	2.45	67.7	80
28.11.91	205.0		13.7	6.49	91.0	330	152	26.2		4.6	0.32	2.1	
28.11.91	295.0		16.9	6.05	502.0	4404	551	787.0		256.0	0.18	31.5	
28.11.91	300.0		16.9	5.95	549.0	4494	552	793.0		255.0	0.15	31.7	
28.11.91	305.0		16.3	5.96	503.0	4524	555	802.0		286.0	0.11	36.3	
28.11.91	335.0		16.8	5.98	483.0	4404	536	775.0		261.0	0.05	29.6	
28.11.91	340.0		16.7	5.95	576.0	4764	563	1000.0		125.0	0.13	15.0	
28.11.91	345.0		16.8	5.96	550.0	4404	540	783.0		260.0	0.15	29.5	
28.11.91	375.0		16.8	5.87	5930.0	4943	565	992.0		180.0	0.29	20.8	
28.11.91	380.0		16.4	5.39	600.0	6112	490	997.0		550.0	0.03	72.3	
28.11.91	385.0		16.1	5.35	661.0	6621	523	1070.0		638.0	0.03	52.7	
28.11.91	430.0		16.1	4.09	1806.0	31460	379	1850.0		10400.0	0.90	1623.0	
28.11.91	490.0			4.23	2409.0	42840	390	2170.0		14500.0	3.74	2220.0	100

Tab.2: Chemical data from the Løkken mine

Water sample	pH	Cond. mS/m	Sulfate mg/l	Cu mg/l	Fe mg/l	Zn mg/l	Cd ug/l	Total N mg/l	Total P ug/l	TOC mg/l
Sulis 1	2,43	495	4340	100	589	57	130	0,4	600	1,8
Sulis 2	2,43	500	4160	106	629	58	140	0,5	640	2,0
Sulis 3	2,45	499	4275	-	-	-	-	0,5	8	2,0
Skorovass	2,73	215	1390	19,3	328	57	120	0,4	270	1,8
Folld.1 (stoll)	2,61	912	11700	170	2510	137	420	1,7	950	12,4
Folld.2(stream)	2,63	488	4629	77	662	74	230	0,5	700	6,3
Kongen	-	-	-	-	-	-	-	1,1	2	2,3
Storwartz	-	-	-	-	-	-	-	0,3	1030	1,3
Fearnley	5,00	410	3375	12,5	187	72	180	0,8	1	3,1
Stordø 1	2,94	167	1050	0,12	166	1,1	1,5	1,1	83	1,6
Stordø 2	2,60	235	1820	0,44	486	1,6	3,2	1,1	960	1,7
Stordø 3	6,12	213	1330	0,006	138	0,3	0,6	1,4	5	0,2
Stordø 4	2,71	177	980	0,28	242	0,9	2,1	0,5	680	1,0
Vigsnes (lake)	4,98	24,4	69	-	-	-	-	2,0	13	1,3
Jacobsbakken	2,95	104	355	0,51	62	3,7	5,0	0,2	230	0,4

Table 3: Chemical data from Norwegian mining sites. Samples were taken in february 1991 (Stordø and Vigsnes) and in may 1991 (all other sites) from surface water inside or right outside the mines.

Water sample (depths, m)	pH	Cond. mS/m	Sulfate mg/l	Cu mg/l	Fe mg/l	Zn mg/l	Cd ug/l	Total N mg/l	Total P ug/l	TOC mg/l
10	3,29	152	750	7,6	13,4	24,0	37,5	8,9	25	1,4
30	4,67	219	1405	2,3	66,9	36,1	35,3	3,2	6	2,4
50	5,26	240	1680	2,8	55,1	38,1	39,4	3,5	6	1,9
70	5,85	269	1770	0,3	82,8	21,2	16,6	3,8	9	2,1
90	4,43	299	2170	5,4	261,0	71,0	58,4	4,4	16	2,8
110	4,30	298	2150	6,8	265,0	76,0	62,6	3,2	17	2,9
120	3,19	173	935	9,0	31,4	32,4	47,3	3,5	100	4,3
130	5,76	263	1800	0,7	92,0	22,7	19,3	5,6	300	2,9
140	3,58	389	3090	26,3	535,0	15,1	330,0	2,6	1110	3,2
170	3,54	401	3190	26,5	517,0	15,1	330,0	2,0	41	1,4

Tab. 4: Chemical data from Vigsnes Coppermine. Water samples were collected in a vertical, water-filled shaft at february 26. 1991.

Results of chemical analyses can be briefly summarized as follows:

- Sulfate is always present in excess in mine waters..

- The levels of metals are variable, and depends strongly on the composition of the ore.

- pH is generally very low in surface water collected inside and right outside mines, but tend to be somewhat higher in water-filled mines.

- Water quality may be highly variable in water-filled mines as shown by data from Løkken (tab.2). A stable situation have been established in this mine with heavily polluted water with high density in the bottom layers, and much less affected water in the surface layers.

3.Enrichment and isolation of pure cultures.

Enrichment cultures were set up in 10 ml-serum bottles, containing 5 ml growth medium under an atmosphere of argon/carbondioxid (80/20). Widdels freshwater medium (WF) for SRB (Widdel & Phennig, 1981), supplemented with trace elements (SL-10) and vitamins was used as basis for all the enrichments. Three different pH-values have been used in the enrichment cultures; pH:7,0, pH:4,8 and pH:4,2. At pH:7,0, the following substrates were used: Na-lactate (20 mM), Na-pyruvate (20 mM), Na-acetate (20 mM), Na-butyrate (10 mM), ethanol (20mM) and Na-formate (30 mM)+Na-acetate (2 mM). At the lower pH-values, ethanol (20 mM) was used as growth substrate.
Growth and sulfide production was observed in enrichment cultures from nearly all sampling sites.

Growing cultures were transferred to fresh medium 2-3 times and diluted in melted agar to obtain pure cultures. Approximately 25 strains of SRB, which all grow in neutral growth medium, have been isolated. Good growth was also observed in several of the acidic enrichment cultures. Large, straight rods dominated and sulfide were produced, indicating that SRB were present. However, the growth in these cultures stopped after one or two tranfers, and no pure cultures of acid-tolerant SRB has been obtained so far.

4.Characterization of strains

4.1.Morphology

Strain	Genus	Cell form	Endospores	Cell size	Motility
L-490	nd	Flexible rod	-	0,6-0,8#5,0-8,0	+
L-200	Desulfovibrio	Vibrio	-	0,8-1,0#2,5-4,0	+
L-380	-------- " --------	--- " ---	-	0,7-0,9#3,0-10	+
L-145	D. maculum	Straight rod	+	nd	+
Su-2	nd	Flexible rod	-	0,6-0,8#4,0-7,0	+
St-31	Desulfovibrio	Small vibrio	-	0,4-0,6#2,0-4,0	+
St-32	-------- " --------	Vibrio	-	nd	+
Vv-FA	-------- " --------	--- " ---	-	0,8-1,0#2,0-5,0	+
Ng-2	-------- " --------	--- " ---	-	0,6-0,8#2nd,5-4,0	+
Rb-1	-------- " --------	--- " ---	-	0,8-1,0#2,5-3,5	+
Rb-e	-------- " --------	--- " ---	-	nd	+
F-2	nd	Flexible rod	-	nd	+

Tab.5: Morphology of SRB-strains isolated from norwegian pyrite mines, as observed by phase contrast microscopy. nd=not determined.

4.2.Substrates utilized

Growth of SRB-strains on different substrates were tested according to table 2. The experiments were conducted in Hungate-tubes with bicarbonate-buffered Widdels freshwater medium supplemented with vitamins (Widdel, 1980). Tubes were inoculated with SRB in late exponsial phase so that the starting concentrations were1-2 10^7 SRB per ml, and incubated in darkness at 20^{o}C.
Growth on hydrogen and carbondioxid was tested in anaerobic culturing tubes (Bellco). A mixture of 80 % hydrogen and 20 % carbondioxid was used, and the tubes were incubated in a horisontal position to allow maximum exchange of gas between the liquid phase and the headspace volume. The gas mixture was replenished at least every second day in tubes with actively growing bacteria.

Substrate	L-490	L-200	L-380	L-145	Su-2	St-31	St-32	Vv-Fa	Ng-2	Rb-1	Rb-e	F-2
H_2+CO_2	-	-	-	nd	-	-	-	-	-	-	nd	-
H_2+CO_2+ac	-	+	+	+	-	+	+	+	+	+	+	-
Formate	-	(+)	(+)	nd	-	nd	(+)	(+)	(+)	(+)	(+)	-
Formate+ac	-	+	+	nd	-	nd	+	+	+	+	(+)	-
Lactate	+	+	+	+	+	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+	+	+	+	+	+
Methanol	-	-	-	-	-	-	-	-	-	-	-	-
Methanol+ac	nd	-	-	-	-	-	-	-	-	-	-	-
Ethanol	-	+	+	-	-	-	+	+	+	+	+	-
1-butanol	-	-	+	-	-	-	-	-	+	+	+	-
Glycerol	nd	-	-	+	+	-	-	-	-	-	-	-
Acetate	-	-	-	-	-	-	-	-	-	-	-	-
Propionate	-	-	-	-	-	-	-	-	-	-	-	-
1-butyrate	-	-	-	-	-	-	-	-	-	-	-	-
Fumarate	nd	-	-	-	-	-	-	-	-	+	-	-
Succinate	-	-	-	-	-	-	-	-	-	-	-	-
Malate	-	-	-	-	-	-	-	-	-	+	-	-
Benzoate	-	-	-	-	-	-	-	-	-	-	-	-
Citrate	nd	-	-	-	-	-	-	-	-	-	-	-
Alanine	-	-	-	-	-	-	-	-	-	-	-	-
Glutamate	nd	-	-	-	-	-	-	-	-	-	-	-
Glucose	-	-	-	-	-	-	-	-	-	-	-	-
Fructose	nd	-	-	-	-	-	-	-	-	-	-	-
Lactose	nd	-	-	-	-	-	-	-	-	-	-	-

Tab.6: Substrates utilized by SRB-strains in growth medium containing sulfate, bicarbonate and vitamins. nd=not determined, (+)=poor growth, ac=acetate.

The flexible rods (L-490, Su-2 and F-2) utilize an extremely narrow range of substrates, including only lactate and pyruvate. The Desulfovibrio-strains (L-200, L-380, St-32, Vv-FA, Ng-2 Rb-1 and Rb-e) utilize the typical "Desulfovibrio-substrates" lactate, pyruvate and ethanol. $H_2 + CO_2$ and formate are utilized if acetate is provided as an additional carbon source. No strains have been shown to grow autotrophically or by disproportion of sulfite/thiosulfate.

Sulfide production differ significantly from growth substrate to growth substrate. This should be kept in mind when a treatment process based on sulfide precipitaion is considered. Only small amounts of sulfide is produced by SRB growing on pyruvate. This is probably because pyruvate has a "degree of reduction" close to that of biomass, which means that the energy demand in the conversion of substrate to biomass is limited. Therefore, a relatively small part of the pyruvate is consumed in the energy-generating, respiratory reaction leading to the production of sulfide. In contrast, much more sulfide is generated when the bacteria are grown with hydrogen or formate as electron donors. Intermediate amounts are produced by ethanol and lactate-oxidizing cultures.

Fermentative growth of the SRB-strains were tested in Widdels freshwater medium without sulfate or any other electron acceptor on the following substrates: pyruvate, lactate, fumarate,

malate, alanine, glucose, fructose, lactose and glycerol. Pyruvate was utilized by all strains tested, while lactate allowed poor growth of most strains. Glycerol was fermented by strains Su-2 and L-145 and fumarate by strain Rb-1.

4.3.Mixed cultures

Mixed cultures of fermentative bacteria and SRB growing on lactose have been obtained from Wallenberg shaft as well as from Raubekken. The Wallenberg-culture consisted of a straight rod and a typical Desulfovibrio, while a coccoid strain and a Desulfovibrio constituted the Raubekken-culture. The coccoid (Rb-k) was isolated as a pure culture, and substrate utilization was tested. It grows extremely well on lactose, glucose and fructose, and also on malate and pyruvate. Ethanol has been detected as a fermentation product by use of Gas Chromatography, and it might also be hypothesed that lactate is produced. Both etanol and lactate are excellent substrates for the SRB-partner.
Such mixed cultures are particulary attractive as model systems for use in prosess studies since they can mineralize more complex compounds than pure cultures of SRB.

4.4.Temperature

Most sulfate-reducing bacteria are mesophilic with temperature optima at 30-35^{o}C. Truely cryophilic species have never been isolated (Widdel, 1988). The effect of temperature on bacterial sulfate-reduction rate can within certain limits be described by the Arrhenius equation. Q_{10}-values between 2,0 and 3,9 have been reported in marine sediments by several authors (Widdel, 1988).

All SRB-strains isolated, are routinely cultured at room temperature. Growth of strain L-200 has been measured at temperatures ranging from 0,5^{o}C to 22,0^{o}C. The apparatus used was an aluminium-block which was heated in one end and cooled in the other and allowed testing of growth at 10 different temperatures simultanously. Hungate-tubes with Widdels freshwater medium and 20 mM lactate were inoculated with an actively growing culture of strain L-200 and incubated in the glycerol-filled holes in the aluminium block in duplicate for 18 days. Growth was measured spectrophotometrically at 600 nm using a Spectronic 21. The temperature variations in the gradient was approximately +/- 0,5^{o}C during the experiment. Results are shown in fig.1.

L-200 grew well at 22^{o}C. At lower tempertures growth ceased gradually, but was measurable even at 3^{o}C (not shown). Microscopical examination at the end of the experiment revealed actively swimming cells down to 5^{o}C. No growth was observed at 0,5^{o}C. The temperature in the water-filled Løkken mine is kept relatively constant at 13-17^{o}C throughout the year, indicating that strain L-200 should be able to grow fairly well. However, low temperatures during the winter season might be an important obstacle to a successful utilization of the sulfate-reduction process in outdoor wetland systems in our part of the world.

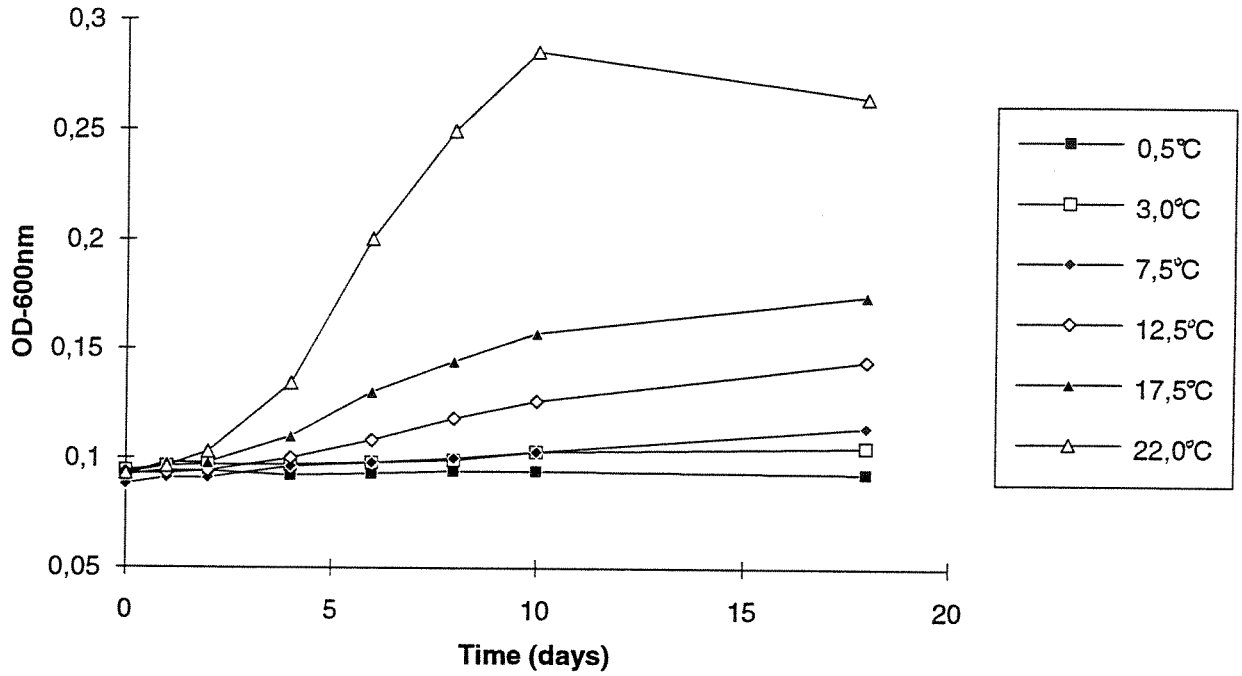

Fig. 1: Growth of strain L-200 at low temperatures. OD=optical density.

4.5.Growth factors

The SRB-isolates are routinely cultured in a medium containing a mixture of 6 vitamins (Widdel, 1980). To see whether these vitamins are required for growth, isolates are now being cultured in medium lacking vitamins. If good growth is observed after three transfers, it is concluded that no obligate vitamin requirements exists. So far, the two Desulfovibrio-strains L-200 and Rb-e, and the fermentative strain Rb-k, have been shown to grow well without vitamins. Other strains are currently being tested.

In a treatment process it is important to avoid costly amendments, and vitamins or other growth factors should therefore not be required by the active microflora..

4.6.Copper sensitivity

Several heavy metals are known to be toxic to microorganisms. Copper is extensively used to prevent undesired growth of bacteria, but it is also an essential micro-nutrient. Some bacterial strains have acquired resistance mechanisms which make them able to cope with elevated levels of this metal. This is true for strains of E.coli, Pseudomonas syringae, Mycobacterium scrofulaceum, Vibrio alginolyticus and possibly others. The mechanisms include intracellular

and extracellular complexation, precipitation and energy-requiring efflux-mechanisms. The active compounds are often plasmid-encoded.

Cu^{2+} is the most toxic form of copper. However, this ion may form soluble complexes with inorganic and organic compounds which are much less toxic. In marine environment it has been shown by several authors that approximately 1 % of the total Cu is present as Cu^{2+}. Most of the remaining 99 % is usually bound in organic complexes and can therefore be regarded as nontoxic to microorganisms.

Somewhat conflicting results have been reported regarding the copper tolerance of SRB. According to Widdel et al. (1987), 0,1 μM Cu^{2+} in the growth medium strongly inhibited growth of Desulfobacter, strain AcKo. On the other hand mine water containing 100 mM Cu^{2+} did not significantly affect sulfate-reduction rates in bench scale treatment experiments as reported by Reynolds et al (1991). Hammack and Edenborn (1992) found that SRB are moderately tolerant to Cu^{2+} and other metal ions. The great differences can probably be attributed to differences in metal speciation and presence/absence of solid phases in the culturing vessels. Once established in a sediment, it seems clear that SRB can take advantage of their ability to produce sulfide and precipitate CuS ($K_{sp}(CuS) = 10^{-38}$). This might be an important mechanism by which SRB survive in strongly Cu-polluted habitates. As far as we know, SRB-strains with specific mechanisms for Cu-resistance has not been found.

As already discussed, it is difficult to compare results from experiments made in different laboratories under different growth conditions. It should therefore be emphasized that it is extremely important to define the growth medium carefully and to interpret the results with care.

Several SRB-strains are currently being tested for Cu-sensitivity and compared to selected type strains (Desulfovibrio vulgaris, Desulfovibrio gigas, Desulfotomaculum orientis) in our laboratory. Growth is tested in medium with and without sulfate to see whether the sulfate reduction process influence sensitivity.
In preliminary experiments it was observed that addition of Cu^{2+} as anoxic $CuCl_2$-solution raised the redox-potensial in the growth medium significantly. The medium was initially reduced by addition of Na-dithionite to a final concentration of 0,3 mM. It was necessary to add approximately 1 mM of extra Na-dithionite to restore the initial redox-potensial after addition of 1 mM $CuCl_2$. Typical results are shown in fig.2. The difference in maximum bacterial counts between respiratory and fermentatively grown cultures is probably due to the extra energy made available by respiration compared to fermentation of pyruvate.

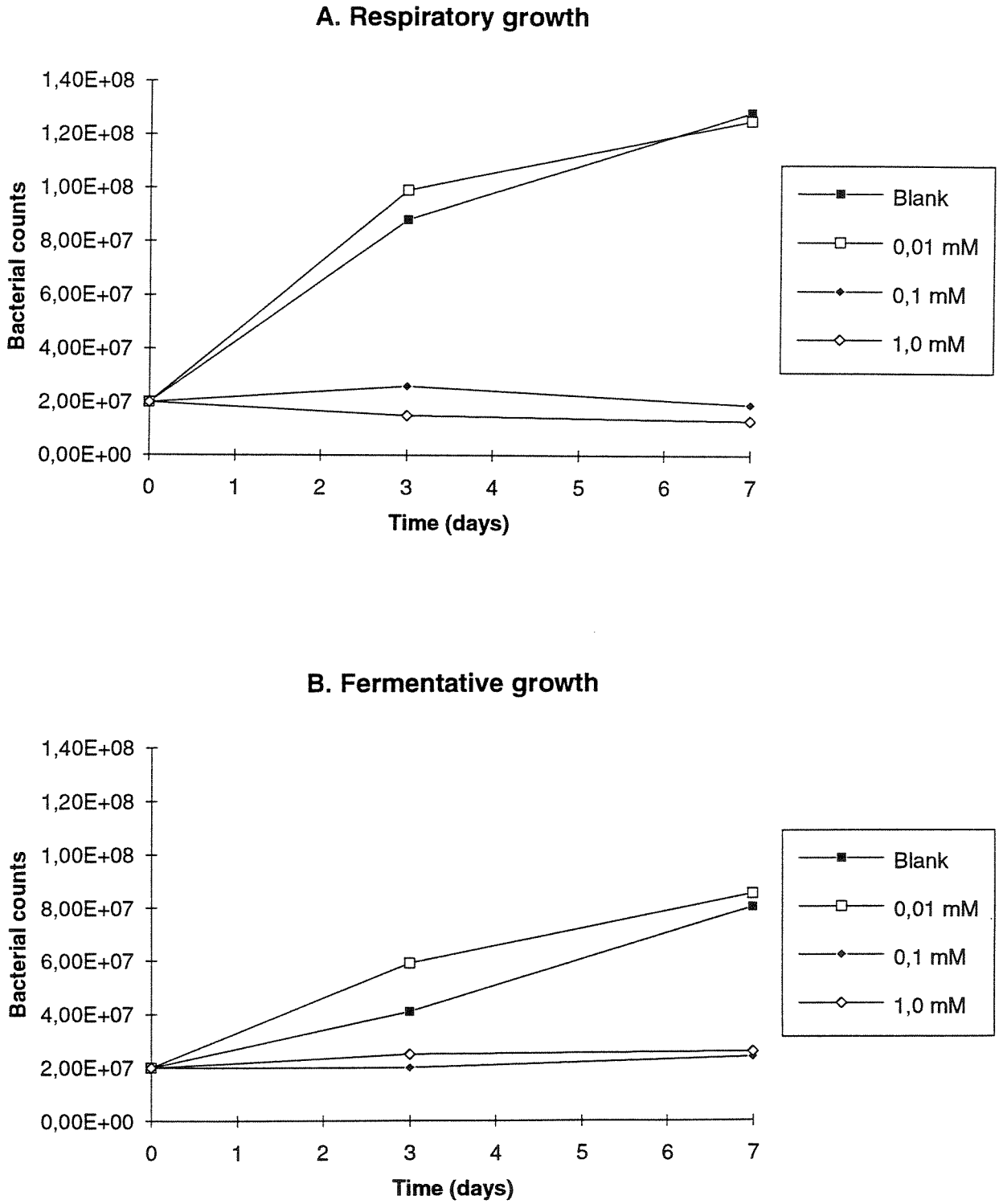

Fig.2. Cu-sensitivity of strain Su-2, cultured in the presence (A) and absence (B) of sulfate in growth medium.

The toxicity of Cu^{2+} to microorganisms may partly be due to inhibition of enzyme activities. Formate dehydrogenase (FDH) and hydrogenase (Hase) in Desulfovibrio vulgaris has previously been shown to be sensitive to 1 mM Cu^{2+} (Fitz and Cypionka, 1991). Hase in D.orientis (Cypionka and Dilling, 1986) and pyruvate dehydrogenase in D. gigas (Niviere et al., 1991) were also sensitive to Cu^{2+}.
Enzyme assays might therefore be an attractive alternative to growth experiments for assessment of toxicity if a correlation between the two approaches could be found.

The effect of Cu^{2+} on two important enzymes present in most SRB-strains was therefore tested. Enzyme activity of FDH and sulfite reductase was measured with standard methods. Formate dehydrogenase catalyze the oxidation of formate with methyl viologen (MV^{2+}) as an artificial electron acceptor. Sulfite reductase catalyze the reduction of sulfite to sulfide, using MV^{+} as artificial electron donor.

Strain Vv-FA retained 33 % of it FDH-activity after incubation with 1 mM $CuCl_2$ for 20 min. Strain L-200 retained 14 % activity after incubation with 1 mM $CuCl_2$ for 30 min. The results indicate that FDH-activity is moderately sensitive to Cu^{2+}. FDH is probably located in the periplasma, and may be more susceptible than intracellular enzymes.
The sulfite reductase-activity of strain Su-2 was not affected by Cu^{2+}. This enzyme is located in the cytoplasma.

5.Immunological studies.

5.1.Production of antisera:

Polyclonal antisera have been prepared against the SRB-strains L-200, Lø-490, St-31, St-32 and Su-2. The antisera will be called anti-L200, anti-Lø490, anti-St31, anti-St32 and anti-Su2.
Vaccines containing approximately 5 10^9 formalin-treated bacteria per ml phosphate-buffered saline (PBS) were prepared and delivered to Haukeland Hospital, Bergen, for antiserum production. Rabbits were injected intravenously with increasing amounts of vaccine and finally sacrified after 3-4 weeks. Freunds Complete or Incomplete Adjuvant were occationally added to the vaccine to enhance immunereponse. Antisera were inactivated at 56°C for 20 min. to eliminate complement factors, sterile-filtered and frozen at -80°C.

5.2.Methods used:

Immunological methods like Immunofluorescence (IF)-microscopy, Enzyme Linked Immunosorbent Assay (ELISA) and Western Blotting (WB) are useful tools for rapid detection and characterization of bacterial strains.
ELISA was used for determination of antiserum titer, which is a measure for antibody concentration in the antiserum. Titer is here defined as the dilution of antiserum which correspond to an A-492 nm-value of 1,00 by ELISA, using the HRP-color developement system. All five antisera were shown to have high titers, ranging from 20 000 to 1 000 000. ELISA can also be used to quantify the degree of homology between surface antigens of different bacterial strains.

WB has so far been used for characterization of anti-Lø490. Both internal antigens and surface antigens are detected by WB. However, only continous epitopes are detected, since the proteins are denaturated by sodium dodecyl sulfate (SDS) during the preparation of samples. WB is also useful for detection of single proteins (enzymes etc) in different bacterial strains.
IF-microscopy was used as a rapid method for comparison of SRB-strains. IF was also used for screening of water samples for the presence of SRB. Only surface antigens are detected when intact cells are prepared for IF-microscopy. The epitopes can be either continuous or discontinuous, since no denaturation occurs.

For a correct interpretation of results obtained by immunological methods, the crossreactivity of the antisera should be known. This work involves testing of antisera against type strains of bacteria.

5.3.Characterization of anti-Lø490 by WB:

Crossreactivity of anti-Lø490 with a selection of type strains of SRB was examined by WB. Species belonging to the genera Desulfovibrio, Desulfotomaculum, Desulfosarcina, Desulfobacter, Thermodesulfobacterium and Desulfuromonas were were included in the test. Species are listed in appendix. Cells were harvested by sentifugation, suspended in SDS-buffer and heated to 95^{o}C for 4 min. Proteins were then separated by SDS-PAGE, transferred to a nitrocellulose membrane by WB and examined for crossreactions with anti-Lø490.

Minor crossreactions were detected with all strains except D. gigas, D. variabilis and T. mobile. The antigen patterns are shown in the appendix. Evidently, strain Lø-490 has a few common antigens with a broad spectrum of sulfate-reducers.

5.4.Comparison of strains by IF:

Antisera were used to compare SRB-strains isolated from norwegian mines with each other and with selected type strains of SRB by IF-microscopy. Intact bacterial cells from pure cultures of the strains were stained with the antisera and thereafter with FITC-conjugated Swein anti-rabbit antibodies. Results are summarized in tab.8.
The results can be summarized as follows:

- No crossreactions were detected with the type strains Escherichia coli B and Pseudomonas fluorescens.

- No crossreactions were detected with the type strains of SRB tested so far (Desulfovibrio gigas, Desulfovibrio vulgaris and Desulfotomaculun orientis).

- Anti-Lø490 recognized strains from Sulitjelma, Stordø, Folldal, Vigsnes Mine and Lake Vigsnesvann. Strains from Sulitjelma, Folldal and Vigsnes Mine were all motile, flexible rods which were morphologically indistinguishable from each other. Endospores has not been observed.
The positive strains from Stordø and Lake Vigsnesvann were both spore-forming, straight rods, distinctly different from the above mentioned isolates.

- Anti-L200 recognized some of the morphologically similar Desulfovibrio-strains isolated from Løkken mine, Raubekken stream and Lake Vigsnesvann.

- Anti-St32 recognized Desulfovibrio-strains from N.Geiteryggen and Raubekken stream.

- Anti-St31 seemed to be highly specific for its homologous strain. This was not surprising since strain St-31 is markedly different from all other strains isolated in pure culture so far.

- Anti-Su2 recognized strains from Løkken mine, Vigsnes mine and Folldal. The reaction pattern resembles anti-L490.

- In addition to the strains listed in tab.8, bacteria present in one of the primary enrichment cultures (Orvdalen Mine) at low pH-values were examined by IF-microscopy. Interestingly, these bacteria were recognized by both anti-Lø490 and anti-L200.

Strain	**Antisera**				
Strain	Anti-L200	Anti-Lø490	Anti-St32	Anti-St31	Anti-Su2
SRB-STRAINS FROM MINES					
L-490	-	+	-	-	(+)
L-200	+	-	-	-	-
L-380	-	-	-	-	-
L-145	-	-	-	-	-
L-295	-	-	-	-	+
L-385	(+)	-	-	-	-
Su-2	-	+	-	-	+
St-31	-	-	-	+	-
St-32	-	-	+	-	-
Vv-FA	(+)	-	-	-	-
Ng-2	-	-	(+)	-	-
Rb-l	-	-	-	-	(+)
Rb-e	(+)	-	(+)	-	-
F-2	-	+	-	-	(+)
V-130	-	+	-	-	(+)
Rb-k_1	-	-	-	-	-
TYPE STRAINS OF SRB					
D.gigas	-	-	-	-	-
D.vulgaris	-	-	-	-	-
D.orientis	-	-	-	-	-
OTHER TYPE STRAINS					
E.coli B	-	-	-	-	-
P.fluorescens	-	-	-	-	-

Tab.8: Crossreactions between antisera and bacterial strains as detected by IF-microscopy. $_1$=not SRB.

5.5.Screening of water samples:

Water samples collected in the Wallenberg shaft during the last year was filtered through 0,2μm Nucleopore filtered, stained with IF-technique and examined in a fluoresence microscope. A mixture of all 5 antisera was used in order to detect a relatively broad specter of SRB. The filteres were also stained with DAPI to determine total number of bacteria present. Very few IF-positive samples were found, and the total numbers were lower than previously found. This indicates that the growth conditions for bacteria in general is less favourable at the moment than previously observed.

6.Chemostate experiments

Chemostates are well suited for physiological as well as ecological studies of microorganisms. We wanted to use chemostates as model systems for continuously fed bioreactors and to perform more basic studies on the physiology and ecology of certain selected strains. Only preliminary results have been obtained so far.

First of all, it was nescessary to gain general experience with the operation of anaerobic chemostates. Chemostates with a liquid volume of 300 ml were chosen. A continuous flow of nitrogen was used to maintain a small over-pressure in the headspace-volume of the chemostate. The medium was Widdels freshwater medium, but a phosphate/citric acid buffer system was used instead of the usual bicarbonate system. This was done to eliminate the need for carbondioxid in the gas phase.
So far a pure culture of strain L-200 and a mixed culture of the strains Rb-k and L-380 have been grown in chemostates. L-200 and L-380 are sulfate-reducers of the Desulfovibrio-type, while Rb-k is a fermentative strain. The chemostates were run by room temperature. Experiences with the two cultures will be briefly discussed:

L-200 was fed with a neutral growth medium containing 10 mM lactate as the sole carbon and energy source. Growth was slow, and the culture tended to be washed out at a dilution rate of 0,02 h^{-1}.
In another experiment, L-200 was cultured in a growth medium with pH 4,6 containing 20 mM formate and 2 mM acetate as growth substrates. pH in the chemostate was found to be 8,37. Although this culture was washed out rapidly at the dilution rate applied, it shows that the sulfate-reducers has a significant potensial to raise pH. Formate is probably particulary well suited as electron donor, since large amounts of sulfide is produced.

Lactose was provided as the only carbon and energy source for the mixed culture of the strains Rb-k and L-380. The dilution rate could be increased to 0,1 (corresponding to a generation time of 10 hours) without washing out the culture. The mixed culture was not so susceptible to oxygen stress as the pure culture of L-200. pH was lowered in the culturing vessel as the dilution rate was increased and vice versa. This can be explained by accumulation of organic acids due to rapid growth of the fermentative strain.

The main problems encountered so far are that the continous cultures are susceptible to oxygen leakage, and that the experiments are time-consuming due to the relatively low growth rates. Our further work with chemostates will be concentrated on the following topics:

- Competion between SRB-strains under different growth conditions

- Stimulatory effects of different waste products on sulfate-reduction.

- Growth of SRB at low pH-values under substrate-limiting conditions.

It will be considered to introduce a solid phase into the culturing vessel in order to improve the stability of the cultures. Use of a layer of sand or gravel has been shown to have good effect (Kuyucak et al, 1991).

7.Effects of brewery- and dairy waste on bacterial sulfate reduction.

The aim of this study was to investigate the effect of different waste products from food industries on bacterial sulfate reduction. Such wastes should be easily degradable, contain all necessary nutrients in appropriate amounts and not contain toxic or inhibitory compounds.They should also be cheap and readily available near the mine.

We chose to test sweet whey, berme and inactivated berme in this experiment. Sweet whey is a biproduct from the manufacturing of white cheeses and contains large amounts of lactose (50-60 g/l) in addition to a significant portion of proteins. pH was 6,58. Sweet whey has previously been shown to stimulate bacterial sulfate reduction in simulated wetlands (Stark et al., 1991). Most of the whey produced in Norway is utilized in the production of brown cheese. However, only about 56 % is used in the world as a whole.
Berme is a waste product from breweries and contains mainly organic acids and large amounts of old and dead yeast cells. pH was 4,71. Inactivated berme is berme in which the yeast cells are killed by addition of "foraform". pH was 4,64. An autochtonous population of fermentative bacteria is supposed to be present in the three waste products. However, phase contrast microscopy showed that total number of bacteria was below 10^6 ml^{-1}.

Two different inocula were used; one was a mixed culture of a fermentative lactose-degrading strain and a sulfate-reducer of the Desulfovibrio-type, while the other one was a pure culture of a Desulfovibrio. Growth and sulfide production was examined in a common anoxic mineral medium (Widdel, 1980), in mine water with pH 2,9 and in mine water where pH was adjusted to 5,0. Mine water was obtained from Løkken mine in may 1991 and was stored in plastic containers for one year before use. pH was 5,13 at the time of sampling. After the storage, pH had dropped to 2,88, probably due to precipitation of ferric iron compounds. To mimic the initial water quality, pH was raised to 5,02 by the use of 1 N NaOH. Chemical analyses were not conducted, but in previously collected samples from the same spot, concentrations of Cu, Zn and Fe averaged 20 mg/l, 300 mg/l and 80 mg/l, respectively.

The experiments were conducted in Hungate-tubes. pH was measured in one of the tubes immediately after inoculation, and these tubes were discarded. pH was also measured at the end of the experiment.
Tubes were incubated at 20°C in dark for 60 days. Growth and sulfide production was examined after 7, 21 and 60 days.

The results are shown in tab.9, and can be summarized as follows:

- Sweat whey stimulated bacterial sulfate reduction when added to a common anaerobic, mineral growth medium. However, if the starting concentration of whey was too high, sulfate reduction was inhibited. This is probably due to rapid growth of fermentative bacteria which cause an almost immidiate drop in pH. Maximum concentrations of free sulfide in the tubes was 5,96 mM.

- Berme stimulated SR. Maximum sulfide production recorded was 6,52 mM. A major disadvantage with berme is the large amount of organic material stored in the yeast cells. This material is not readily available for the fermentative bacteria or the sulfate-reducers.

- No significant growth or sulfide production was observed when mine water was used instead of anaerobic growth medium. The low pH-values are probably the main inhibitory factor. This result is not too promising for the in-situ treatment concept.

- No sulfide production was observed in uninoculated tubes, indicating that SRB are absent in sweet whey and berme.

- In all tubes with mineral medium except control tubes, other morphological types of bacteria than the inoculated ones appeared. Several of these became dominating.

- pH was lowered during the experiment in all tubes with mineral medium except control tubes. Acidification was most evident in tubes where no growth of SRB had occured.

Medium+substrate+inoculum	Start-pH	Final pH	Sulfide,21days	Sulfide,60days
Control(WF+dest.water+L-200)	6,79	6,82	0	0
Control(WF+dest.water+Co)	6,81	6,87	0	0
WF+whey+L-200	6,92	3,94		0,33
WF+whey+Co	6,9	3,67		0,88
WF+whey		3,61		
WF+whey(1:5)+L-200	6,93	6,49	5,96	5,51
WF+whey(1:5)+Co	6,91	6,51	5,49	4,57
WF+whey(1:5)		5,98		
WF+berme+L-200	6,7	5,34		5,45
WF+berme+Co	6,68	5,32		6,52
WF+berme		4,77		
WF+inact.berme+L-200	6,55	5,31		1,14
WF+inact.berme+Co	6,58	5,5		5,87
WF+inact.berme		4,89		
WF+sup.berme+L-200	6,63	5,65	5,69	5,56
WF+sup.berme+Co	6,72	5,5	4,91	4,23
WF+sup.berme		4,62		
WF+sup.inact.berme+L-200	6,6	4,86	1,19	1,2
WF+sup.inact.berme+Co	6,58	5,17	2,73	3,1
WF+sup.inact.berme		4,59		

Tab.9. Changes in pH and sulfide production during growth experiment in anaerobic medium supplemented with sweat whey and berme (brewery waste) as carbon sources.

8.Summary

Samples have been collected in several abandoned pyrite mines in Norway for chemical analysis and enrichment of sulfate-reducing bacteria. SRB seems to be ubiquitous in sediments affected by acidic drainage, and have been found in both of the two water-filled mines which have been sampled. No SRB could be isolated from oxic surface water inside and outside mines.

A selection of isolated strains have been characterized morphologically, physiologically, ecologically and immunologically. Their exact taxonomic position remain to be clarified, but members of the genera Desulfovibrio and Desulfotomaculum have been identified. All isolates grow well on pyruvate and lactate, and these compounds seem to be the sole carbon and energy sources utilized by some strains. H_2, formate and ethanol serve as excellent electron donors for the Desulfovibrio-strains.

Copper sensitivity is comparable to a type strain of SRB (D.gigas), indicating that special mechanisms for copper detoxification probably not exist in the strains tested so far. More strains are currently being tested. The Cu-levels found in the Wallenberg mine at the moment do not preclude growth of SRB.

pH-tolerance of the two strains tested is also comparable to type strains, and no growth was observed at pH-values below 5,5 in batch culture. These observations indicate that growth in affected habitates may be restricted to sediments and biofilms where the microenvironment is more favourable.
pH tolerance will be tested in chemostat under carbon-limitation to find out whether undissociated organic acids could be responsible for the observed growth inhibition at low pH-values.

In a treatment process it will be necessary to add a cheap and readily available organic waste product to support growth of SRB. The effect of sweat whey (dairy waste) and berme (brewery waste) on bacterial sulfate-reduction was examined in laboratory experiments. Both wastes stimulated SR in growth medium, but not acidic mine water. Low pH-values is the likely explanation for this.

Four possible lines of technological developments have previosly been suggested:

1) In situ treatment in water-filled mines

2) SRB bioreactor design for separation of metal sulfides.

3) Integration of a SRB unit process into calcium precipitation and biosorption technology.

4) Biosorption in cheap biomass for polishing and recycling.

The *in situ* treatment and development of a SRB reactor will together with further laboratory tests be the major areas of research in the Nordic Industry Fund project the next years. Promising results have recently been achieved in the US with anaerobic operaton of artificial wetlands and simple bioreactor systems, as reported by several authors. Spent mushroom

compost have been widely used as a solid matrix and source of organic carbon to support growth of sulfate-reducing bacteria in these experiments. pH is typically raised from 2,5-3 in inflowing mine water to circumneutral values in the effluent, and most metals are removed effectively.
Based on these positive results, it should be considered whether similar approaches could be interesting in norwegian mines.

9.References

Arnesen, R.T., E.R. Iversen, S.T. Kallquist, M. Laake, T. Lien and B. Christensen. Monitoring water quality during filling of the Løkken mine; A possible role of sulfate-reducing bacteria in metals removal. In: Proceedings of the Sec. Int. Conference on the Abatement of Acidic Drainage, Montreal, Canada, 16-18 sept. 1991, 3:201-217.

Christensen, B. and S.T. Kallquist. Progress report to Nordic Industial Fund, sept. 1991.

Christensen, B. and M. Laake. Progress report to Nordic Industrial Fund, sept. 1992.

Danneberg, S., M. Kroder, W. Dilling and H.Cypionka (1992). Oxidation of H_2, organic compounds and inorganic sulfur compounds coupled to reduction of O_2 or nitrate by sulfate-reducing bacteria. Arch. Microbiol. 158:93-99.

Dilling W. and H. Cypionka (1990). Aerobic respiration in sulfate-reducing bacteria. FEMS Microbiology Letters, 71:123-128.

Dvorak, D.H., R.S. Hedin, H.M. Edenborn and P.E. McIntyre (1992). Treatment of metal contaminated water using bacterial sulfate-reduction: Results from pilot-scale reactors. Biotechnolgy and Bioengeneering, Vol. 40, pp. 609-616.

Fitz, R.M. and H. Cypionka (1991). Generation of a proton gradient in Desulfovibrio vulgaris. Arch. Microbiol. 155:444-448.

Fukui, M. and S. Takii (1990). Survival of sulfate-reducing bacteria in oxic surface sediments of a seawater lake. FEMS Microbiology Ecology 72:317-322.

Guyre, R.A., A. Konopka, A. Brooks and W. Doemel (1990). Microbial sulfate-reduction in acidic (pH 3) strip-mine lakes. FEMS Microbiology Ecology 73:193-202.

Hammack, R.W. and H.M. Edenborn (1992). The removal of nickel from mine waters using bacterial sulfate reduction. Appl. Microbiol. Biotechnol, 37:674-678.

Kleinmann, R.L.P., R.S. Hedin, H.M. Edenborn. Biological treatment of mine water - an overview. In: Proceedings of the Sec. Int. Conference on the Abatement of Acidic Drainage, Montreal, Canada, 16-18. sept. 1991, pp27-42.

Kuyucak, N., R.J. Mikula and K.G.Wheeland. Interpretation of properties of lime sludge generated by different processes. In: Proceedings of the Sec. Int. Conference on the abatement of acidic drainage, Montreal, Canada, 16-18. sept. 1991.

Maree, J.P and W.F. Strydom (1985). Biological sulphate removal in an upflow packed bed reactor. Water Res. Vol.19, No.9, pp. 1101-1106.

Maree, J.P and W.F. Strydom (1987). Biological sulphate removal from industrial effluent in an upflow packed bed reactor. Water Res. Vol.21, No2, pp. 141-146.

Niviere, V., A. Bernadac, N. Forget, V.M. Fernandez and C.E. Hatchikian (1991). Localization of hydrogenase in Desulfovibrio gigas cells. Arch. Microbiol. 155:579-586.

Reynolds, J.S., J.L Bolis, S.D. Machemer and T.R. Wildeman. Sulfate-reduction in a constructed wetland. Preprint; Presented before the Division of Environmental Chemistry, American Chemical Society, Atlanta, GA, US, 14-19 april, 1991.

Stark, L.R, W.R. Wenerick, P.G. Wuest, F.M. Williams, S.E. Stevens jr.; "Adding a carbon supplement to simulated treatment wetlands improves mine water quality". In Proceedings of the Sec. Int. Conference on the Abatement of Acidic Drainage, Montreal, Canada, sept. 16-18, 1991, vol.2; 465-483.

Tuttle, J.H., P.R. Dugan, C.B. MacMillan, C.I Randles (1969). Microbial dissimilatory sulfur cycle in acid mine water. Journal of Bacteriology, 97:594-602.

Tuttle, J.H., P.R.Dugan, C.I. Randles (1969). Microbial sulfate reduction and its potensial utility as as acid mine water pollution abatement procedure. Appl. Microbiol. 17:297-302.

Widdel, F. (1980). Anaerober abbau von Fettsauren und benzosaure durch neue isolierte arten sulfat-reduzierenden bakterien. Doctoral thesis, Univ. of Gøttingen.

Widdel, F. (1988). Microbiology and ecology of sulfate- and sulfur-reducing bacteria. In: Biology of anaerobic microorganisms. Ed. by A.J.B. Zehnder. Wiley & Sons Inc.

10.Appendix

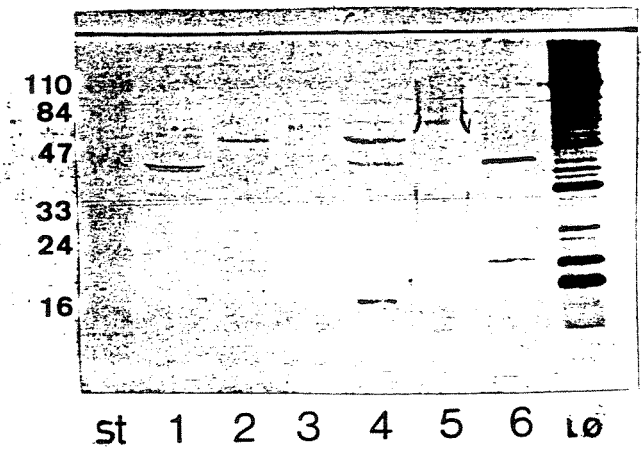

Fig. 3. Type strains of SRB compared to strain Lø-490 by Western Blot. Anti-Lø490 was used as primary antiserum. Lane 1:Desulfovibrio vulgaris(NCIMB 8303), lane 2: D.desulfuricans,Sylt 3 (NCIMB 9335), lane 3: D.desulfuricans,Essex 6 (NCIMB 8307), lane 4: D.desulfuricans,Norway 4 (NCIMB 8310), lane 5: D.desulfuricans, El Agheila (NCIMB 8380), lane 6: Desulfovibrio sp. (DSM 2056), st=BioRad Low Range Molecular Weight Standard, Lø= strain Lø490.

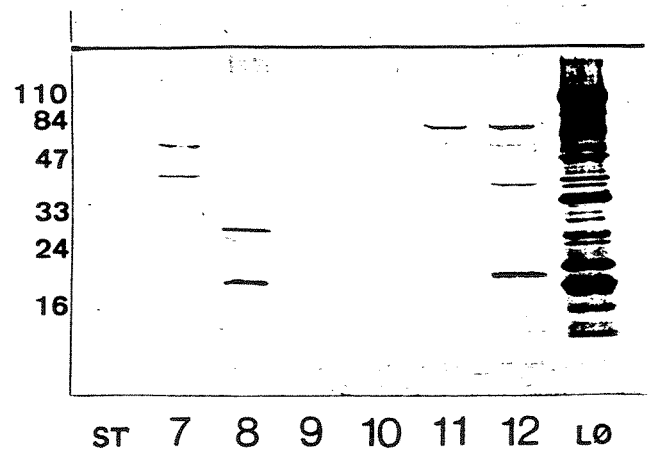

Fig.4. Type strains of SRB compared to strain Lø-490 by WB. Anti-Lø490 was used as primary antiserum. Lane 7: Desulfovibrio baarsii (DSM 2075), lane 8: D. sapovorans (DSM 2055), lane 9: D. gigas (DSM 496). lane 10: Desulfosarcina variabilis (DSM 2060), lane 11: Desulfobacter postgateii (DSM 2034), lane 12: D. curvatus (DSM 3379), st=BioRad Low Range Molecular Weight Standard, Lø= strain Lø-490.

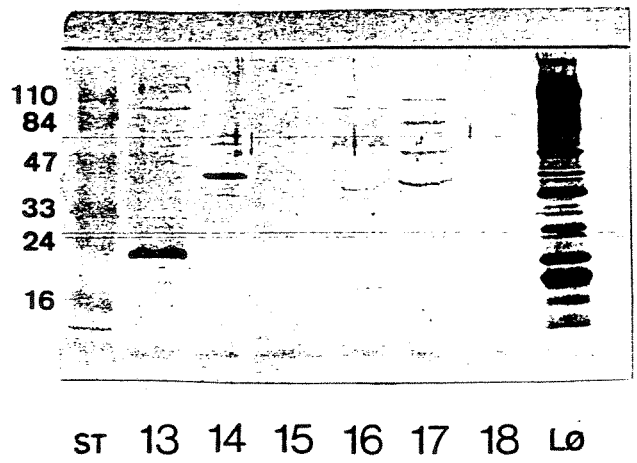

Fig. 5. Type strains of SRB compared to strain Lø-490 by WB. Anti-Lø490 was used as primary antiserum. Lane 13: Desulfotomaculum nigrificans (DSM 574), lane 14: Thermodesulfobacterium mobile (DSM 1276), lane 15: T. commune (DSM 2178), lane 16: Desulfuromonas acetoxidans (DSM 684), lane 17: Strain 4303 (Desulfovibrio-isolate from the North Sea), lane 18: Strain G-4701 (Desulfovibrio-isolate from the North Sea), st=BioRad Low Range Molecular Weight Standard, Lø= strain Lø-490.

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