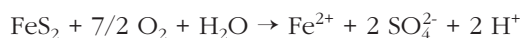


The role of bacteria in degradation of exposed massive sulphides at Citronen Fjord, North Greenland: project 'Resources of the sedimentary basins of North and East Greenland'

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The multidisciplinary research project 'Resources of the sedimentary basins of North and East Greenland' was initiated in 1995 with financial support from the Danish Research Councils (Stemmerik *et al.*, 1996). The Citronen Fjord zinc prospect (Fig. 1) discovered by Platinova A/S in 1993 is by far the largest sulphide occurrence known in North Greenland, and is currently being investigated as a potential exploitable resource. However, the mining and processing of sulphide ores can create serious pollution problems in the surrounding terrestrial and aquatic ecosystems by exposing large amounts of sulphidic material to atmospheric oxygen and 'attack' by mineral-oxidising bacteria. At lower latitudes, the slow abiotic oxidation of metal sulphides is known to be significantly accelerated by bacterial attack. A microbiological investigation of the Citronen Fjord zinc deposit was initiated in the summer of 1995 to investigate the role bacteria might play in oxidation of sulphidic material in High Arctic areas. This is a joint project involving the Danish Environmental Research Institute (Department of Arctic Environment) and the University of Aarhus (Department of Microbial Ecology).

Pyrite is the dominant sulphide mineral in the Citronen Fjord deposit (Kragh *et al.*, 1997). Pyrite oxidises when exposed to atmospheric oxygen and meteoric water, and acid, sulphate and iron are released:



At Citronen Fjord, oxidation of the exposed sulphides has produced impressive, vividly coloured gossans (Figs 1, 2). These gossans offer a rare opportunity to study microbial and chemical sulphide oxidation processes, as well as the microbial diversity in a still undisturbed environment. The present investigation represents a zero-line study of microbial activity, and will provide an important reference standard with respect to the potential effects of mining of the Citronen Fjord zinc deposit on microbial processes.

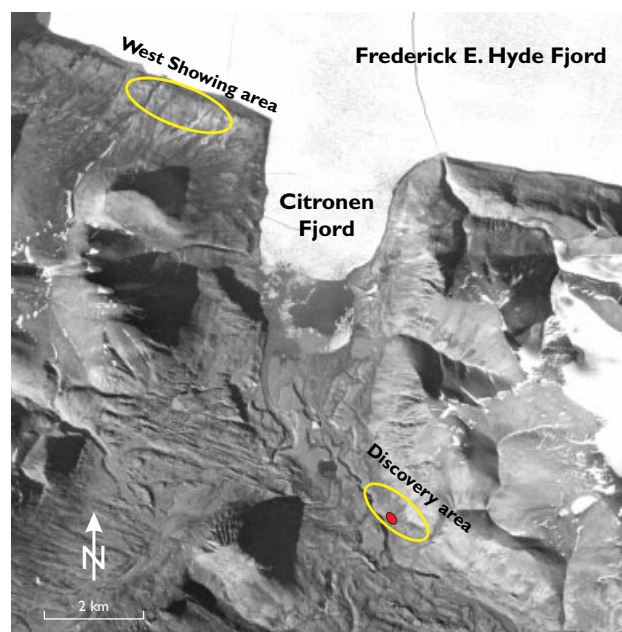


Fig. 1. Vertical aerial photograph of the Citronen Fjord area. Major gossans occur within the 'Discovery' and 'West Showing' areas. Microbiological investigations have been focused on 'gossan C', indicated with a red dot. For regional location and geological setting see Kragh *et al.* (1997). Photo: 874E 1096, 23rd July 1978; Kort- og Matrikelstyrelsen, Denmark.

The project addresses two main subjects: (1) the physiology of microbes living in this extreme environment; and (2) the role of bacteria in degradation of exposed metal sulphides. Detailed physiological experiments and tests of isolated microbes have recently been conducted in the laboratory and the results are discussed briefly below.

The role of bacteria in the degradation of sulphidic material has been investigated in a combination of *in situ* measurements and laboratory studies. High priority was given to evaluation of the total *in situ* oxidation of sulphidic material in the gossans, including abiological and biological processes. The relative impor-



Fig. 2. View to the east-south-east over the gossans in the 'Discovery area', with 'gossan C' in centre of photograph. Note the intense ochre staining of the river bed below the gossan. The height of the gossan is about 25 m.

tance of bacterial and chemical oxidation will be evaluated on the basis of future laboratory experiments combined with the results obtained from total oxidation rate measurements and analyses of collected water samples.

Microbiological studies

Background

Micro-organisms are divided into two basic cellular types, *eucaryotes* and *procaryotes*, that are characterised by fundamental differences in cell structure. Eucaryotic cells form the higher *protista* that include fungi, yeasts and green algae, whereas procaryotes form the lower protista that comprise bacteria.

Microbes are a large and diverse group of organisms that are capable of living as single cells in nature, and are generally capable of reproduction and energy production independently of other cells. In order to understand the immense metabolic diversity, it is necessary to be familiar with the terms *lithoautotrophic*, *organo-*

heterotrophic and *mixotrophic*. Some bacteria can produce energy by modifying inorganic compounds such as minerals, ferrous iron and sulphur (lithotrophs), and assimilate carbon dioxide as their sole source of carbon (autotrophs). Other bacteria and the higher protista can derive energy and carbon from a variety of organic compounds (organoheterotrophs). In addition, some bacteria have the capacity to combine both strategies (mixotrophs).

Identification of bacteria

Most of the bacterial physiology data at present available were obtained from laboratory experiments on material collected during a short visit to Citronen Fjord in July 1995, and on cultures isolated from this material. Sampling was confined mainly to oxidised ore from 'gossan C' (Fig. 1) and sedimentary material in freshwater streams draining this gossan (Fig. 2).

Initial laboratory studies were focused on confirming the existence of bacteria in the collected samples. The material was suspended in tubes containing a medium suitable for growth of mineral-oxidising bac-

teria supplemented with a radioactive growth substrate ($^{14}\text{CO}_2$ or ^{14}C -glucose). The tubes were incubated in a temperature gradient block at 1–3°C intervals within the temperature range 0–35°C. Microbial growth was identified by counting cell-bound radioactivity as a measure of carbon incorporation by lithoautotrophs and organoheterotrophs respectively. Microbial growth and respiration were identified at temperatures in the 0–32°C range, and microscopic analyses of incubated material revealed the presence of both lithoautotrophic bacteria and higher protista (Langdahl, 1995).

Culture studies

The temperature characteristics for the growth of bacteria capable of catalysing the oxidation of ferrous iron have been the focus of several studies (e.g. Ferroni *et al.*, 1986; Berthelot *et al.*, 1993), mainly because bacteria are used commercially in the processing of sulphide ores. Most of the ferrous iron-oxidising bacteria that may be involved in mineral degradation processes are described as *mesophilic* or *thermophilic*. Mesophiles and thermophiles include bacteria that have maximum activity in the temperature ranges of 30–37°C and 40–45°C, respectively. A few strains have been found to be active at temperatures down to as low as 2°C (Leduc *et al.*, 1993). At Citronen Fjord, temperatures in excess of 2°C (i.e. above the assumed lower limit for bacterial ferrous iron oxidation) are only reached in short periods during the summer, and it was therefore significant that results obtained from analyses of enriched and pure cultures reveal the presence of lithoautotrophic Fe^{2+} -oxidising bacteria in the gossan samples. Despite the low ambient temperatures, the bacteria seem to be metabolically very active in the Citronen Fjord gossans.

Detailed physiological studies of the lithoautotrophic bacteria (Fig. 3) are being carried out in co-operation with the Deutsche Sammlung von Mikroorganismen (DSMZ), Braunschweig, Germany. DSMZ uses 16S rRNA sequence analyses in order to taxonomically classify the bacteria. The provisional results indicate that the lithoautotrophic Fe^{2+} -oxidising bacteria are members of the well-described mesophilic species *Thiobacillus ferrooxidans*.

Oxygen consumption measurements

During the 1996 field season, the emphasis of the work was on *in situ* measurements of oxygen consumption and detailed collection of ore and water samples in

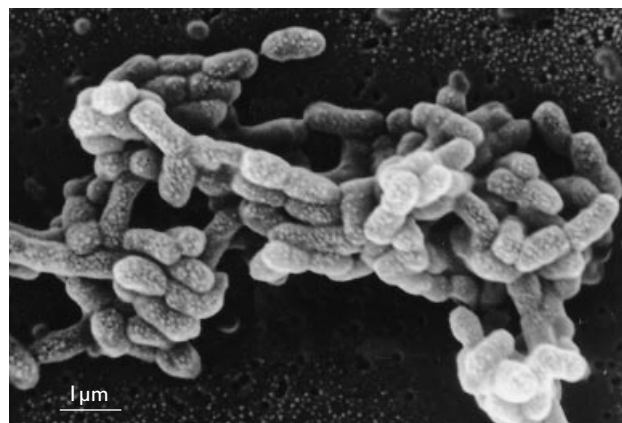


Fig. 3. Scanning electron photomicrograph of ferrous iron-oxidising bacteria isolated from gossan material from the Citronen Fjord zinc deposit.

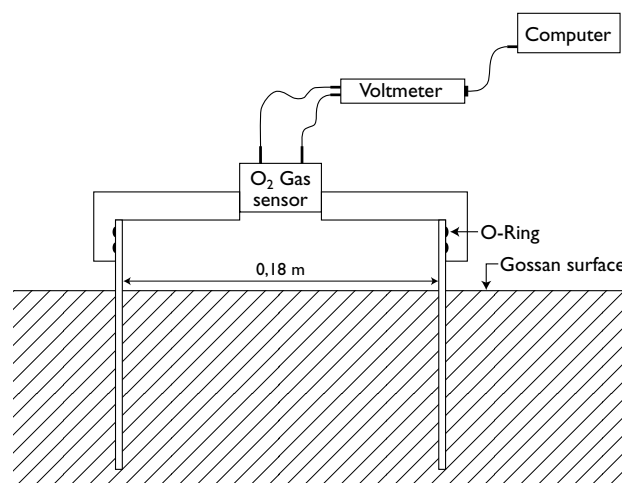


Fig. 4. Details of the closed gas reservoir used for the *in situ* measurements of oxygen consumption.

order to evaluate the total oxidation of sulphidic material. The contaminated effluent observed below the gossan area contains high concentrations of acid, sulphate, and dissolved metals (Fig. 2), which is a result of the transport of oxygen into the gossan and complex interactions between the oxidative chemical and biological reactions within the material.

As the oxidation of sulphides proceeds, atmospheric oxygen is consumed. The oxygen consumption rates were determined by measuring the decrease in oxygen concentration in a closed gas reservoir covering the gossan surface. An aluminium cylinder was manually driven into the gossan leaving the top of the column above the surface (Figs 4, 5). An oxygen sensor sealed into an aluminium cap was placed on top of the column to form the closed gas reservoir at the gossan sur-



Fig. 5. *In situ* measurement of oxygen consumption rates on 'gossan C'. The view is north-west with the mountain at about 1000 m a.s.l.

face during measurements. Oxygen concentrations were recorded every five minutes during a one hour period using a voltmeter connected to the electrochemical gas sensor. As oxygen is consumed in the gossan, the oxygen concentration in the chamber decreases and can be expressed in terms of oxygen flux across the gossan surface (Elberling & Nicholson, 1996).

Preliminary data processing of the oxygen consumption measurements indicates that the oxidation of sulphidic material is surprisingly high, and is in fact comparable to rates obtained from mining areas at lower latitudes. These high oxidation rates are in agreement with the extreme natural environmental conditions observed downstream of the gossan, including low pH-values (1–3) and iron and sulphate concentrations above 10 g/l.

Future work

Physiological characterisation of the isolated bacterial strains continues, and will be focused on the mecha-

nisms by which the bacteria survive prolonged exposure to sub-zero temperatures in the Citronen Fjord area. Are the bacteria capable of producing spores or other types of resting 'bodies' less susceptible to the cold, or are they capable of surviving in a freeze-dried form? These microbiological investigations will be innovative and will contribute important new information on mineral-oxidising bacteria.

The isolation of ferrous iron-oxidising bacteria was carried out using culture enrichment techniques. Unfortunately, these techniques selectively promote growth of the microbes best suited to the conditions in the laboratory, although attempts at conditions analogous to the gossans were established. As a consequence, the bacteria obtained are not necessarily the most important bacteria *in situ*. In order to elucidate the significance of the isolated bacterial strains, physiological studies will be supplemented with DNA analyses. Analyses of DNA products from sulphidic material collected during 1996 field work will be compared to amplification products from the isolated bacterial species by gel electrophoresis. The technique may be extended

to investigate bacterial diversity in ore material collected from other comparable environments. An interesting question is whether the bacterial population found at Citronen Fjord is similar in physiology and numbers to that of populations found in areas where sulphide ore is being, or has been mined.

The present study indicates that microbes can be almost omnipresent in natural environments, even in environments we might instinctively consider devoid of life. It is known that micro-organisms may number billions per cubic centimetre of material in favourable environments. The obvious question to be resolved is the number of bacteria per cubic centimetre in the 'inhospitable' gossan material from the Citronen Fjord area.

Acknowledgement

The aerial photograph is published with courtesy of the Kort- og Matrikelstyrelsen, Copenhagen, Denmark (permission A. 200/87).

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