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Geomicrobial processes in the subsurface: A tribute to Johannes Neher's work

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Abstract

For almost 50 years, Johannes Neher's research emphasized the significant role of microbes in geological processes. He has been searching for microbial evidence in the formation of silicates, such as cornelian, jasper, moss agate, chalcedony, and chrysocolla, as well as quartzites, itacolumnites, diamonds, dendrites on calcite, silicate horizons in limestone beds, and dolomite phenocrysts that formed in a biotite gneiss. Johannes Neher has never published his research and, thus, has caused opposition and disbelief amongst scientists. Two examples of his discoveries which are of fundamental significance in geology are briefly presented in this paper: (1) bacterially mediated precipitation of dolomite, and (2) microbial dissolution of quartz in an Alpine environment.

Keywords: Quartzite; Iron crystallite; Dolomite; Bacterium; Fungus; Biodegradation

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1. Introduction

Geological microbiology or geomicrobiology is basically the study of microbial processes which occur in modern sediments, in ground waters circulating through sedimentary and igneous rocks, and in weathered earth crust [1]. Already more than 150 years ago it was recognized that microbes play an important role in geologic processes. Ehrenberg [2], for example, observed an association of the bacterium Gallionella ferruginea with ochreous deposits of bog iron, and Winogradsky [3] discovered that Beggiatoa oxidizes H₂S to elemental sulfur. Further discoveries included the significance of bacterial sulfate reduction in the formation of sulfur deposits

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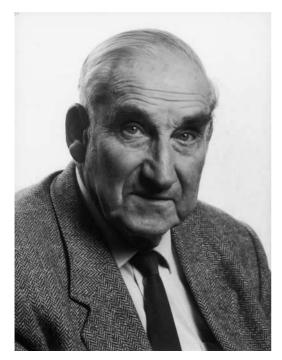


Fig. 1. Johannes Neher at the age of 80.

[4,5] and the important role of microbes associated with the precipitation of CaCO₃ [6–8]. Thus, at the beginning of this century, various important geomicrobial processes were already receiving the attention of microbiologists.

Düggeli was one of the first Swiss scientists to show interest in the area of geomicrobiology. He focused primarily on bacteriological studies in calcareous rocks [9]. Since the end of the forties, a wide spectrum in the area of geomicrobiology has been introduced to Swiss science by Johannes Neher who emphasized the significance of microbes in rock formation and destruction, however, with great opposition of geologists, biologists and chemists.

2. Biography of Johannes Neher

Johannes Neher (Fig. 1) was born on October 17th, 1906. He originates from a distinguished family, connected with the iron mines and metallurgy plant near Sargans, in eastern Switzerland. He grew up in the small ancient town of Maienfeld, surrounded by vineyards. He studied geology at the

ETH (Swiss Federal Institute of Technology) in Zürich, with Rudolf Staub as his supervisor. He wrote his diploma thesis on part of the Tauern tectonic window in Austria. Later, he conducted research on snow and avalanches and served as an army geologist during World War II.

In the mid-thirties, Johannes Neher started to study the Splügener Kalkberge, a highly complicated mountain group in Grisons. Despite repeated prodding by successive professors, he never completed his Ph.D. thesis. He was not interested in obtaining academic degrees and he disliked writing. Fortunately, many of his results are embodied in the magnificent Andeer map sheet published together with Viktor Streiff and Heinrich Jäckli [10,11]. Johannes Neher stayed on at the geology department, where he became the guardian of the institute tradition, beloved by all of the several student generations who knew him. Some private means allowed him to live modestly, but independently. In 1988, he was honoured by becoming a permanent guest of the ETH.

During his studies on the Triassic carbonate rocks of his field area, Johannes Neher became interested in dolomitization and silicification phenomena. He did pioneering work in isolating small, dolomitized fossils, especially gastropods and crinoids, through slow and controlled solubilisation of the rock material by dilute acetic acid.

Johannes Neher started to notice structures within sedimentary and even low-grade metamorphic rocks, which presented clear indications of organic origin. A chance encounter with the biochemist Ernst Rohrer, who had been obliged to leave Czechoslovakia for political reasons, proved to be very productive. Rohrer introduced Neher to new techniques, such as phase contrast microscopy. Together, they huddled for entire nights over the microscope, becoming more and more excited about their new observations.

Around 1949, Johannes Neher presented some of his results to biologists at the ETH. The botany professors remained skeptical, and some of them even expressed their disbelief. This was a traumatic experience for Johannes. He withdrew to his study and became less and less communicative with the outside world. Even a visit by NASA scientists did little to change his attitude. He continued to conduct his experiments and to assemble an impressive collection

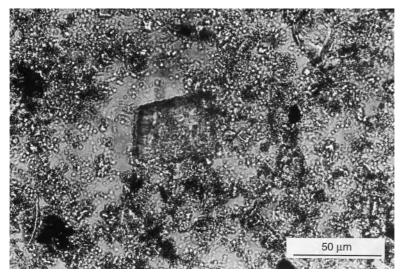


Fig. 2. Photomicrograph of a well developed dolomite crystal (large crystal in the center) which formed mediated by bacterial cultures. It was the first experiment in which dolomite was experimentally precipitated (around 1955).

of photographic documents, a few examples of which are presented in this paper.

Since the early fifties, Johannes Neher started various projects in the field of geomicrobiology. One of his preferences was to study silica minerals, such as cornelian, jasper, moss agate, chalcedony, and chrysocolla. He believed strongly that microbes were involved in the formation of these minerals. Other projects in which he had been searching for microbial evidence comprised the formation of quartzites, itacolumnites, diamonds, dendrites on calcites, silica horizons in limestone beds, and dolomites that formed in a biotite gneiss. He also carried out a number of experiments with microbial cultures showing the ability of these organisms to dissolve materials such as stainless steel, aluminium, and even gold. Unfortunately, most of these studies were never published.

Johannes Neher is a first-class observer, in the field as well as in the laboratory. He has a wide grasp of optical and SEM microscopy, and his photomicrographs are most impressive. However, due to his isolation, he did not have the opportunity to work under aseptic conditions, so that the possibility of contamination must be examined in each case.

In the following, two unpublished studies are presented which Johannes Neher carried out in the late fifties and in the early seventies, respectively. They are examples of spectacular findings in terms of geologic aspects and are of wide interest as they contribute to controversial geologic problems associated with microbial activities in the subsurface. However, Johannes probably never recognized the full significance of his observations.

3. Bacterially mediated dolomitization

At the beginning of the 20th century, geologists recognized that there was a non-uniform distribution of dolomite throughout geologic times. They proposed that past conditions may have varied from the present, thus explaining the scarcity of dolomite found in modern sediments. During approximately the past 30 years, however, minor amounts of dolomite have been found forming in several modern environments, and various thermodynamic, kinetic and hydrological factors have been proposed to explain its formation [12]. Only very recently, a possible significance of microbes for the formation of dolomites at low temperatures was experimentally shown by Vasconcelos [13] and Vasconcelos et al. [14]. In these experiments, ferroan dolomite crystals of less than 1 µm were precipitated in the presence of sulfate reducing bacteria from the genera of Desulfovibrio. However, more than 40 years ago Johannes

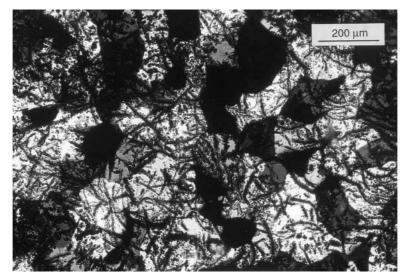


Fig. 3. Thin-section photomicrograph of filamentous microbes in quartzite as indicated by the blocky pattern in the background formed by hypidiomorphic quartz crystals. The filaments were later identified as fungi of the class Basidiomycetes. Crossed nicols.

Neher and Ernst Rohrer had already conducted an experiment in which bacterially mediated dolomite was precipitated.

In 1958 and 1959, Neher and Rohrer documented the presence of dolomite in a biotite gneiss which had been recovered from a drill core from a depth

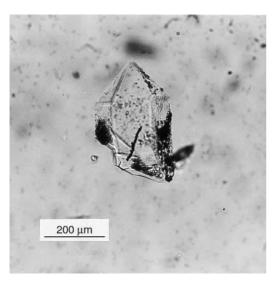


Fig. 4. Photomicrograph of a well developed quartz crystal with an inclusion of a fungal filament (center). Such filaments bored into the quartz by dissolving SiO₂.

of 160 m near Koblenz, northern Switzerland [15,16]. They found bacterial colonies forming dark rims around individual dolomite crystals, suggesting that the presence of these micro-organisms was responsible for the degradation of quartz, feldspar, and mica and for the formation of dolomite occurring in the gneiss. Johannes Neher did not show much interest in this rather odd deposit of dolomite in this crystalline environment. However, he was very excited about the discovery of the presence of bacteria at a depth of 160 m and wanted to convince the scientific world that bacteria are present and able to grow at depth, even within gneisses. In order to prove the existence of these subsurface bacteria, Johannes Neher and Ernst Rohrer started to grow bacteria isolated from the biotite gneiss.

In their initial report, of which an english version is published here for the first time, they described their discoveries as follows:

"Microscopical investigations of several thin sections of a biotite gneiss indicate the presence of micro-organisms in this rock. The existence of bacteria and fungi in different, partially deep lying rock formations has been known for a long time. Repeatedly, reports and observations of fossil bacteria have been published [17–19]. It is, however, debatable whether these organisms are still alive. Particularly the modern oil bacteriology shows that bacteria

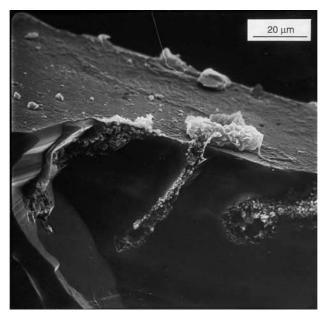


Fig. 5. SEM photomicrograph of the face of a quartz crystal with a subvertical boring. The fungal filament which bored into the quartz has disappeared. The organic material excreted by the microorganisms (on the surface and along the walls of the boring track) has mineralized into an iron phase.

are present and alive at great depths [20–24]. Only the culturing of these bacteria can yield proof for the existence of living microbes in the investigated biotite gneiss."

Johannes Neher and Ernst Rohrer described their experiment as follows: "Small pieces of the gneiss were etched with dilute HCl (10%) for 4 hours. Afterwards, they were put into 5% formalin for 30 minutes and washed with sterile distilled water. Under aseptic conditions the gneiss was broken with a chisel and pieces of the gneiss were put onto different culture media. They were incubated for 4 weeks at temperatures between 20°C and 65°C, mostly without success. The only culture media that showed microbial growth had a composition similar to that of Bushnell and Haas [25]:

$\overline{MgSO_4}$	0.06%
CaCl ₂	0.002%
K_2HPO_4	0.15%
KH_2PO_4	0.1%
conc. Fe ₂ Cl ₆ solution	6 gtt.
distilled H ₂ O	ad 100

At temperatures of 45°C and 50°C a slight turbidity was observed, formed by coccoid bacteria. For further experiments we attempted to simulate conditions similar to those in the gneiss. Therefore, pieces

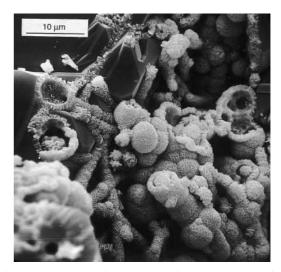


Fig. 6. SEM photomicrograph showing encrusted spherical bodies forming chain-like structures on quartz surfaces in quartzite. The chains probably represent former fungal filaments.

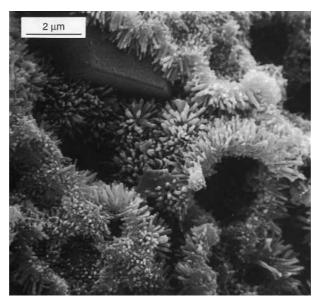


Fig. 7. SEM photomicrograph of encrusted spherical bodies in quartzite. The encrustations consist of radially grown needle-like goethite crystals (FeOOH).

of the biotite gneiss [15] were powdered and dissolved with hot circulating dilute HCl. Surplus HCl was removed by using ion exchangers Dowex No 1 and No 3 [26–29]. Subsequently, the solution was saturated with carbon dioxide.

Inoculation of the different culture media consisting of silicic acid [30,31], slabs of non-glassy porcelain [32], and blocks of quartz [33], was carried out using standard methods. 4 to 6 petri dishes containing culture media were put into a closed glass vessel which was kept in an oil bath to keep the temperature constant. The vessels were evacuated, rinsed and filled with carbon dioxide.

After 14 days, the petri dishes were checked for the first time. The porcelain slabs did not show evidence of bacterial growth. The silica media kept at temperatures of 40°C, 45°C, and 50°C showed the beginning formation of bacterial colonies (diameter of the colonies was about 50 µm) and the quartz blocks incubated at 45°C, 50°C, 55°C, and 60°C showed a rapid growth of organisms. After two months, crusts developed in the cultures colonizing the quartz blocks. After having grown bacteria from the biotite gneiss, we think it is proven that there are living microbes in this rock."

Because it was important for Neher and Rohrer to

prove that there are really living organisms at 160 m depth, they did not pay much attention to the crusts formed. A later examination by X-ray diffractometry (XRD) showed that these crusts essentially consisted of dolomite with crystal sizes of up to 60 μ m (Fig. 2). For the present-day geologic community the understanding of microbially mediated formation of dolo-

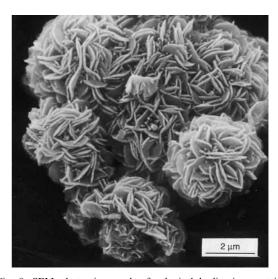


Fig. 8. SEM photomicrograph of spherical bodies in quartzite which are encrusted by rosette-arranged platy hematite (Fe_2O_3).

mite is of great importance, because the origin of dolomite has been a controversial topic in geology for more than 150 years [12]. Unfortunately, the microbially mediated dolomite precipitation remained a by-product of Johannes Neher's and Ernst Rohrer's experiment and became forgotten.

4. Fungal dissolution of quartz and precipitation of iron phases

The effect of microbes on the dissolution of silica and the nucleation of iron crystallites has been reported from numerous environments, such as organic-rich ground water [34,35], hydrothermal environments [36-38], from a desert area [39], and from laboratory experiments [40]. In contrast to these descriptions which are generally associated with elevated temperatures, Johannes Neher documented similar processes going on at low temperatures in an Alpine environment covered by snow for almost 6 months per year. In the fifties, he discovered a yellowish quartzite lens within the Triassic Hauptdolomite Formation of the Austroalpine nappes near the Bernina Pass, Grisons, Switzerland. Interestingly, other quartzite lenses in the same formation did not have this conspicuous colour feature. By this observation, Johannes Neher has illustrated that the 'yellow' quartzites are heavily attacked by filamentous microbes (Fig. 3). However, he did not know the origin of the filaments.

These filaments typically occur parallel to mechanically weak zones in the quartzite. Within these zones, they are found both along edges of adjacent quartz crystals and on quartz faces. Occasionally, filaments occur within vein quartz crystals, into which they bored subvertically from the crystal face by dissolving the SiO₂ (Figs. 4 and 5). They are approximately 3-9 µm in diameter and up to 300 µm long. They consist of a hollow internal part (approx. 1 µm in diameter) and an encrusted rim. SEM obervations show that most of the organic filament material has disappeared, leaving an encrusted etch mark on the quartz surfaces. The encrustations often consist of spherical bodies forming a chain-like structure along the former filament (Fig. 6). They show a variety of crystal structures and consist mainly of needle-like goethite (FeOOH)

(Fig. 7) or rosette-arranged platy hematite (Fe_2O_3) (Fig. 8). These iron phases account for the unique color of the quartzite.

Recent studies show that the variety and arrangement of the spherical iron crystallites along the filaments have a striking similarity to calcium oxalate crystals arranged along different fungal filaments cultured in the laboratory [41]. These calcium oxalates apparently formed in association with the fungal secretion of oxalic acid in the presence of calcium ions. Also filaments found to attack quartzites were recently determined as fungi of the class Basidiomycetes (F. Graf, pers. commun.). Quartz dissolution seems to be accelerated and controlled by organisms [42], such as fungi, if they produce high concentrations of oxalic acid. Therefore, it is assumed that quartz dissolution and iron crystallite formation in the quartzites investigated are associated with the presence of oxalic acid secreted by fungal filaments.

5. Discussion

The two projects summarized in this paper are of fundamental geological significance, but have never received the attention and the acknowledgement of the scientific community.

Firstly, many of the ancient carbonates consist of dolomite. Thus, their formation, which is still speculative today, is of major interest in carbonate sedimentology. Ironically, Johannes Neher was not interested in the dolomite which his experiments produced. Had this aspect of the experiments been persued this would have been the first study to contribute to understanding the principle of dolomite formation. Unfortunately, most of the rock material which Neher and Rohrer had used in their experiments is now lost. Therefore, we cannot reconduct the experiments under similar conditions. Vasconcelos [13] and Vasconcelos et al. [14] suggest that microbes play an important role in dolomite formation or at least create chemical conditions in a micro-environment in which dolomite precipitates. This suggestion leads to the question whether such chemical conditions existed during the formation of the large ancient dolomite deposits. A reevaluation of Johannes Neher's work could contribute significantly to this question and stimulate further research.

Secondly, dissolution of silicates is important in the weathering of rock and in cycling silicon in nature. Within the pH range of most natural waters, silicate dissolution is extremely slow. However, at high concentrations of multifunctional organic acids, such as ascorbic, citric, and oxalic acid, quartz dissolution is accelerated and its solubility is controlled by microorganisms [35], [43].

Silicates exist in monomeric form as well as polymeric form. Microbes assimilate silica only in its monomeric form. Thus, the biogenic dissolution of quartz and its depolymerization, the detailed mechanism of which is still not understood, are of fundamental significance in the biological silica cycle [44,45]. Organisms, such as fungi, which degrade silicates, are important mediators for those organisms which assimilate and concentrate silica, i.e. diatoms, and they are dominant agents for rock weathering.

With his spectacular discovery of quartz dissolution by fungal filaments in the yellow quartzites near the Bernina Pass more than twenty years ago, Johannes Neher greatly contributed to a field of geomicrobiology which is still in an early stage of development. His illustrative investigations clearly demonstrate the important role of microbes in the weathering of quartz, particularly in an Alpine environment. However, he has left the detailed revelation of these processes to a new generation of scientists.

6. Conclusion

Johannes Neher did not approach and follow science in an orthodox way. With his respect for the importance for living things he was convinced that microbial life plays an important role in the formation and decomposition of rocks which are generally considered to have formed abiotically. Therefore, he concentrated on the search for biological evidence in all types of rock. With his convincing illustrations and experiments, he demonstrated that microbes are really important in rock formation and weathering. However, as a geologist his knowledge of chemical and biological processes was insufficient and a collaboration with other scientists, such as microbiologists, was impossible for many reasons. Thus, he was never able to understand the details of the geomicrobial processes investigated. As a consequence, he was never accepted by the scientific community. Nevertheless, his discoveries remain spectacular. We can not yet foresee the geological significance of all of Johannes Neher's projects. However, they will serve as a base for a wide spectrum of research in the field of geomicrobiology for a future generation of scientists.

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