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The Use of ESEM in Geobiology

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1. Introduction

Geobiology is an interdisciplinary field of research that explores the interaction between the biosphere and the geosphere and/or the atmosphere. It involves researchers from numerous fields such as paleontology, microbiology, mineralogy, geochemistry, biochemistry, sedimentology and genetics. Geobiological research cover a wide range of areas like, for example, the origin and evolution of life, environmental microbiology, microbe-mineral interactions, molecular ecology and detection of biomarkers. It is responsible for at least two major subdisciplines: geomicrobiology (the study of microbe-mineral interactions) and astrobiology (a discipline focused on the conditions for life in the universe, including the search for life on other planets).

A major part of most geobiological research is focused on interactions between microorganisms and minerals or other substrates. This includes everything from microbes in soil and sediments, via fossilized microorganisms in rock and minerals, to extremophiles at hydrothermal vents. There are numerous methods, protocols and instruments that are used for this kind of research but one of the most basic methods and commonly used is the Scanning Electron Microscope (SEM). SEM is easy operated and can give high resolved images down to micro meter size, which is a requirement when analysing microorganisms. Coupled with, for example, an energy dispersive X-ray spectroscopy (EDS or EDX) detector it becomes a valuable tool for elemental analysis which is a critical part of geobiological research. Microbe-mineral interactions most commonly result in micro-sized biomineralizations or amorphous precipitates that may contain important information about the metabolism and life-cycles of the microbes, redox chemistry in the microbial habitat, and paleoenvironmental conditions.

Geobiological samples usually involve living species of microorganisms or fragile materials like fossilized microorganisms, organic matter or hydrated minerals like clays that collapse in conventional SEM. Environmental Scanning Electron Microscopy (ESEM) is a modification of conventional SEM originally developed for the study of biological samples but has also become more frequently used within geobiological related research. With its gaseous environment in the specimen chamber as well as other technical applications ESEM makes it possible to study wet and uncoated specimens in their natural state. This is a substantial advantage opposed to conventional SEM.

The main difference between ESEM and SEM is that the first has a specimen chamber where the specimen can be imaged while gas is present. The specimen chamber is designed to maintain water in its liquid phase and for that a minimum water vapour pressure of 609 Pa (6.09 mbar or 4.579 Torr) is required at O^{o} C. This creates new possibilities: A) Hydrated specimens can be examined in contrast to SEM where specimens are desiccated by the vacuum. Thus biological specimens can be maintained fresh and live. B) Specimens do not require preparation techniques used in SEM such as the deposition of a thin gold or carbon coating. Such techniques sometimes require vacuum and can disturb the samples. Biological samples also need to be dehydrated before coating which is a time consuming process. The gas in ESEM is electrically conductive due to the ionization, which prevents that negative charge accumulates and this is the reason why specimens do not need to be coated prior to examination. Thus, with ESEM specimens can be examined faster and more easily, without complex and time consuming preparation methods and without modifications or in worst case damage to the sample surface by preparation work and exposure to vacuum. The aim with this paper is to give a brief background to geobiology and ESEM, and to show the advantages of ESEM over conventional SEM in the study of geobiology.

2. Geobiology: The link between geology and biology

Geobiology as an independent discipline is relatively new and has attracted a lot of attention during the last decade with the result of an increased number of active researchers, foundation of international scientific journals as well as centers and institutions worldwide devoted to geobiological research. However, the link between life and geological processes can be traced back as far as the foundational text in modern geology by James Hutton (1788). He documented quite ordinary observations that anyone could have done about erosion of land into the oceans by rivers and the presence of fossilized shells in sedimentary rocks in the mountains of Scotland. What Hutton managed to do was to put these observations in a context where he questioned the surface of Earth as a sustainable habitat for life. In the early part of the 20th century Vernadsky (1926) further explored the connection between life and geological processes, and Baas Becking (1934) coined the term geobiology and outlined many geobiological processes much as we see them today.

The last decades have involved a growing awareness of the close connection between the physical world and life sciences. Earth as a system is complex and not as black and white as previously thought. The traditional way when studying the Earth system in dividing it into separated disciplines like geology, biology, chemistry or physics are not always the most practical approach. An interdisciplinary perspective and awareness when looking at the Earth system is almost a requirement to understand it and move forward in Earth sciences. Geobiology is a result of interdisciplinary thinking within geology and biology and their subdisciplines, and geobiology as a science has shown that there is no distinct boundary between the both. They are tightly connected and interact with each other on many levels, both at the present but also throughout Earth´s history (Knoll, 2003). The evolution of life has been intimately connected with the mineral evolution (Hazen et al., 2008), the rise of continents (Rosing et al., 2006), emergence of the aerobic biosphere (Melezhik et al., 2005), formation of fossil fuel and ore formations (Southam & Saunders, 2005). Ever since their emergence on Earth microbes have played an important role as geological agents involved in mineral growth and dissolution, rock and mineral weathering and alteration, mobilization of metals, cycling of

elements in the ocean and the lithosphere, metabolism of hydrocarbons and transformation of organic carbon, fractionation of stable isotopes etc (e.g. Lindsay and Brasier, 2002; Tice and Lowe, 2004; Lowe and Tice, 2007; Furnes et al., 2008). But it is not only biological activity that influences geological processes. It works both ways and geological processes control and influence the microbial ecology as well, something that is explicit in, for example, extreme environments (Huber et al., 2007). The discovery of the subsurface biosphere has deepen the knowledge of life´s distribution, adaptability and variety (Pedersen, 1993). Earths surface is no longer the limit for habitability. The subsurface is just as colonized and may contain as much as one third of the Earths biomass (Gold, 1992; Staudigel et al., 2004). Life in extreme environments and in the subsurface further show that Earth itself may not be the limit for life but that it may extend beyond. Astrobiology has shown that the conditions for life exist on other planets as well, and that life could have originated or been transported there, and possibly be able to sustain (Farmer and Des Marais, 1999).

3. Environmental Electron Scanning Microscope (ESEM)

3.1 History

Experimental approaches to examine specimens in chambers filled with water or atmospheric gas with conventional and scanning transmission types of electron microscopes were reported of as early as the 1940s (Ardenne and Beischer, 1940; Abrams and McBain, 1944; Swift and Brown, 1970; Parsons et al., 1974). Such experiments used different kinds of "environmental cells" where gas was introduced temporarily during the examination, however, neither of these experiments succeeded in creating a stable environmental cell for routine analysis. In 1970 the first images of wet specimens in an SEM were published by Lane (1970), who injected a jet of water vapor over the point of observation at the specimen surface. The gas diffused in the chamber without any damage to the instrument. The need for differentially pumped chambers to allow for the transfer of the electron beam from the high vacuums in the gun area to the high pressures in the specimen chamber forced developments during the 1970s. Shah and Beckett (1977) reported of differentially pumped chambers to maintain botanical specimens conductive for signal detection, and Robinson (1974) reported of improvements by combining a backscattered electron detector with differential vacuum pumping and introduction of water vapour around 600 Pa at the freezing point of temperature. In 1978, Gerasimos Danilatos, a Greek-Australian physicist started to work with Robinson at the University of New South Wales in Sydney, Australia, and designed the original ESEM that were operable at room temperature and high pressures up to 7000 Pa (Danilatos and Robinson, 1979). During the 1980s and early 1990s Danilatos developed and optimized the design of the ESEM (Danilatos, 1981, 1985, 1988, 1990a, b; Danilatos and Postle, 1983). He reported the construction of an ESEM capable of working at any pressure from vacuum up to one atmosphere, optimization of the use of differential pumping systems combined with electron backscatter detectors, the idea of the environmental gas itself as detection medium, and the invention of the gaseous detection device (GDD).

3.2 Description of ESEM

The functioning of an ESEM is in many ways identical to a conventional SEM and it is assumed that the reader is familiar with the operation of a SEM. Basically, an ESEM is a

SEM that can operate at the low pressure of a usual SEM through to, at least, the pressure required to observe liquid distilled water. An ESEM, just like a SEM, employs a scanned electron beam and electromagnetic lenses to focus and direct the beam on the specimen surface. A very small focused electron spot is scanned in a raster form over a small specimen area and the beam electrons interact with the specimen surface layer and produce various signals. These signals are collected with appropriate detectors and can be monitored in the form of images, graphs, digital recordings etc. Beyond these common principles, the ESEM deviates substantially from a SEM in several respects briefly outlined below.

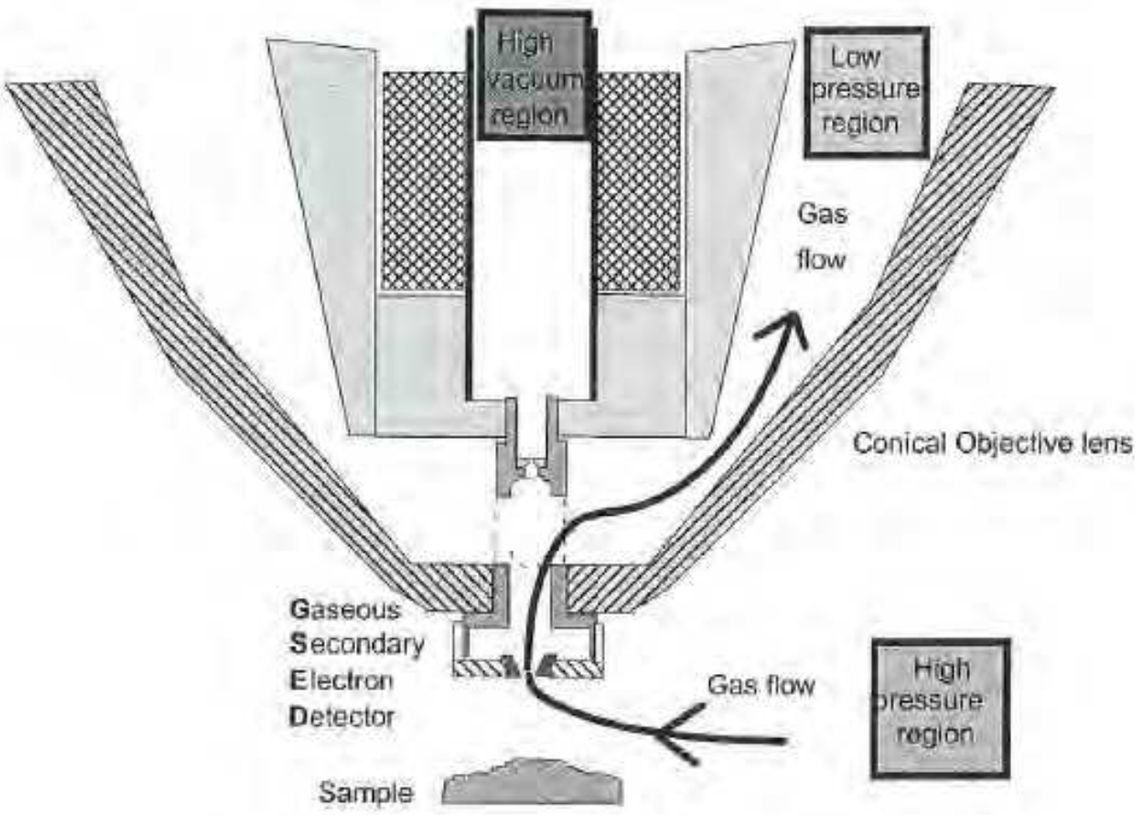


Fig. 1. Schematic illustration of an ESEM lens. Cross section showing the high vacuum region and low pressure region as well as the direction of the gas flow. From the XL30 Options Manual, FEI and Philips (1998).

3.2.1 Separated regions

The ESEM must have, just like a conventional SEM, a high vacuum region (usually with pressure less than 10^{-2} Pa) for the generation and focusing of the electron beam. In a SEM the high vacuum region and the region of the specimen is the same but in an ESEM these two regions must be separated (Fig. 1). The specimen chamber in an ESEM is designed to maintain water in its liquid phase and for that a minimum water vapour pressure of 609 Pa (6.09 mbar or 4.579 Torr) is required at O^{o} C.

3.2.2 Differential pumping

The basic principle of an ESEM is, thus, to have a high pressure and gaseous specimen chamber, and a high vacuum electron optics area separated from each other but still connected to allow for the transfer of the electron beam. The two regions are separated by at least two small pressure limiting apertures, one aperture that separates the high vacuum region of the electron gun and an intermediate cavity. The second aperture separates the intermediate cavity and the high pressure specimen cavity. Gas leaking from the specimen chamber through the aperture to the intermediate cavity is instantly removed by a pump system. This is called differential pumping. Gas that escapes further into the high vacuum area of the electron optics is similarly removed by a pump to maintain required vacuum. Additional pumping stages may be added to achieve an even higher vacuum in the electron optics area.

3.2.3 Electron beam transfer

An electron beam generated in the vacuum of the upper column will on its way through the intermediate cavity and in the specimen chamber come in contact with an increasing amount of gas molecules. This will result in a gradual loss of electrons due to electron scattering by the gas molecules and eventually total loss of the beam. However, the electrons are scattered over a broad skirt-like area around the focused spot and since the skirt width is orders of magnitude greater than the spot width, the skirt only contribute background noise and the amount of electrons in the original focused spot is enough for imaging of the specimen. The remaining electron beam is, however, only a fraction of what it was in the upper column and merely enough for imaging. The particular conditions of pressure, distance and beam voltage is crucial for signal detection and the operation of an ESEM is centered on refining the instrument for optimum performance and achieving precision for the instrument to operate close to its physical limit (Danilatos, 2009). By doing this it is possible to use an ESEM in much the same way as a SEM. Secondary and backscattered electrons, X-rays and cathodoluminescence is generated as in a SEM and can be detected with slight modifications to the detectors. The main difference regarding detectors is that the conventional secondary electron detector of SEM, the Everhart-Thornley detector, can not be used in the presence of gas and thus the gaseous detection device (GDD) has been developed. The principle of the GDD is that the environmental gas itself is used for beam transmission and as a detector of the electrons (Danilatos, 1997, 1990a), compared to the Everhart-Thornley detector where light guide the transmitted electrons. In a GDD the signals emanating from the beam specimen-interaction interact with the surrounding gas in the form of gaseous ionization and excitation. The ionized gas is then collected by electrodes and the signal is amplified for its purpose.

3.2.4 Specimen charging

In conventional SEM negative charge is accumulated as the electron beam impinge on the surface of the specimen. This tends to deflect the electron beam from the scanned point with the result of charging artefacts on the image which greatly disturbs the imaging and analyses. This is normally eliminated in conventional SEM by coating the specimen prior to examination by a thin layer of usually gold or carbon. The gas in ESEM is electrically conductive due to the ionization, which prevents that negative charge accumulates and this is the reason why specimens do not need to be coated prior to examination.

3.2.5 Disadvantages

Even though ESEM involve several substantial advantages over conventional SEM there are a few disadvantages. Some of these disadvantages can be limited by instrument design and the disadvantages differ between various instrument manufacturers. The main disadvantage is the distance in the specimen chamber over which the electron beam remains usable in the gaseous environment. The useful distance is a function of accelerating voltage, beam current, nature and pressure of the gas, and of the aperture diameter used. The distance varies from ~10mm to less than 1mm depending on the gas pressure. Another result of the limitation of useful specimen distance is the limitation of magnification. At very high pressure the distance becomes so small that the field of view is limited by the aperture width. The vacuum in a conventional SEM result in a superior magnification range compared to an ESEM.

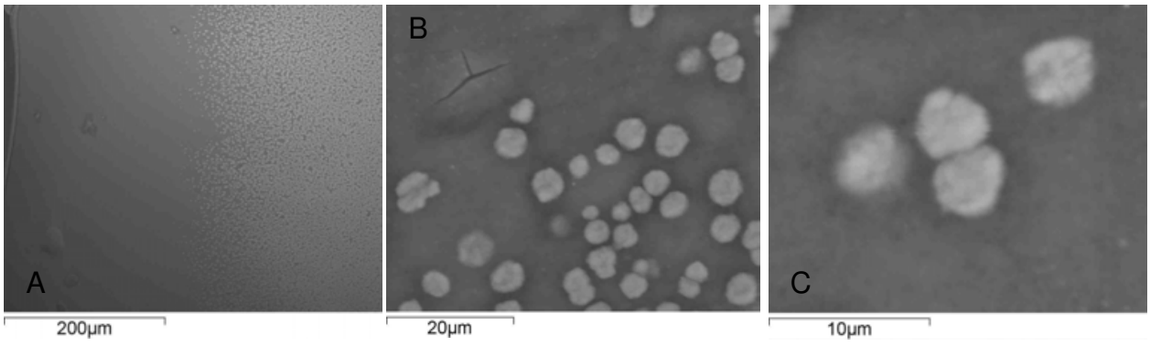


Fig. 2. ESEM images of plant-growth-promoting rhizobacteria. A) Image showing an overview of the bacteria grown on an agar plate. B) A close up showing the coccoidal morphology of the bacteria. C) Image showing mitosis.

The presence of gas may also generate various disturbances in certain applications, like, for instance, the resolution of the image. This issue can, however, be limited by altering chamber pressure and accelerating voltage. It is needed for each instrument to find the most useable combination and correlation between the parameters.

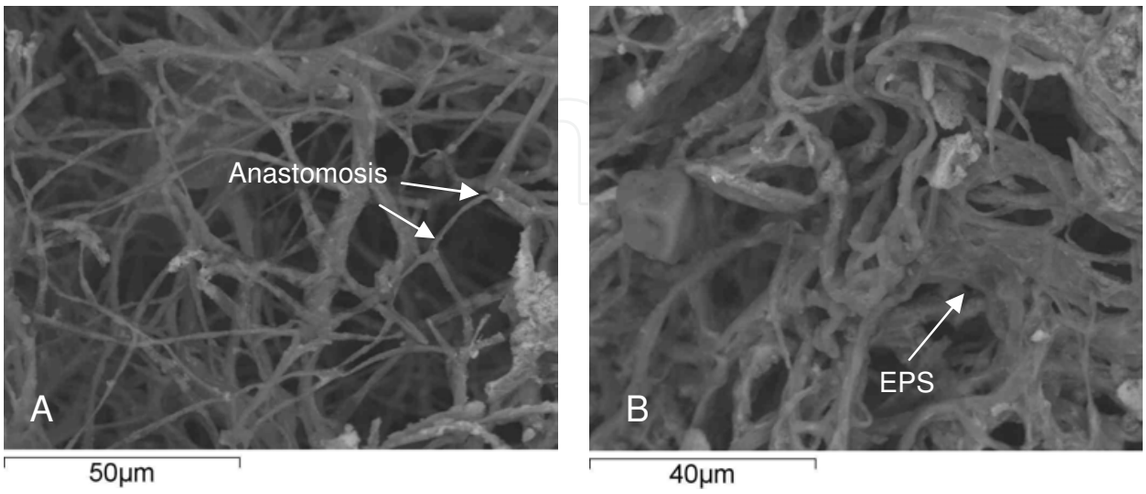


Fig. 3. ESEM images of an ectomychorrizal fungus. Note the frequent branching and anstomoses between branches. In B EPS has been precipitated in the fungal mycelium.

4. The use of ESEM in geobiology

As mentioned in sections 1 and 2 geobiological research is performed at the intersection where geology and biology meet. One could ask why the use of ESEM in geobiology differs from conventional geological or biological use of ESEM, and of course, they sometimes overlap. Microorganisms are studied with ESEM within microbiology (e.g. Bergmans et al., 2005; Ahmad, 2010), and minerals, sediments and substrates is studied with ESEM within traditional geology (e.g. Donald, 2003; Reed, 2005; Huiming et al., 2011). However, the main difference is that within geobiology the interaction of these two fields is examined. Geobiology represent the point at which life starts to interact with the physical world and the outcome of this is usually very fragile such as living, encrusted or fossilised microorganisms, amorphous, hydrated minerals or substrates like clays, and combinations of these like biomineralisations or mineral trapping biofilms and EPS (extra cellular polymeric substances). ESEM has been used in various geobiological studies (e.g. Little et al., 1991; Douglas and Douglas, 2000; Nealson et al., 2002; Hallberg and Ferris, 2004; Waters, 2008), and in the following sections we will try to illustrate some of its applications. In example I live microorganisms (bacteria and fungus) from soils collected at various locations in Sweden will be studied. In example II drilled rock samples from the oceanic seafloor in the Pacific Ocean will be used to illustrate the exploration of the deep subseafloor biosphere and how the interaction between microorganisms and mineral substrates can be studied, and also how sensitive hydrated minerals can be if they are not treated in a proper way.

4.1 Instrument

In this study an XL30 environmental scanning electron microscope with a field emission gun (XL30 ESEM-FEG) was used. The ESEM was equipped with an Oxford x-act energy dispersive spectrometer (EDS), backscatter electron detector (BSE) and a secondary electron detector (SE). The acceleration voltage was 20, 15 or 10 kV depending on the nature of the sample and the instrument was calibrated with a cobolt and a carbon standard. Peak and element analyses were made using INCA Suite 4.11 software.

The high vacuum mode was in some tests used as an equivalent to the conditions of a conventional SEM.

4.2 Example I: Live microorganisms

Microorganisms used in geobiological related research is usually collected from natural environments, isolated and grown in laboratory for further studies or experiments. This is a time consuming and expensive procedure but beyond the scope of this paper and, thus, will not be described in further detail.

First of all, we have used a plant-growth-promoting rhizobacteria. These bacteria have been isolated from soil samples where they existed in symbiosis with fungi and then grown on an agar plate (Fig. 2). With the aim of ESEM we are able to view the bacteria at micrometer scale and study their morphology. The bacteria are coccoidal and their main diameter range between ~1 to 3 μm. It is also possible to observe such feature as mitosis (cell division) (Fig. 2C).

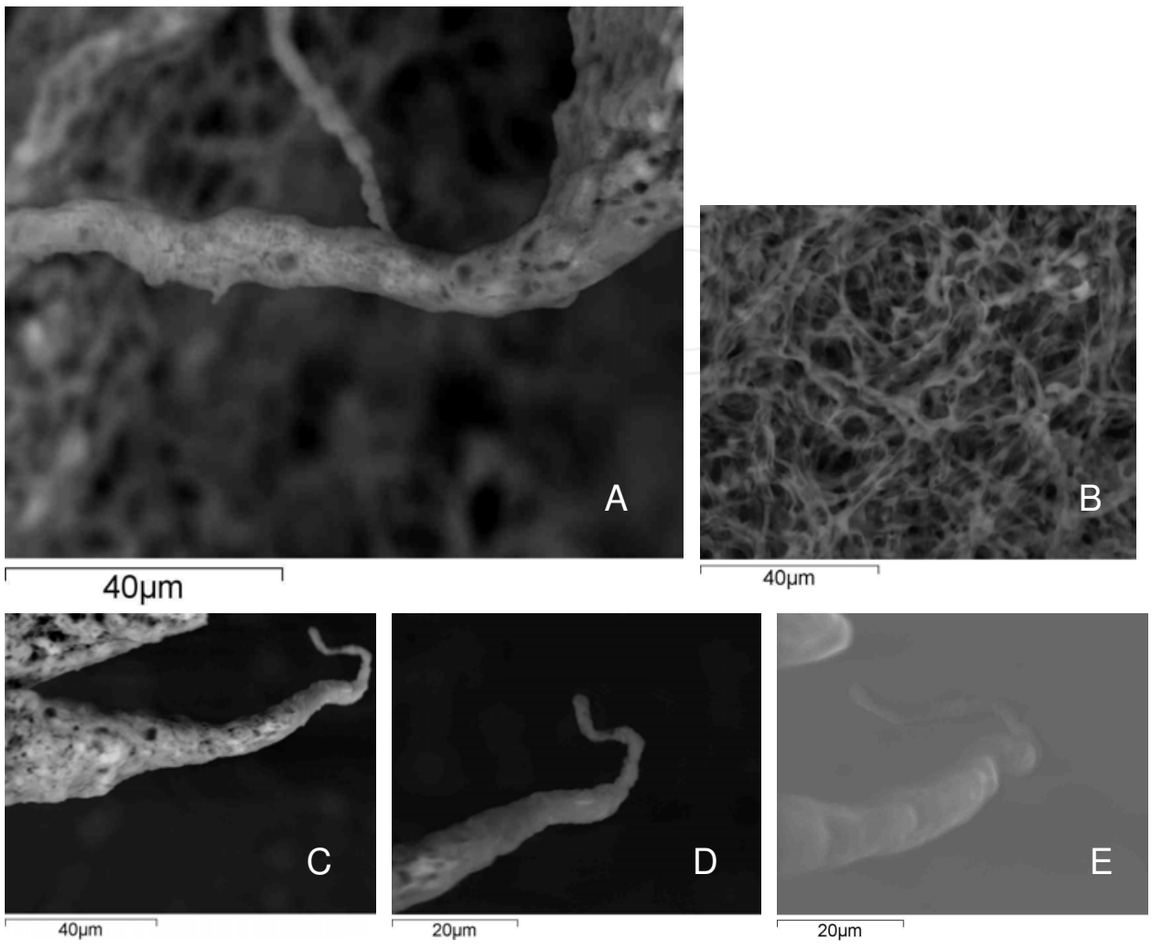


Fig. 4. ESEM images of *Suillus Granulatus* grown in a liquid media. A) Close up of a hypha. B) An overview image showing the mycelium. C-E) Images showing the same hyphae at various conditions: C) low vacuum (2mbar), D) 1mbar, and E) wet mode.

Secondly, an ectomychorrizal fungus grown on an agar plate has been used (Fig. 3). This fungal mycelium show characteristic fungal morphologies as frequent branching hyphae and anastomosis between branches (Fig. 3A). The diameter of the hyphae varies between a few micro meter to ~10 µm. It is also possible to see production of EPS on the fungal mycelium (Fig. 3B).

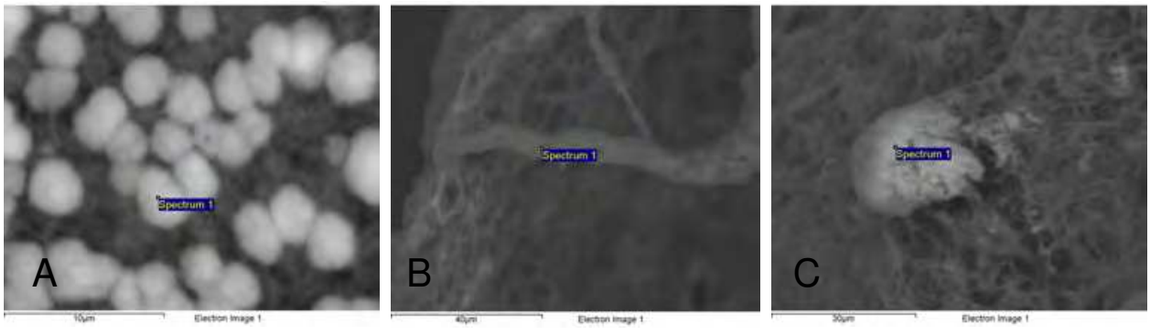
Thirdly, a fungus, *Suillus Granulatus*, grown in liquid media has been used. It is a fungus with traditional fungal morphology (Fig. 4). It is characterized by long, curvi-linear hyphae, 3-10 µm in diameter and several hundred µm in length, creating a complex mycelium. Biomineralizations are frequently occurring in this mycelium and ESEM mode makes it possible to analyse them with EDS without coating, which may disturb the analysis (Fig C in Table 1).

Figures 4C-D show one of the issues that may occur with ESEM. The presence of gas in the specimen chamber results in a blurry and unfocused image at high magnifications. To achieve the best quality of the images we experimented by altering the pressure in the specimen

chamber. Figures 4C-E illustrate how the quality of the images change with varied pressure. Wet mode resulted in poor contrast, high vac resulted in charging artifacts, but the best image quality was achieved with low vacuum at 2mbar. At that pressure and in that mode a high quality image could be produced as well as an element spectrum that is of great advantage. We tried to document the microorganisms in an ordinary SEM as an illustrative example but failed to produce images due to charging artefacts. However, conventional SEM is not a realistic option to study these samples. The microorganisms would collapse and to prevent that a dehydration process and coating would be required, which is time consuming and would destroy the samples and a lot of information that they contain.

4.2.1 EDS analysis

Element analysis with EDS is also possible to perform in ESEM mode on the live microorganisms and associated biomineralizations without gold or carbon coating (Table 1). In live material and in biomineralizations analyses of the carbon content is sometimes of highest priority and would be impossible to do with carbon coating.



	A	B	C
C	24.72		
O	21.71	57.87	46.63
Na	14.18	1.81	
Mg		1.95	
P	2.79	20.38	22.08
S	1.65	1.64	
K	8.96	6.12	3.36
Cl	25.98	0.91	
Ca		4.45	0.99
Fe		4.87	26.94
Total	100.00	100.00	100.00

Table 1. EDS data in wt %. A) A plant-growth-promoting rhizobacteria, B) *Suillus Granulatus*, C) biomineralization in the hyphal network of *Suillus Granulatus*. It is difficult to identify the mineral but it appears to be a Fe and P-rich oxide. Normally a mineral phase with such high Fe content needs to be coated before analysed by EDS.

4.3 Example II: Hydrated minerals and fossilized microorganisms

Samples of subseafloor basalts from the Emperor Seamounts in the Pacific Ocean, drilled and collected during Ocean Drilling Program (ODP) Leg 197, are used to present the advantages of ESEM in the study of hydrated minerals, fossilized microorganisms and interactions between the both. For a detailed description of the sampling sites, mineralogy and biogenicity of the microfossils see Ivarsson et al. (2008a, b) and Ivarsson and Holm (2008). Briefly, the samples consist of veins and vesicles in basalts. These veins and vesicles are partly filled by hydrothermally formed secondary mineralisations of calcite, zeolites and clays (Fig. 5). The vein walls are usually coated with a montmorillonite phase ($(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2 \cdot nH_2O$) and successively with carbonates ($CaCO_3$) or zeolites species such as phillipsite ($K_2(Ca_{0.5}, Na)_4(Al_6Si_{10}O_{32}) \cdot 12H_2O$), chabazite ($Ca_2(Al_4Si_8O_{24}) \cdot 12H_2O$), and tetranatrolite ($Na_{16}(Al_{16}Si_{24}O_{80}) \cdot 16H_2O$). Zeolites and calcite seldom occur in the same void probably due to local differences in the composition of the hydrothermal fluids but they do exist in the same system with interconnected veins and vesicles. In addition, several of these veins and vesicles contain complex networks of fossilized filamentous microorganisms (Figure 6A-C). These microfossils are composed of a similar montmorillonite phase as is found on the vein walls.

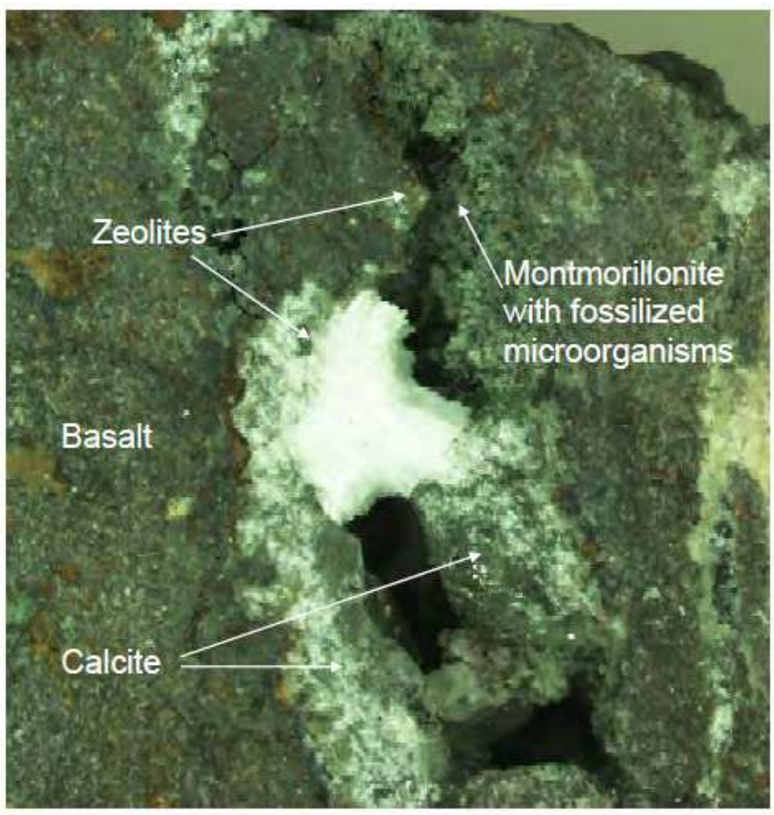


Fig. 5. Optical micrograph. A vein in basalt from the Emperor Seamounts in the Pacific Ocean, drilled and collected during Ocean Drilling Program (ODP) Leg 197. The vein contain secondary mineralisations of calcite, zeolites and montmorillonite (clay), and fossilized microorganisms on the vein walls.

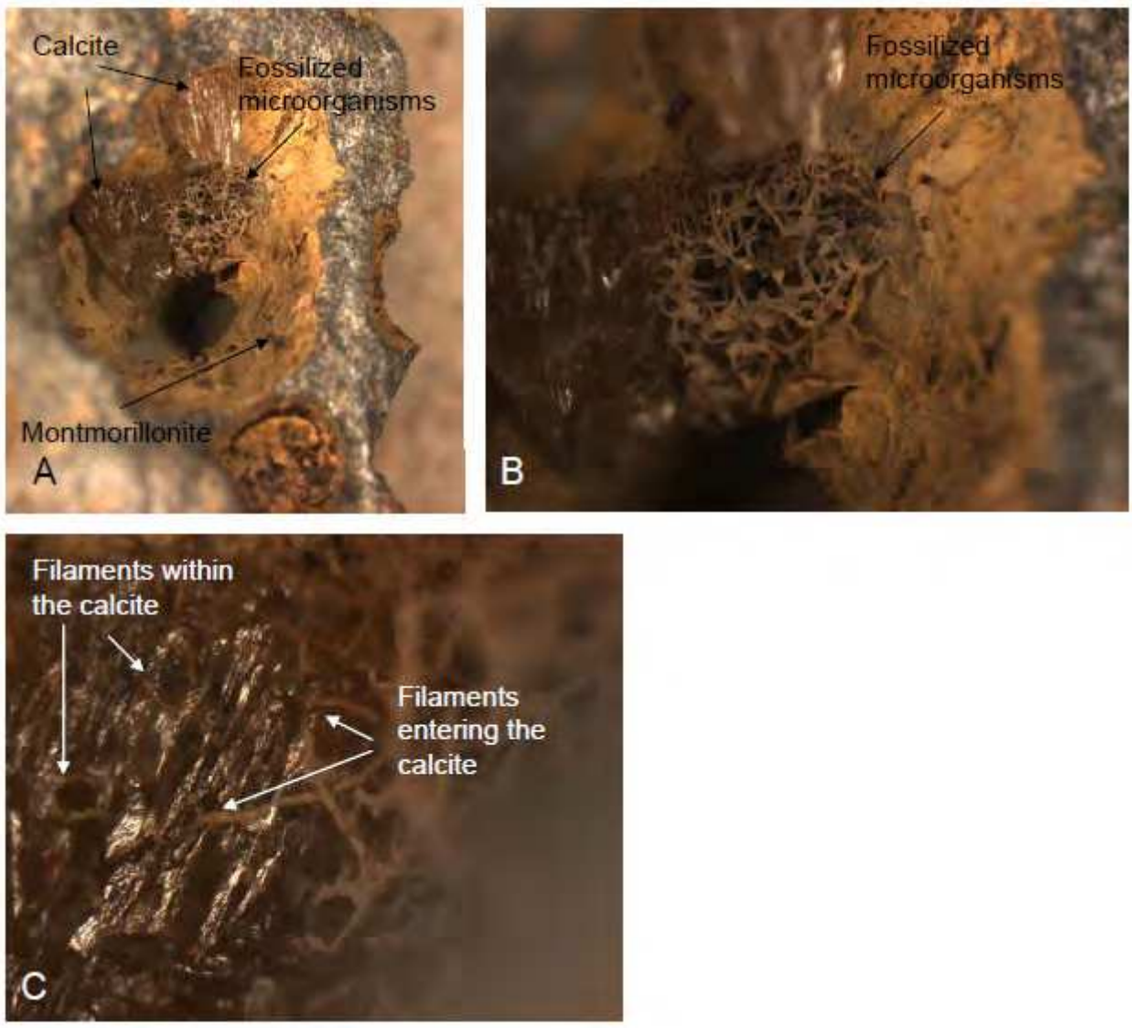


Fig. 6. Optical micrographs. A) Showing a vesicle in basalt from ODP Leg 197 with calcite crystals and montmorillonite grown on the vein walls and an assemblage of fossilized microorganisms. B) Close up of the network of fossilized microorganisms seen in A. C) Image that shows how the fossilized filaments have penetrated into the mineral substrate.

4.3.1 Preparation

Due to the nature of the samples with partly filled veins or vesicles, and the fragile nature of the microfossils the samples are not prepared as thin sections but the veins and the vesicles are sawed to small cubes (~1x1 cm in diameter) from the original drill cores. Attempts were made to expose as much as possible of the content of the vesicles but differences in height are difficult to avoid and the samples are far from being as horizontal as the surface of thin sections. Coating these samples is also not an option due to their nature as sawed cubes and due to the fragile nature of the fossilized microorganisms.

The zeolites and the clays are both hydrated minerals and contain crystalline H_2O. Analysing them in SEM would result in desiccation and eventually crystal collapse.

However, by studying the minerals with a gaseous atmosphere in ESEM they maintain their mineral structure and collapse is avoided. This is a substantial advantage over conventional SEM. Another advantage is that the samples do not need to be coated, which in this case is impossible due to the sensitivity of the samples.

4.3.2 Fossilized microorganisms

The ESEM analysis of the fossilized microorganisms gives us a much more detailed description of the morphology, occurrence, preservation and composition of these structures than optical microscopy do (Fig 7). The filaments are up to several hundred µm in length and 5- 20 µm in width depending on where in the network they occur, smaller diameter closer to the attachment in the minerals and wider diameters further from the attachment. They branch frequently and in some cases anastomoses between branches occur. They consist of an inner part and an outer part. Both the inner and the outer part are usually 5 to 10 µm in width depending on the total diameter of the filament. They mainly consist of a clay phase that compositionally corresponds to montmorillonite. It is possible to see that the filamentous networks are attached directly onto the vein walls but also penetrating calcite or zeolite crystals. The morphology and occurrence of these fossilized microorganisms resolved by ESEM correspond to fungal morphology rather than filamentous prokaryotes, thus, with the aim of ESEM it is possible to characterize the microfossils and determine what type of microorganisms they once were.

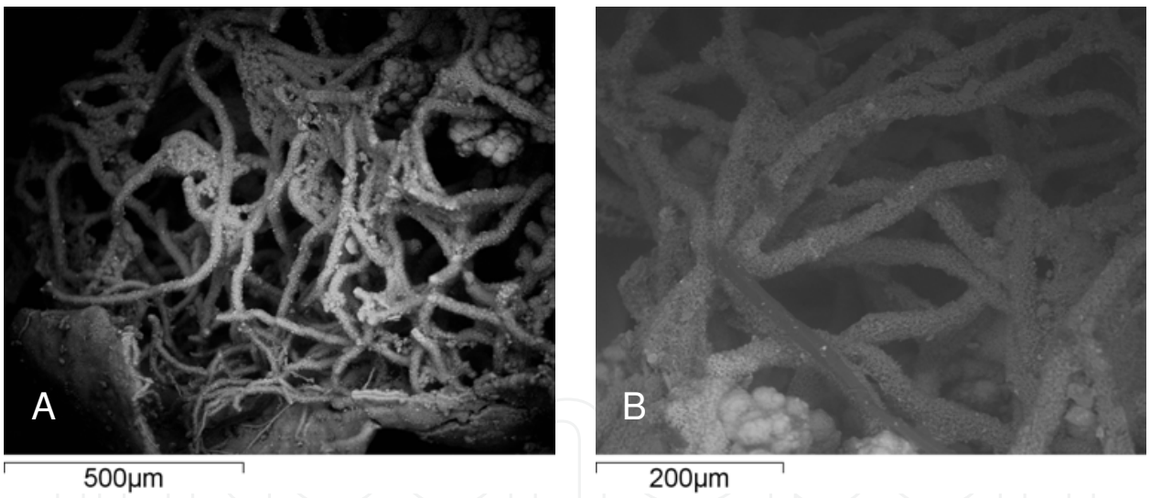


Fig. 7. ESEM images. Images showing how the fossilized microorganisms occur in the veins.

4.3.3 Hydrated minerals

The minerals in the investigated veins and vesicles consist of clays and zeolites, two groups of minerals that contain crystalline H_2O in their crystal structure. The ESEM images show how the montmorillonite looks on a micro meter scale and it allow relative good EDS analyses despite the differences in focal depth (Fig. 8, Table 2). Montmorillonite form in aqueous environments thus the fact that the microfossils consist of montmorillonite indicate that the microorganisms lived while the vesicles were circulated by hydrothermal fluids. This makes it possible to constrain a time window when the microorganisms existed in the

system – while the hydrothermal system was active and the volcanism still was active. This is a strong argument for interpreting the microfossils as syngenetic with the rock, the secondary mineralizations and the hydrothermal activity, and not being a modern contaminant.

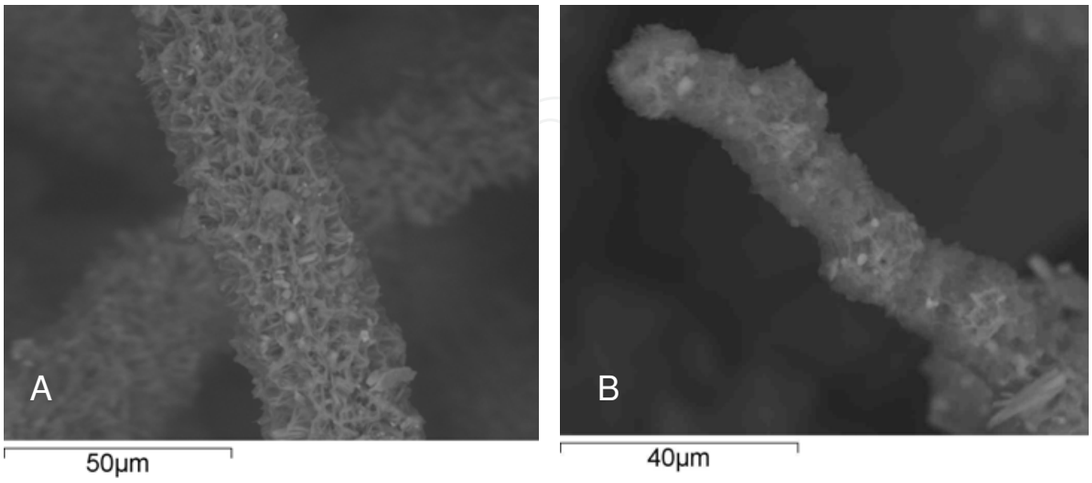
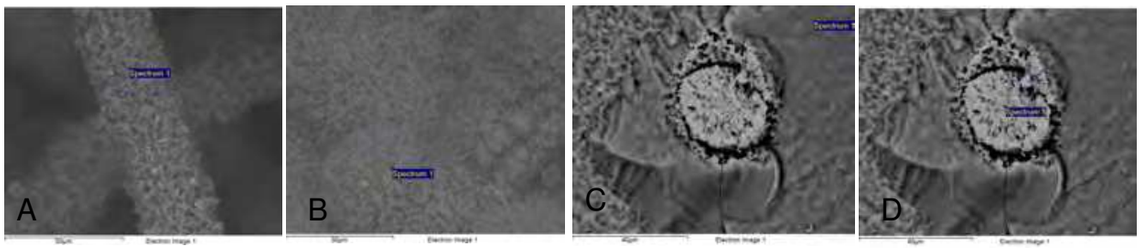


Fig. 8. ESEM images of fossilized microorganisms. A) ESEM image showing the well crystalline clay phase the microfossils consist of. B) ESEM image after the microfossil have been subject to high vacuum. Note how the clay phase has been desiccated and appear collapsed.



	A	B	C	D
Mg	7.96	3.65		11.40
Na			3.61	0.84
Al		2.99	12.27	8.43
Si	18.95	9.65	30.13	22.62
K			1.15	0.44
Ca		1.07	4.37	1.12
Fe	32.36	46.26		9.48
O	40.73	36.37	48.47	45.67
Total	100.00	100.00	100.00	100.00

Table 2. EDS data in wt%. A) Close up of a fossilized microfossil showing the structure of the clay phase. B) The clay phase of the vein walls. C) Zeolite. D) Cross section of a fossilized microorganism that dissolved the zeolite producing a tunnel structure while it was alive.

Tests were done in high vacuum to analyse these mineral phases but failed due to charging artifacts. However, ESEM mode made it possible to produce high resolved images and EDS analyses. Figure 8 illustrate how a fossilized filament consisting of montmorillonite looks like in ESEM and how a fossilized filament look like after being subject to high vacuum mode which is equivalent to SEM mode.

4.3.4 Microbe-mineral interactions

In optical microscopy it was possible to observe that the microorganisms had penetrated the zeolite and to some extent the calcite crystals during their existence with the result of long micro sized tunnel structures. Microbially produced cavities or tunnel-like structures in minerals are either produced by mechanical force or by chemical dissolution (McLoughlin et al., 2010). It is not possible to determine in optical microscopy how the tunnel-structures in our samples have been produced, however, in ESEM images it is possible to see that the minerals are clearly dissolved at the margins of these structures (Fig. 9). It is also possible to view dissolved patches on the mineral surface where the microfossils have been attached but for some reason been removed (Fig. 9B). Several types of microorganisms are known to produce long, curvi-linear structures in minerals due to directed dissolution of the minerals by the production of acids and siderophores. The cause of mineral dissolution could be just to expand the microbial habitat but there could also be other causes like searching for elements or compounds within the minerals that the microorganisms could use for their metabolism. In subseafloor environments iron and manganese are elements with redox potential that microorganisms commonly use (Edwards et al., 2005). Iron oxidising autotrophic bacteria as well as manganese oxidising heterotrophic bacteria are common at Seamounts in the Pacific Ocean i.e. the Loihi Seamount which is the active seamount of the Emperor-Hawaiian chain today (Emerson and Moyer, 2002; Templeton et al., 2005). The production of tunnel structures in volcanic glass from subseafloor pillow lavas have also been interpreted to be caused by the oxidation of iron since volcanic glass contain high amounts of reduced iron easy to be oxidised by microbes. However, zeolites do not contain iron or any other element that could be used by microorganisms. Thus the question remains why the microorganisms once dissolved the zeolites to such an extent. One explanation could be that they actually did obtain elements or compounds that they could use in their metabolism by boring through the minerals. Zeolites are well known for their capacity to adsorb various elements and compounds like metals, hydrocarbons and molecular hydrogen within their crystal framework of molecular-sized channels (Sheta et al., 2003; Langmi et al., 2003). Zeolites are frequently used in industrial processes as ion exchangers, catalysts and molecular sieves, and it is most likely that zeolites in subseafloor settings adsorb compounds like Fe, CH_4 or H_2 from hydrothermal fluids which microorganisms can scavenge when dissolving the minerals. The question is whether zeolites can adsorb enough compounds and elements with redox potential to make microbial mining worth the effort or not.

In conclusion, it was possible by the aim of ESEM to characterize the fossilized microorganisms, perform element analyses of the hydrated minerals and study the interaction between the microorganisms and the mineral phases which gives us information about the living conditions of the microorganisms and perhaps even their metabolism, which is interesting in a geobiological context.

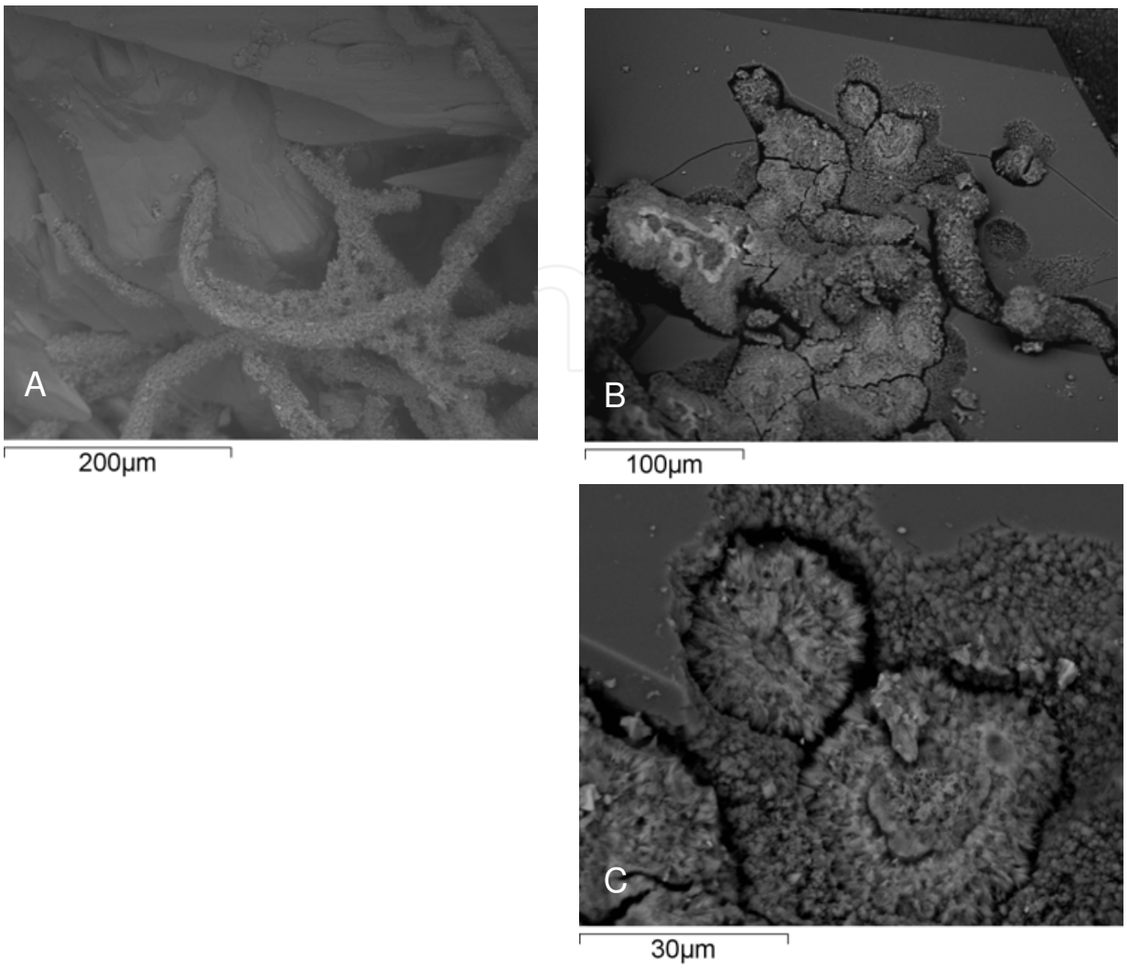


Fig. 9. ESEM images. Images showing the interaction between the mineral substrate and the fossilized microorganisms. In B and C it is possible to see that the microorganisms produced the tunnels in the mineral by dissolution. In B dissolved patches are viewable where fossilized microorganisms have been attached but later removed.

5. Concluding remarks

The ambition with this chapter was to show the advantage of ESEM in geobiological related research over conventional SEM. We have choosen samples that represent that specific intersection in nature where biology meets geology. We have seen how live microorganisms collected and isolated from soil samples can be viewed and analysed in ESEM. We have seen how the deep subseafloor biosphere can be explored by the study of fossilized microorganisms in rock samples. We have further seen how microorganisms interact with their close environment by, for instance, dissolving minerals to scavenge elements for their metabolism, but we have also seen how microorganisms form minerals by precipitation. All this have been made possible by the use of ESEM. The samples used in this study are extremely sensitive and fragile and procedures for ordinary SEM analysis such as desiccation and coating would have substantially damaged the samples. We have in this chapter used modern or quite young samples (even though the samples from the Emperor

Seamount are 48Ma) but a large part of geobiological research is carried out on samples much older and, thus, much more fragile than this. The oldest traces of life on Earth are about 3.5 Ga and need to be handled with extreme care (Schopf, 1993). The same is for extraterrestrial samples, such as putative microfossils in meteorites (McKay et al., 1996) or near future sample return from Mars (MEPAG, 2008). Thus, ESEM is an instrument with great future potential and we hope that we with this chapter have shown its capability within geobiological research.

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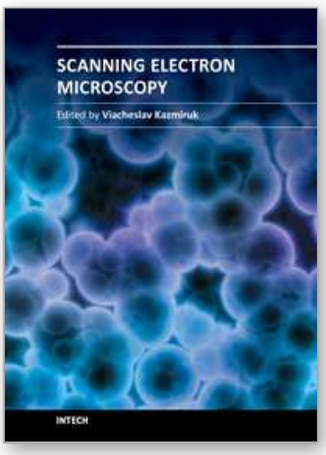

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Today, an individual would be hard-pressed to find any science field that does not employ methods and instruments based on the use of fine focused electron and ion beams. Well instrumented and supplemented with advanced methods and techniques, SEMs provide possibilities not only of surface imaging but quantitative measurement of object topologies, local electrophysical characteristics of semiconductor structures and performing elemental analysis. Moreover, a fine focused e-beam is widely used for the creation of micro and nanostructures. The book's approach covers both theoretical and practical issues related to scanning electron microscopy. The book has 41 chapters, divided into six sections: Instrumentation, Methodology, Biology, Medicine, Material Science, Nanostructured Materials for Electronic Industry, Thin Films, Membranes, Ceramic, Geoscience, and Mineralogy. Each chapter, written by different authors, is a complete work which presupposes that readers have some background knowledge on the subject.

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