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Microbial processes in glaciers and permafrost

A literature study on microbiology affecting groundwater at ice sheet melting

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October 2009

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Contents

Abstract

A repository for spent nuclear fuel will remain for hundred thousands of years. During this period, several ice ages will most likely take place. To understand the effect of melt water from ice sheets on the repository, the microbiological processes of oxygen reduction has to be elucidated. This report is a compilation of the present knowledge about biological activity in glacier environments. These environments consist of many different parts which have their own biological character depending on the prevailing physical and chemical conditions. There are, for example, ice sheets and glaciers, glacial streams and rivers, soil and water beneath the ice, soil and water in front of and beside ice sheets and glacier and deep groundwater beneath the ice. The microbiological processes of importance are consumption of oxygen by aerobic microorganisms, anaerobic organisms and their reduced metabolites, like sulphide, acetate and methane, which can act as reducing agents in biological or chemical oxygen reduction. The lithotrophic type (inorganic energy source) of metabolism is important in these cold environments. There are also microbiological processes important to radionuclide transport and the production of complexing agents, biological colloids and biofilms.

The study of microbial processes in glacier and ice sheet environments is still a young scientific niche. The studies have so far mostly been concentrated to ice surfaces and the subglacial environment. The most important findings from the literature study are as follows. Primary production is ongoing in snow cover and on ice surfaces of glaciers and ice sheets. The production is dependent on the location, because of temperature and solar radiation, but also on the prevailing state of the glacier. On surfaces and in the snow cover, heterotrophic microorganisms consume oxygen and organic material. In surface ice structures anaerobic conditions may occur. The subglacial environment is very active with several types of microorganisms, both aerobic and anaerobic. The oxygen concentration in the subsurface varies depending on whether the surface water has been transported over a long or short residence time. The pro-glacial environment including rivers and lakes, are also very microbiologically active.

Recent research has demonstrated that microorganisms are more active than previously expected in permafrost environments. This research is increasing due to the effect of global warming on microbial activity in permanently frozen environments.

The groundwater beneath the ice sheets and glaciers still needs to be explored. There will be increasing knowledge from the ongoing research project in Greenland (Greenland Analogue Project). The chemical composition of the groundwater and the subsurface microbiology is therefore still unknown.

Sammanfattning

Ett förvar för utbränt kärnbränsle kommer att finnas kvar i hundratusentals år. Under den tidsperioden kommer ett flertal perioder med nedisningar förmodligen att äga rum. För att förstå hur smältvatten från isen kan påverka förvaret måste de syreförbrukande mikrobiologiska processerna tas i beaktande. Den här rapporten sammanfattar den nuvarande kunskapen om biologisk aktivitet i glaciärer och inlandsisar. Dessa miljöer består av ett flertal delar med olika mikrobiologisk karaktär beroende på de rådande kemiska och fysiska förutsättningarna. Miljöerna är bland annat glaciärer och inlandsisar, glaciärbäckar, jord, vatten och djup grundvatten under isen, jord och vatten framför en glaciär. Mikrobiologiska processer av vikt för syrekonsumtion är aerob metabolism, anaeroba mikroorganismer och deras reducerade metaboliter såsom sulfid, acetat och metan. Dessa ämnen fungerar som reducerare i syrereduktionen som kan var både kemisk och biologisk. Det finns också mikrobiologiska processer som är viktiga för transport av radionuklider, produktion av komplexbildande ämnen, biologiska kolloider och biofilmer.

Mikrobiella processer i glaciärer och inlandsisar har inte studerats speciellt länge, endast i mellan 20 och 30 år. Studierna har till största delen varit koncentrerade till överdelen av isområdena och miljön under dem. De viktigaste slutsatserna från litteraturstudien är följande. Primärproduktion pågår i snötäcken och på isarna. Storleken och hastigheten av produktionen är beroende av temperatur och solinstrålningens intensitet och därför också av var isarna är belägna. Glaciärernas tillstånd är också avgörande. På isytorna finns också heterotrofa mikroorganismer som konsumerar organiskt material med reduktion av syre. Inuti isblock och i djupa fickor i isen pågår anaeroba mikrobiella processer. Miljön under isarna är mycket aktiva med många typer av mikroorganismer, både aeroba och anaeroba. Syrekoncentrationen i den här miljön varierar beroende på om det glaciära ytvattnet har transporterats under en kortare eller längre tid. Miljöerna framför en glaciär, till exempel bäckar och sjöar, har också stor mikrobiell aktivitet.

Nyare forskning har visat att mikroorganismer är aktivare än förväntat i permafrost miljöer. Forskningsinsatserna inom det här området ökar eftersom den globala uppvärmningen påverkar den mikrobiella aktiviteten i de permanent frusna områdena.

Inga rapporter eller artiklar om den kemiska sammansättningen av grundvattnet under glaciärer och inlandsisar kunde hittas vid litteratursökningen. Vilken typ av grundvatten och hur det har påverkats av avsmältningen är därför okänt. Det samma gäller för mikrobiologin i de här grundvattnen.

1 Introduction

1.1 Background

A repository for spent nuclear fuel will remain at its most hazardous for a few hundred thousand years, over which several ice ages will probably take place. The melting of glacier ice will increase the infiltration of dilute oxygenated water, which could theoretically reach the repository. Such an event would threaten the function of the repository barriers, the bentonite clay by the dilute groundwater and the copper canisters by possible dissolved oxygen in the water /SKB 2006/. The effect on the bentonite will not be discussed here, but the possible effects of dissolved oxygen, in terms of microbial processes extending to a depth of 500 m, will be studied.

The main cause of oxygen depletion in the surface environment and shallow groundwater in terrestrial environments is the biodegradation of organic material by aerobic microorganisms. In addition, aerobic methanotrophic and lithotrophic microorganisms consume oxygen in their metabolisms. Oxygen concentration in shallow groundwater and its variation over the year has been investigated as a part of the site description of the proposed repository site at Olkiluoto, Finland /Pedersen et al. 2008/. The oxygen concentration varied between investigated boreholes and seasons, and the greatest depth at which oxygen could be measured was 16 m.

One argument for oxygen reaching a repository depth of 500 m cites the lack of organic material in glacial meltwater. Until recently, it was believed that glacial systems were abiotic /Gibbs and Kump 1994/. An increasing number of studies now verify the opposite, and a recent review by /Hodson et al. 2008/ thoroughly summarizes current knowledge of organisms and biological processes in glacial environments. Studies of glaciers and ice-sheets around the world have demonstrated that microorganisms and other organisms thrive not only in snow cover and surface ice environments, but that organisms can also be found *in* the ice at various depths and in subglacial environments.

The present report deals with the following environments:

- ice sheets and glaciers,
- glacial streams and rivers,
- regolith beneath the ice,
- regolith in front of and beside ice sheets and glaciers,
- deep groundwater beneath the ice.

Important microbial processes discussed include:

- active, aerobic microorganisms that are important to oxygen reduction,
- anaerobic microorganisms and their metabolic products, such as sulphide, acetate, and methane,
- microbial processes important to radionuclide transport and the production of complexing agents, biological colloids, and biofilms.

This report summarizes the present state of knowledge of microbial processes in different parts of glaciers and ice sheets. The report includes studies of seven different environments:

- the snow cover,
- the ice surface,
- the ice sheet,
- glacial and ice channels,
- the subglacial environment,
- soil and groundwater in front of and along the sides of glaciers and ice sheets,
- deep groundwater beneath glaciers and ice sheets.

The report also presents a brief compilation of data regarding microbial processes in permafrost.

1.2 Description of the influence of different metabolic groups of microorganisms

1.2.1 Metabolic groups of microorganisms

Microbial decomposition and organic material production depend on the energy sources and electron acceptors present /Madigan and Martinko 2006/. Organic carbon, including methane, and reduced inorganic compounds, including hydrogen, are possible energy sources in the subterranean environment.

During the microbial oxidation of these energy sources, microbes preferentially use electron acceptors in a particular order (see Figure 1-1): first oxygen, and thereafter nitrate, manganese(IV), iron(III), sulphate, sulphur, and carbon dioxide. Simultaneously, fermentative processes supply the metabolizing microorganisms with, for example, hydrogen and short-chain organic acids (see Figure 1-2).

As the solubility of oxygen in water is low, and because oxygen is the preferred electron acceptor of many bacteria that use organic compounds in shallow groundwater, anaerobic environments and processes usually dominate at depth in the subterranean environment but anaerobic environments can also be found locally on a micro-scale in aerobic environments. In environments containing small amounts of organic material, the lithotrophic microorganisms play an important role in oxygen consumption. These organisms oxidize reduced inorganic compounds such as ferrous iron, manganese(II), ammonium, sulphide, sulphur, methane and hydrogen. For example, microbial activity can increase the rate of oxidation of ferrous iron to ferric iron by as much as 10^6 times /Singer and Stumm 1979/. Many of these organisms use carbon dioxide as their carbon source and thus are autotrophs (many of which can use organic carbon sources as well). Table 1-1 shows examples of lithotrophic and methanotrophic microorganisms and the energy-converting redox reactions.

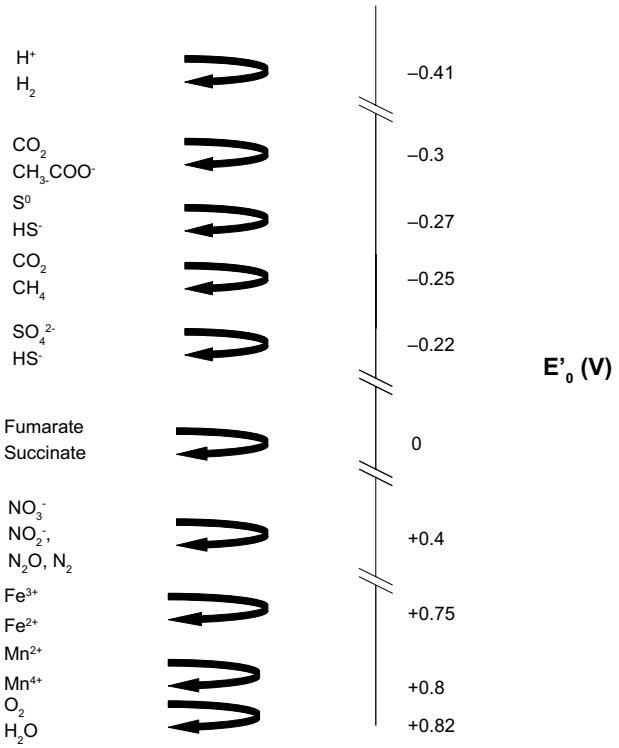


Figure 1-1. *Some redox couples involved in microbial processes.*

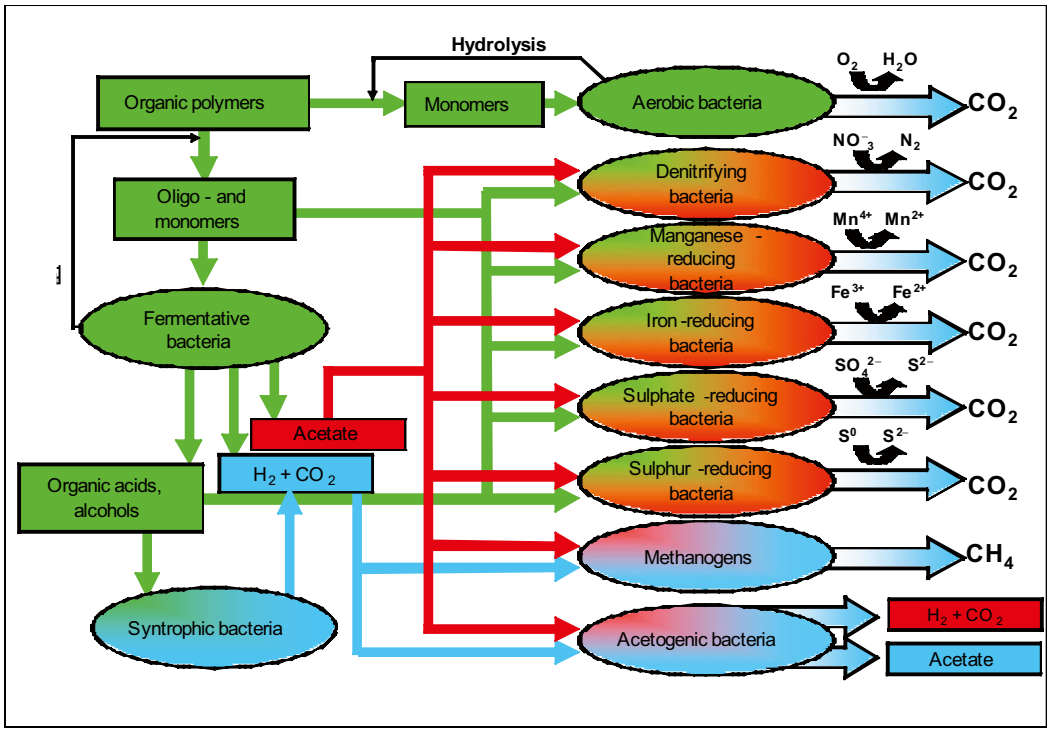


Figure 1-2. *Possible pathways for the flow of carbon in the subterranean environment. Organic carbon is respired with oxygen, if present, or else fermentation and anaerobic respiration occur using an array of different electron acceptors.*

Table 1-1. Aerobic lithotrophic microorganisms and the energy-converting redox reactions.

Hydrogen bacteria	**$2H_2 + O_2 \rightarrow 2H_2O$**
Methanotrophic bacteria	$CH_4 + O_2 \rightarrow CO_2 + 2H_2O$
Sulphur bacteria	$2HS^- + 2H^+ + O_2 \rightarrow 2S^0 + 2H_2O$
Sulphur bacteria	$2S^0 + 3O_2 + 2H_2O \rightarrow 2SO_4^{2-} + 4H^+$
Nitrifying bacteria	$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$
Nitrifying bacteria	$2NO_2^- + O_2 \rightarrow 2NO_3^-$
Iron bacteria	$4Fe^{2+} + 4H^+ + O_2 \rightarrow 4Fe^{3+} + 2H_2O$
Manganese bacteria	$2Mn^{2+} + O_2 + 2H_2O \rightarrow 2MnO_2 + 4H^+$

Different microbial groups influence the environment in different ways, depending on what metabolic group is dominant. Table 1-2 lists the activities of these groups and their possible effects on their environment. These microbial processes generally lower the redox potential, E_h. Most of the microbially mediated reactions will not occur in a lifeless groundwater environment without the presence of the catalysing enzymes of the microorganisms. However, the concentrations of reduced electron acceptors alone will not reveal when, where, or the rate at which the individual microbial processes occur.

1.2.2 Other aspects of microbial diversity

There are several ways to categorize microorganisms and some of the expressions used in the present report are described here. A microorganism is defined as an organism that cannot be seen without the magnification of a microscope. The cell size of microorganisms is approximately 1–10 µm. Viruses are smaller, approximately 0.01–0.1 µm; the taxonomic position of viruses is still unclear, and they are not included in the "universal tree of life".

Table 1-2. Activities and effects of the different physiological groups of microorganisms found in granitic groundwater.

Metabolic groups of microorganisms	Activity	Effect on the environment
Aerobic respiration	Oxidation of organic material or inorganic compounds by oxygen reduction	Depletion of oxygen and organic material Lowering of redox potential
Anaerobic respiration	Oxidation of organic material along with the reduction of compounds other than oxygen	See below for each specific group of bacteria
Iron-reducing bacteria	Oxidation of organic material along with ferric iron reduction	Depletion of organic material and ferric iron Increase in ferrous iron concentration and alkalinity Lowering of redox potential
Manganese-reducing bacteria	Oxidation of organic material along with manganese(IV) ion reduction	Depletion of organic matter and manganese(IV) Increase in manganese(II) concentration and alkalinity Lowering of redox potential
Sulphate-reducing bacteria	Oxidation of organic material along with sulphate reduction	Depletion of organic matter and sulphate Increase in sulphide concentration and alkalinity Lowering of redox potential
Methanogenesis		
Heterotrophic methanogens	Convert short-chained organic material to methane and carbon dioxide	Depletion of organic material Increase in methane gas and carbon dioxide (alkalinity) concentrations Redox not influenced
Autotrophic methanogens	Oxidation of hydrogen gas and reduction of carbon dioxide to methane gas	Depletion of hydrogen gas and alkalinity Increase in methane gas concentration Redox lowered
Acetogenesis		
Heterotrophic acetogens	Convert organic material to acetate	Depletion of organic material other than acetate Increase in acetate concentration Redox not influenced
Autotrophic acetogens	Oxidation of hydrogen gas along with reduction of carbon dioxide to acetate	Depletion of hydrogen gas and alkalinity Increase in acetate concentration Redox lowered

Prokaryotes and eukaryotes

Prokaryotes are organisms without a cell nucleus. The genetic material in these organisms is one double-stranded DNA molecule that is anchored to the cell membrane. Organisms in the domains *Bacteria* and *Archaea* belong to the Prokaryotes.

Eukaryotes are organisms that have their genetic material in a nucleus surrounded by a membrane. This group includes all organisms except the *Bacteria* and *Archaea*; the domain is called *Eukarya.*

Gram-positive and Gram-negative Bacteria

One group of organisms in the domain *Bacteria* has a type of cell wall differing from that of other organisms in this domain. The Gram-positive bacteria have a thick cell wall made of peptido-glycan outside the cell membrane, while Gram-negative bacteria have a thin cell wall and an additional membrane outside the cell wall. Many Gram-positives have the ability to form spores, which is a survival mechanism especially in dry environments. All Gram-positives are found in the cluster called *Firmicutes* in the universal tree of life (see Figure 1-5).

The universal tree of life

The information in the DNA sequence can be used to create a phylogenetic tree of the organisms living on the Earth today (Figure 1-3). For this purpose, one part of the DNA molecule is used, the part that encodes for 16S of the ribosome (for prokaryotes) or 18S (for eukaryotes); these sequences

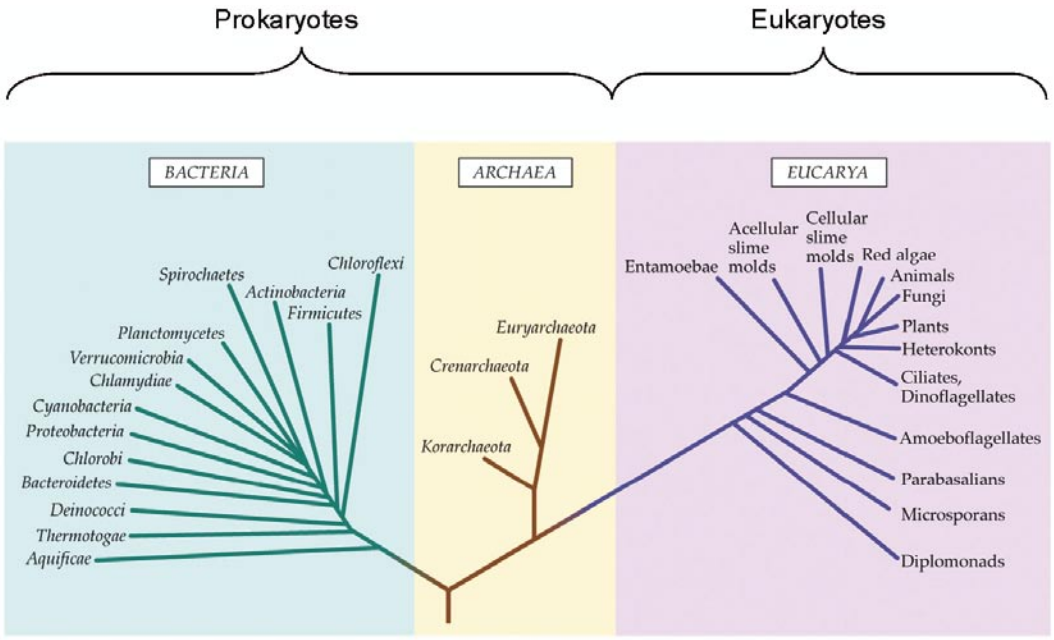


Figure 1-3. *The universal phylogenetic tree. The tree is derived from the comparative sequencing of 16S or 18S RNA.*

are denoted the 16S rRNA gene or the 18S rRNA gene. In all cells, ribosomes are the structures in which proteins are synthesized. By statistically comparing the similarity of these DNA sequences, a phylogenetic tree can be made (see Figure 1-3). It can be seen that the organisms cluster into the three domains, *Bacteria*, *Archaea*, and *Eucarya*, as described above. This approach was first suggested and applied by /Woese et al. 1985/.

The 16S or 18S rRNA genes in natural samples can be used to describe the organisms present in a sample; the method is described below.

1.3 Methods

This section describes the methods used in the literature cited in the report. There are of course an abundance of other methods that can be applied in the study of glacier and ice-sheet microbiology but they will not be described in the report. The use of many different methods makes it difficult to compare the results.

1.3.1 Total number of cells (TNC)

This direct method for enumerating all cells in a sample is briefly described in the following.

Water samples are taken aseptically in sterile flasks or tubes. The samples are preserved by adding formaldehyde to a final concentration of 2–4% (v/v).

In the laboratory, a portion of the sample is filtered onto a black polycarbonate filter with a pore size of 0.2 µm. The cells are then stained with a fluorescent dye, often acridine orange, which specifically binds to the nucleic acids, RNA and DNA, in the cells. The cells can then be counted under a fluorescence microscope. If so called ultra-small cells or viruses are to be counted a filter with smaller pore size, down to 0.02 µm, has to be used /Noble and Fuhrman 1998/.

The total number of cells corresponds linearly to the viable biomass in groundwater /Eydal and Pedersen 2007/. The TNC method is a simple and direct way to determine the living biomass in a system. Since the cells are directly viewed, a clear picture of the cell sizes and number of dividing cells is also obtained.

Fluorescence microscopy can also be used to directly study photosynthesizing microorganisms, since the pigments involved in photosynthesis are auto-fluorescent. The microscope must be equipped with a proper set of cut-off filters to select the required wavelength.

1.3.2 Viable cell counts

This is an indirect way to estimate how many cells are viable in a sample. Briefly described, the cells are distributed on a growth medium of solidified agar, i.e. agar plates. To obtain a cell number between 30 and 300 on one plate, several dilutions of a sample have to be inoculated on a plate. The composition of the medium can be varied and should mimic the environment from which the sample comes, but with the addition of energy and carbon sources and macro- and micronutrients. The agar plates are then incubated at an appropriate temperature. Growth on the agar surface appears as bacterial colonies, small dots of growing cells. The same approach can be used to grow anaerobic microorganisms, but in that case the plates are incubated in an anoxic atmosphere. Under such conditions, mostly fermenting and nitrate-reducing organisms grow, so it is necessary to carefully select the substrate and carbon source and add sufficient amounts of nitrate to such cultures.

The viable count method has many disadvantages. A natural sample contains many different microorganisms with different metabolisms, making it impossible to use only one growth medium to grow all organisms present in a sample. The glacial environment, for example, is a low-nutrient and dilute milieu. That the organisms found in it are adapted to these conditions is nicely indicated by the results of /Christner et al. 2000/, where the microorganisms grew best on agar plates containing low concentrations of substrate and nutrients (see section 2.5).

1.3.3 Enrichment cultures and the most probable number method (MPN)

To grow more of the range of microorganisms present in a natural sample, one can use enrichment cultures. These cultures, made using selective media and incubated under selective conditions, are used to isolate specific microorganisms. For example, if nitrogen-fixing microorganisms are desired, a growth medium without any nitrogen source other than nitrogen gas in air is used. The cultural conditions then will select for microorganisms able to fix atmospheric nitrogen, and one will obtain an enrichment culture of these organisms. Other enrichment cultures of interest are for fermenters, anaerobic organisms (e.g. nitrate-, iron-, manganese-, and sulphate-reducers), and methanogens. Other organism groups amenable to such a cultivation method would be aerobic lithotrophs, such as ammonium-, iron-, manganese-, sulphide-, and sulphur-oxidizing microorganisms. In addition, organisms able to degrade various kinds of pollutants can be found by using enrichment cultures.

Enrichment culturing is a qualitative method and yields no information as to whether the identified organisms dominate a given environment or constitute a minor part of the population. The principle of the enrichment method can be used quantitatively in the most probable number method (MPN). In this method, a ten times dilution series of the sample is made until there is less than one cell per millilitre in the last dilution. After that, five or ten tubes are inoculated from each dilution and incubated. When the cultures have been incubated, growth or no growth in the tubes is registered. The most probable number of cells in the sample can then be calculated statistically from the results /Greenberg et al. 1992, Hallbeck and Pedersen 2008/. Using a set of different growth media for most of the metabolic groups present in the environment of interest, the growth results will be much more representative of the actual population investigated. Investigations of populations in Fennoscandian Shield groundwater containing up to nine different growth media indicated that up to 14.8% of the total number of cells could be cultivated /Hallbeck and Pedersen 2008/. This is higher than would be predicted in light of the “great plate count anomaly”, which claims that only under one percent of all microorganisms present can be cultivated /Haveman and Pedersen 2002, Hallbeck and Pedersen 2008/.

1.3.4 Molecular biology methods

To describe the biological diversity in an environmental sample, the information in the DNA of the cells can be used, the DNA sequence of the 16S rRNA gene being used most frequently. One of the most important things to consider in molecular biology work is the risk of contamination; researchers must preserve the sample properly if they cannot extract the DNA immediately. The choice of DNA extraction method is also important since, for example, Gram-positive and Gram-negative bacteria need different extraction methods.

Brief description of the method

DNA is extracted from the sample. If few cells are available, the DNA must be amplified using the polymerase chain reaction (PCR). This reaction amplifies the desired part of the DNA but in the same proportions as the original sample. The result of this step is a mixture of 16S rRNA genes from all organisms in the sample. In the next step, the DNA is sorted by either cloning or gel electrophoresis.

Cloning

In this step, one copy of the 16S rRNA gene from the mixture is incorporated into an *E. coli* cell by means of a vector, often a plasmid, which is a small ring of DNA that can integrate the 16S rRNA gene DNA sequence. The plasmid is then taken up by one *E. coli* cell, which is grown into colonies on an agar plate. During growth, the plasmid is copied into all new cells, meaning that the single copy of the 16S rRNA gene extracted from the sample has been multiplied up to a thousand million times. The desired DNA sequence can be extracted from the *E. coli* cells and sequenced.

Sequence comparison

The acquired sequences are then compared with sequences deposited in public databases, such as Genebank or EMBL. The database sequences can either come from pure cultures or be clone sequences from environmental samples. Information from pure cultures is the most informative regarding the metabolism and environmental requirements of the organisms. Clone sequences, depending on how close they are to those of known species, are less informative but can give clues as to the diversity of the sample. When an acquired sequence is most similar to a clone sequence in the database, the cloned sequence often comes from an environment similar to that of the studied sample.

Restriction enzyme digestion and gel electrophoresis

Another molecular method used in articles cited in this report is the restriction fragment length polymorphism (RFLP) method. Briefly stated, this method starts with the extraction and amplification of the 16S rRNA gene by PCR with a fluorescent label at one end of the sequence. The PCR products from different samples are then digested by different restriction enzymes. These enzymes cleave the DNA fragment into specific base-pair combinations and result in DNA fragments of different lengths. The fragments are then separated by gel electrophoresis depending on the length and thus the weight of the fragments. By comparing the patterns of the fragments, different samples can be compared. No identification of specific organisms can be made using this method.

1.3.5 Comparison of geochemistry and stable isotope studies

In their metabolisms, microorganisms are isotopically selective against compounds containing the heavier isotopes of an element in a process called isotopic fractionation. The isotopic composition of a compound in an environment thus provides a characteristic signature determined by the microbial processes in play. For example, the residual sulphate in an environment in which sulphate reduction occurs is heavier that the chosen sulphur standard, while the sulphide produced is lighter. This phenomenon is used in environmental studies to elucidate what microbial processes have been ongoing. Of course, the environment also must be chemically characterized. The problem with stable isotope studies is that there are many processes that fractionate isotopes, for example, evaporation. It is therefore difficult to draw conclusions from isotope values alone. Another drawback is that the isotopic composition of the environment does not indicate when the fractionation occurred. For a more complete discussion on the influence of microorganisms on isotopic fractionation see /Clark and Fritz 1997/.

Conclusion

To gain reliable information about microorganisms and microbial processes in an environment, combining microbiological and molecular biology methods gives the best result. Good geochemical data are also necessary to have a clear outline of the ongoing microbial processes.

2 Microorganisms in glaciers, ice sheets, and permafrost: a literature study

2.1 Organism types

2.1.1 Eukaryotes

Eukaryotic organisms are mostly found in snow cover, in cryoconite holes, which are water-filled cylindrical melt holes on the ice surface (see section 2.4), and surface biofilms on ice sheets. Algae (including green algae), protists (including diatoms, phytoflagellates, fungi, and ciliates) and a small number of micro-invertebrates (including nematodes, rotifers, tardigrades, and turbellaria) are eukaryotes that have been found in these environments /Takeuchi et al. 1998, Yoshimura et al. 1997, Hodson 2006a, Vincent et al. 2004, Hawes et al. 1993, De Smet and van Rompu 1994, Shain et al. 2001/.

2.1.2 Prokaryotes: Bacteria and Archaea

Of the prokaryotes, it is mostly organisms belonging to *Bacteria* that are found in glacial and permafrost environments. The following studies used both cultural and molecular methods to examine such organisms: /Sharp et al. 1999, Tranter et al. 2004, Willerslev et al. 1999, Grasby et al. 2003, Foght et al. 2004, Skidmore et al. 2005, Bhatia et al. 2006, Christner et al. 2006, and Cheng and Fought 2007/. The identified *Bacteria* are heterotrophs (both aerobic and anaerobic) and fermenters, though autotrophs and lithoautotrophs, especially where they occur in the subglacial environment, are increasingly attracting attention. /Gaidos et al. 2004/ found indications of autotrophic activity in lake water and sediment in a subglacial lake on Iceland. The anaerobic heterotrophs include nitrate-, iron(III)-, and sulphate-reducing bacteria /Skidmore et al. 2000, Foght et al. 2004/. Archaea have been very rarely found in glacial environments; /Hodson et al. 2008/ claimed that reliable detection has been reported twice by /Battin et al. 2001/ in an Alpine glacier and once in a meltwater lake sediment sequence on Ross Ice Shelf.

2.1.3 Viruses

The occurrence of viruses has been reported in cryoconite holes, water-filled cylindrical melt holes on the ice surface (see section 2.4), in Svalbard, Norway /Säwström et al. 2007, Anesio et al. 2007/. Since viruses are the most abundant members of aquatic microbial communities, they are likely to be found in all environments that harbour microorganisms. Viruses play a significant role in recycling nutrients and carbon in the ecosystems to which they belong. The presence of a great many viruses in a studied system is an indication of living and active microorganisms, since viruses need metabolizing microorganisms to infect and to be able to proliferate. In some references, viruses are reported as virus-like particles (VLP).

2.2 The glacial and ice-sheet environment

2.2.1 Availability of water

All life requires liquid water. The availability of water in a glacier depends on the heat budget at given points and the distribution of drainage channels in the ice. Because of this, water can exist as channels, films, or veins and pockets at grain interstices or boundaries /Paterson 1994/. The heat budget of melting ice is controlled by physical phenomena such as solar radiation and air temperature.

The combined heat budgets of surface, internal, and basal thermal regimes affect the distribution of ice at the pressure melting point. This point varies from one glacier to another depending on the relative importance of heat fluxes from the surrounding environments.

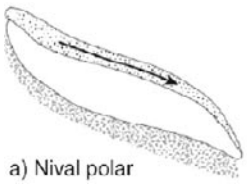
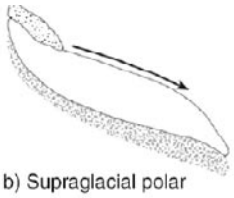
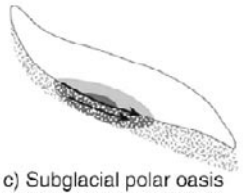
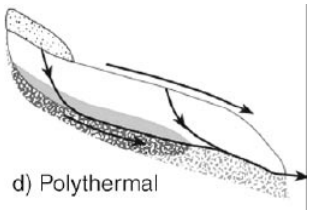
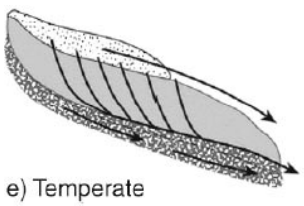
2.2.2 Glacier classification

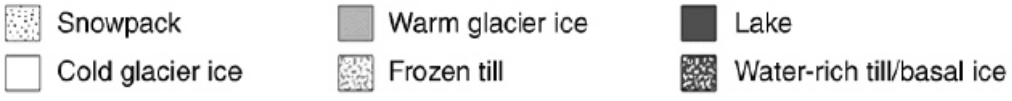
Depending on the variation of heat fluxes from below and above the glacier, and the ice dynamics, the glacier will be subject to various thermal regimes, which is seen in the ecosystem structure in the glacier. Table 2-1 lists five different thermal types of glaciers, together with their key habitats and representative examples around the world; this material was compiled by /Hodson et al. 2008/.

A glacier has several parts, as depicted in Figure 2-1. It can be covered with wet snow, like the nival polar type described in Table 2-1, or the glacial surface may hold meltwater lakes and warm and/or cold ice. There can be subglacial lakes, crevasses, or moulins with fractures, and subglacial channels.

Active microbes have been found in all types of glaciers, but their abundance and activity are greater in polythermal and temperate types. Two key glacial ecosystems have so far been identified: one inhabiting the glacier surface, i.e. the supraglacial system, and the other the ice–bed interface, i.e.

Table 2-1. Schematic of glacier thermal regimes and their most important habitats. Hydrological transfers are indicated by the arrows. Examples of and key references for each type of habitat assemblage are given in the right-hand column.

Glacier type	Key habitats	Examples	References
 a) Nival polar	Wet snow	Tuva Glacier, S. Orkney Island, Antarctica[1]	1. /Hodson 2006/
 b) Supraglacial polar	Glacier surface	– McMurdo Dry Valley glaciers, Antarctica[2–5] – Austre Brøggerbreen, Svalbard, Norway[6,7] – White Glacier, Nunavut, Canada[8]	2. /Christner et al. 2003b/ ; 3. /Porazinska et al. 2004/; 4. /Tranter et al. 2004/; 5. /Wharton et al. 1981/ 6. /Kaštovská et al. 2005;/ 7. /Hodson et al. 2005/; 8. /Mueller and Pollard 2004/
 c) Subglacial polar oasis	Wet snow Water-rich till/ basal ice Subglacial lake	Subglacial Lake Vostok, Antarctica[9–12] Ice stream C, Antarctica[13]	9. /Christner et al. 2001/; 10. /Karl et al. 1999/; 11. /Priscu et al. 1999/; 12. /Siegert et al. 2001/ 13. /Lanoil et al. 2004/
 d) Polythermal	Wet snow Glacier surface Warm glacier ice Water-rich till/ basal ice	Midtre Lovénbreen, Svalbard, Norway [6,7,14–17] Finsterwaldenbreen, Svalbard, Norway[18] Johan Evans Glacier, Ellesmere Island, Nunavut, Canada[19,20]	14. /Anesio et al. 2007/; 15. /Mindl et al. 2007/; 16. /Säwström et al. 2002/; 17. /Wynn et al. 2006/ 18. /Wadham et al. 2004/; 19. /Bhatia et al. 2006/; 20. /Skidmore et al. 2000/
 e) Temperate	Wet snow Glacier surface Warm glacier ice Water-rich till/ basal ice	Haut Glacier D'Arolla, Switzerland[21–23] Franz Joseph and Fox Glaciers, New Zealand[24]	21. /Bottrell and Tranter 2002/; 22. /Sharp et al. 1999/; 23. /Tranter et al. 2002/; 24. /Foght et al. 2004/.



the subglacial system /Hodson et al. 2008/. A third ecosystem has also been described, the englacial system, which exists *inside* glacial ice; the activity of microorganisms is lower here than in the two other ecosystems /Abyzov 1993/.

An overview of current knowledge of the glacial ecosystems is presented below.

2.3 The snow cover

Many studies of snow microbiology have been conducted in temperate environments with seasonal snow cover. One of the most studied phenomena in this environment is snow algae, which usually comprise microalgae and/or phytoflagellates. These organisms are present also in glacier snow cover. The greatest difference between seasonal snow cover and that on glaciers is the amount of litter and other debris, which is much greater in seasonal snow cover in temperate environments because of the presence of vegetation.

Snow cover on a glacier supplies the rest of the glacial environment with inoculi, nutrients, and water /Hodson et al. 2008/. It is also important to the development of the subglacial drainage system and partially governs the integrated glacial ecosystem. /Amato et al. 2007/ determined the number of cells in the snow cover of Kongsvegen, a polythermal glacier at Spitsberg, Svalbard. The accumulated snow layer of the glacier contained 2×10^4 cells mL^{-1} with no significant variation with depth, from 0 to 60 cm. Over the course of one summer, the cell number increased to 2×10^5 cells mL^{-1}. The same investigation determined the diversity of aerobic heterothrophic microorganisms, and the populations of both Gram-negatives and Gram-positives resembled those of microorganisms found in other arctic environments. The population was able to degrade the short-chain organic acids, propionate, acetate, and formate, which are common in Arctic snow. /Hodson et al. 2005, Hodson 2006a, and Jones 1999/ have studied the importance of nitrogen cycling in melting polar glacial snow packs. The rate of this process is slower than in snow from temperate environments because of the lack of litter and debris, as stated above. /Takeuchi et al. 1998 and Yoshimura et al. 1997/ studied the dominant organisms in snow cover and bare glacial ice cover in the Himalayas. They found that the dominant organisms in the accumulation area, the specialized snow algae (*Trochiscia* sp.), became less dominant at the snow line and were replaced by a specialist for the ice environment, in this case, *Cylindrocystis brébisonii.*

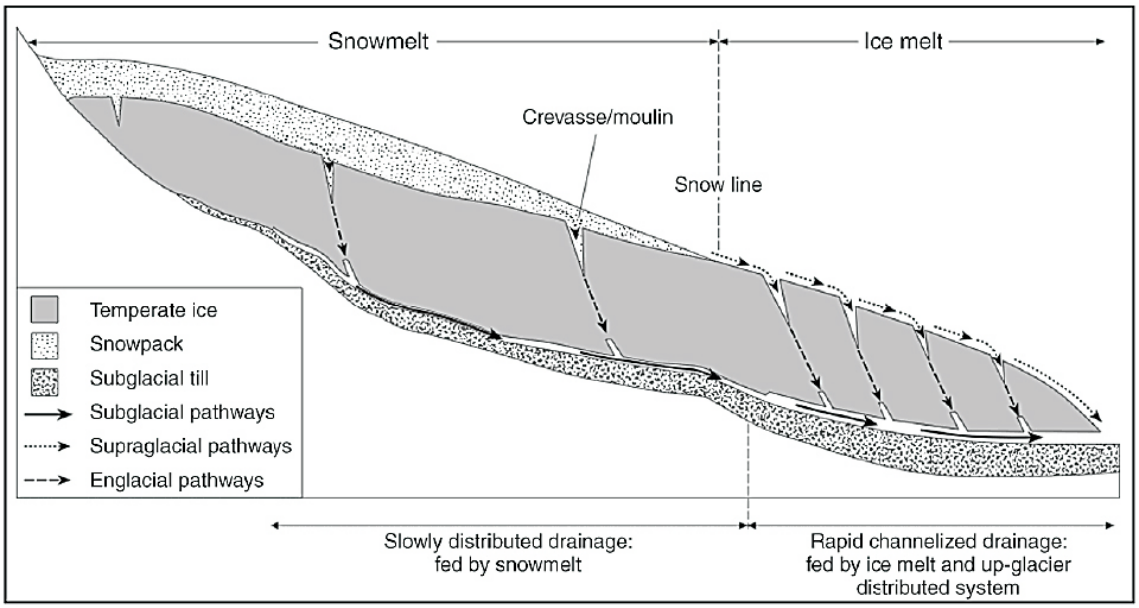


Figure 2-1. *Glacier drainage system structure (modified from /Tranter et al. 1996/). Efficient channels or conduits underlie parts of the glacier bed that are ice covered, while the subglacial drainage system beneath the snow-covered upper glacier is far less efficient and is distributed over broad areas of the glacier bed (offering more opportunities for the assimilation of nutrients derived from the snowpack and crustal sources). From /Hodson et al. 2008/.*

In Antarctica, snow algae often grow intensely following snow melt and fertilization from ornithogenic emissions that volatilize from penguin and other bird guano /Hodson 2006a/. Cell counts in snow from the sub-Antarctic Signy Island indicated cell numbers ranging from 5×10^3 cells mL^{-1} of coloured snow to just 1–2 cells mL^{-1} of "clean" snow. /Fogg 1967, in Vincent 1988/ found a carbon fixation rate of approximately 10 mg C m^{-2} d^{-1} at 0°C at Signy Island, which is lower than that of typical sea ice ecosystems /Vincent 1988/.

The snow cover is one important source of organic material developed via photosynthesis. Since the production of organic material is seasonal, organic material from the snow will accumulate in the ice in winter. During deglaciation, the organic material will follow glacier meltwater and will be metabolized in aerobic or anaerobic systems in the glacier and in the groundwater. This is a rather simplified picture of the snow microbiota and because of the shortage of studies of the heterotrophic activity in the snow cover of glaciers and ice sheets the carbon cycle in such systems is not yet fully understood /Hodson et al. 2008/.

2.4 The supraglacial ice

The surface of a glacier or an ice sheet can be a productive area, and photosynthetic organisms can form mats on the ice surface. Figure 2-2 shows the cover of organic matter on the ice surface of the Midtre Lovénbreen glacier, Svalbard, Norway. The most spectacular and probably most important structures in this part of the glacier are cryoconite holes, formed when solar-heated dark organic and inorganic debris melts into the ice. A cryoconite hole can be open to the atmosphere, closed by an ice lid part of the year or year around, or submerged (see Figure 2-3).

The diameter of these holes varies considerably from two to 120 cm /Tranter et al. 2004/ in Antarctic glaciers, while their depths range from a few to 80 cm /Tranter et al. 2004, Hodson et al. 2008/. In glaciers in the McMurdo Dry Valley, Antarctica, extreme holes can be 5 m deep and 30 cm in diameter /Fountain et al. 2004/. In these glaciers, half of the cryoconites are connected with the rest of the hydrological network in the supraglacial ice. These holes are covered with ice lids up to 36 cm thick that can persist for up to 10 years.

Cryoconite holes in the Arctic seem to be more inter-connected than holes in the Antarctica. This difference between the two environments results in different community structures and dynamics. /Mueller and Pollard 2004/ demonstrated that the community structure in the Canada Glacier, Antarctica, with up to 50% closed cryoconite holes, varied with environmental conditions inside the holes, while the community in the White Glacier, Axel Heiberg Island, Nunavut, Canada, was continuously reset because of meltwater flushing. The closed cryoconite holes, for example, contained ammonium concentrations ranging from 5 to 140 µg L^{-1} and nitrate concentrations from <5 up to 255 µg L^{-1}. The holes in the White Glacier contained ammonium concentrations up to 13.5 µg L^{-1} and nitrate concentrations up to 33 µg L^{-1}.

In summer 2000 and 2001, the photosynthetic rate was measured in the water and in the benthic communities in cryoconite holes in the Midre Lovénbreen glacier in Spitzbergen, Norway /Säwström et al. 2002/. In this glacier, the cryoconite holes covered approximately 6% of the glacier surface. The photosynthetic activity was highest at the bottom of the holes, where it reached 0.63–157 µg C L^{-1} h^{-1}, compared with 0.34–10.56 µg C L^{-1} h^{-1} in the water phase. The community consisted of bacteria, virus-like particles (VLP), cyanobacteria, and hetero- and autotrophic nanoflagellates. The total amount of nitrogen in both water and bottom material was 15–33 mg L^{-1} and the total amount of phosphorous 2.4–3.6 mg L^{-1}. The water contained from 1.00×10^4 to 4.50×10^4 mL^{-1} bacterial cells and the bottom material from 4.67×10^4 to 7.07×10^4 mL^{-1} bacterial cells. The VLP/bacteria ratio was highest in the bottom material at approximately 8, while in the water the ratio was 0.24. This study also recognized that the meltwater on the glacier surface contained well-developed biofilms, in which the photosynthetic rate was 0.6–8.33 µg C L^{-1} h^{-1}.

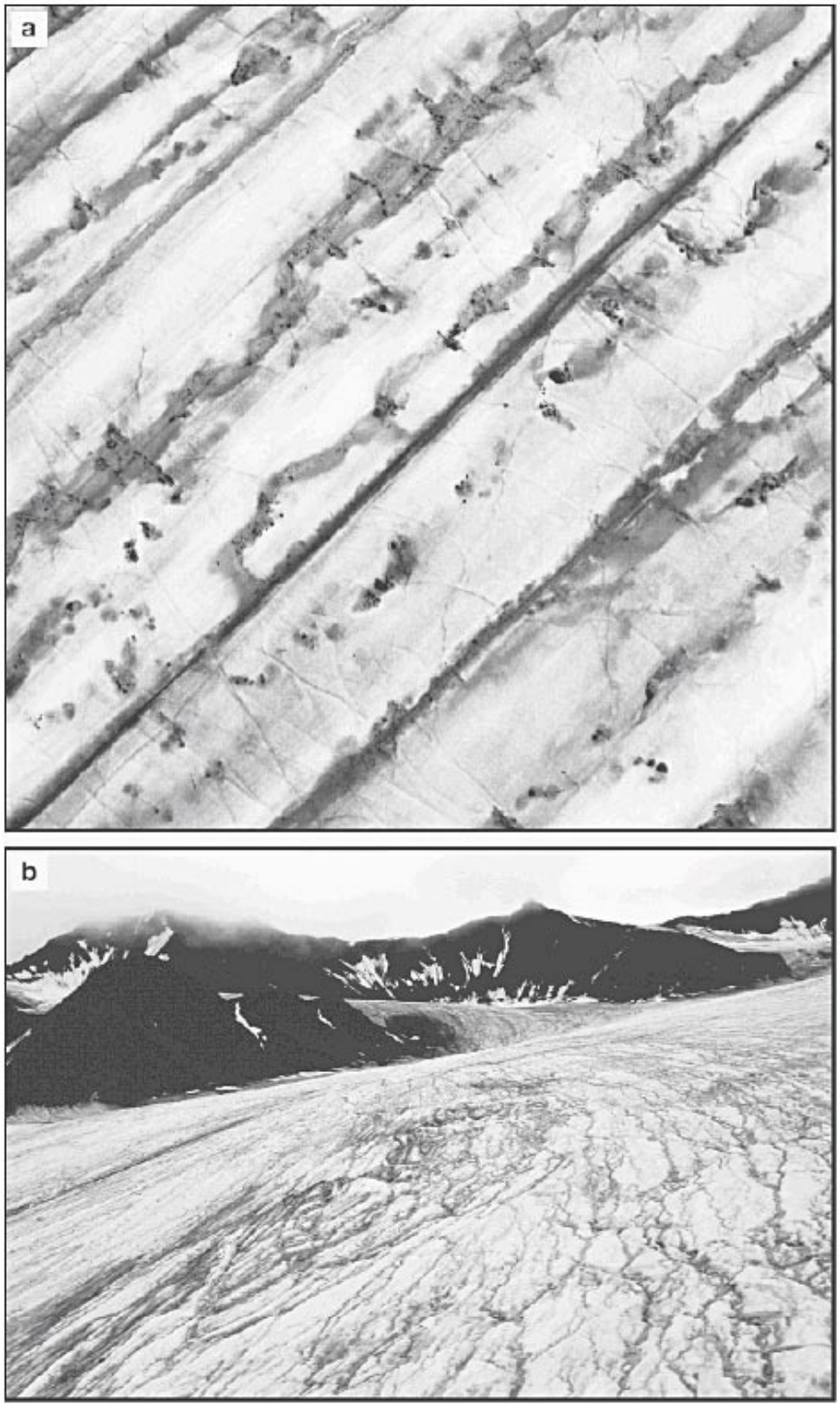


Figure 2-2. *The darkening of supraglacial ice by organic matter on Midtre Lovénbreen glacier, Svalbard, Norway. (a) Aerial photograph of an area approximately 100 m². Cryoconite holes (clusters of small, dark circles) and cryoconite accumulation in streams (some of which follow linear, structural features on the ice surface) are both discernable. (b) Oblique photograph of streams on the same part of the glacier; note the exceptionally dark accumulation of predominantly cyanobacteria in the central part of the image (deposits here are controlled by ice foliation patterns), giving way to hydrologically controlled deposits to the right-hand side. From /Hodson et al. 2008/.*

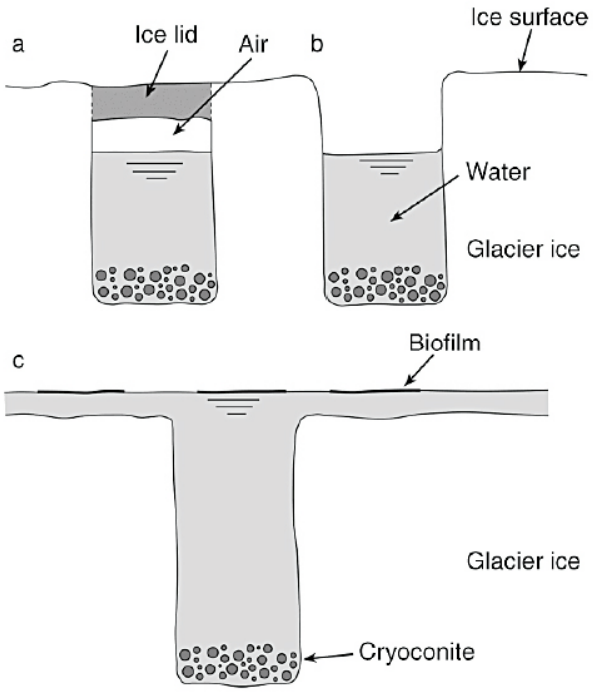


Figure 2-3. *Anatomy of a cryoconite hole, showing (a) closed, (b) open, and (c) submerged morphologies. From /Hodson et al. 2008/.*

On almost any Svalbard glacier (see Figure 2-4) there are areas containing entombed cryoconite holes, giving a mantle with a cracked, hummocky surface. This surface forms blocks with anoxic cores /Hodson et al. 2008/. Under the thick dark layer, the temperature will rise and allow high respiratory rates. The microbial life in cryoconite holes in Svalbard glaciers seems to be limited by phosphorous and not by nitrogen or carbon /Säwström et al. 2007/. There are a few reports of viruses in cryoconite holes /Bratbak et al. 1990, Maranger et al. 1994, Anesio et al. 2004/. These viruses take part in the ecological web and release nutrients to the environment when lysing microbial cells. One study found that there were fewer viruses per microbial cell in the cryoconite holes in Svalbard glaciers than in other aquatic systems, but that the number of virus-infected cells was higher /Säwström et al. 2007/.

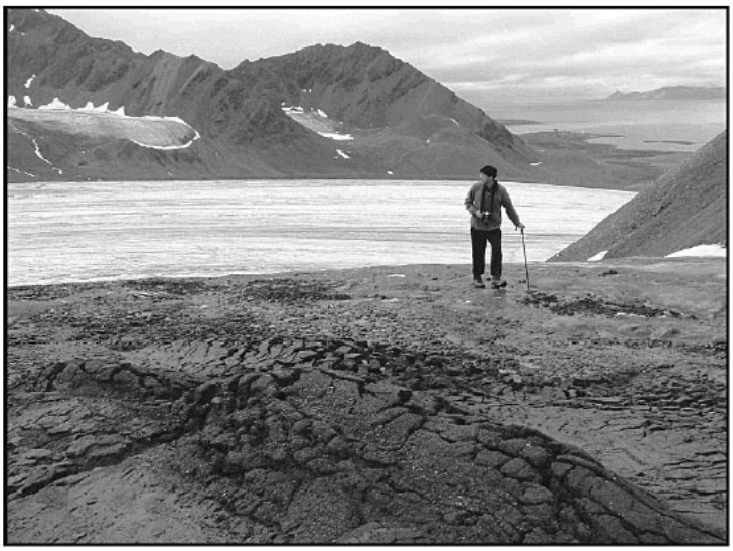


Figure 2-4. *A cryoconite mantle on Midtre Lovénbreen glacier, Svalbard, Norway (foreground). The cracked, hummocky surface results in the formation of distinct blocks whose cores are anoxic. From /Hodson et al. 2008/.*

/Pearl and Priscu 1998/ studied the special ice covering the permanently ice-covered Lake Bonney in Dry Valley, Antarctica. Here, dark soil particles from the surrounding ice-free valley floor blow onto the lake's surface and start to melt down into the ice matrix. The microbial populations in these systems consist of a complex cyanobacterial–bacterial community conducting photosynthesis, nitrogen fixation, decomposition, and biogeochemical zonation. Both cyanobacteria and eubacteria were responsible for nitrogen fixation while the carbon dioxide was fixed by cyanobacteria only. The photosynthesis was stimulated by nitrogen (NO_3^-) and phosphorous (PO_4^{3-}) and nitrogen fixation by phosphorous and iron ($FeCl_3$ + EDTA).

The supraglacial environment with cryoconite holes is one of the most important sources of organic material in ice sheet and glacier systems. The glacier surface becomes increasingly populated by photosynthetic organisms as shown in Figure 2-4, and the system is already anoxic at the surface. During deglaciation, production will increase due to increased temperature, and the organic material will follow the meltwater into the ground and act as the substrate for both aerobic and anaerobic microorganisms.

2.5 The englacial environment

/Abyzov 1993/ was the first to report the presence of microorganisms in bulk ice. He filtered melted ice from depths as great as 2,750 m in the ice core from Vostoc Station in Antarctica. Furthermore, /Abyzov et al. 1998/ tested cells from deep Antarctic ice for viability by measuring the uptake of a ^{14}C-labelled protein hydrolysate. Using this rather crude method, they found a decline in uptake with sample depth. /Karl et al. 1999/ enumerated the microorganisms in ice cores from a depth of 3,603 m, from accretion ice in Lake Vostoc, Antarctica, finding between 2×10^2 and 3×10^2 bacterial cells mL^{-1} of melted ice. The amount of lipopolysaccharide-A in the meltwater was in the same order as the cell numbers and suggested a predominance of Gram-negative bacteria. Uptake studies using ^{14}C-labeled acetate and glucose demonstrated that acetate was the preferred carbon and energy source of the microbial population. /Priscu et al. 1999/ examined an ice core from a depth of 3,590 m from the same accretion ice Lake Vostoc as /Karl et al. 1999/ described. These researchers found from 2.8×10^3 to 3.6×10^4 cells mL^{-1} in the meltwater from the ice core. From sequencing the 16S rDNA gene in extracted DNA from the cells in the melted ice, they concluded that the sequences belonged to the *beta-Proteobacteria*, *Acidovorax* and *Comamonas*, and from the *Afipea* subgroup of the *alpha-Proteobacteria.* In addition, one clone was associated with the *Actinomycetes*, a genus common in soil. The DOC (Dissolved organic carbon) in the 3,590-m core was 0.51 mg L^{-1} of melted water.

/Christner et al. 2000/ carefully sampled ice cores of different ages from seven locations around the world, two sites in Greenland, one site in China, one site in Peru, and three sites in Antarctica. The samples were inoculated on solid media and incubated aerobically, i.e. the total count method was used, and the cells grew better on media containing low nutrient levels. Cells incubated at 10°C needed over 100 days of incubation before growth could be detected, while cells incubated at 25°C started to grow after 30–60 days. By partially sequencing the 16S rRNA gene (the 515 to 1,492 nucleotides of the *Escherichia coli* nucleotide numbering), the microorganisms were provisionally identified. They mostly belonged to spore-forming and non-spore-forming genera in the group of Gram-positive bacteria. As well, fungi were cultured from all locations but not identified. The age of the ice had no effect on the number of cells that could be cultured. /Sheridan et al. 2003/ analyzed the 16S rRNA gene from enrichment cultures inoculated with meltwater from a Greenland glacier ice core; the water sample came from 3,042 m and was considered to have frozen 100,000 years ago. The results indicated that DNA from many bacterial phylogenetic groups could be identified, such as alpha-, beta-, and gamma-*Proteobacteria*, *Thermus*, *Bacteriodes, Eubacterium*, and *Clostridium.* The authors claimed that these organisms had stayed viable for 100,000 years. The ice contained some sediments and the cell number in the meltwater was 6×10^7 cells mL^{-1}. The research group has reported similar results from other parts of the Greenland glacier ice core /Miteva et al. 2004, Miteva and Brenchly 2005/.

Eukaryotic DNA has been extracted from englacial ice. /Willerslev et al. 1999/ was able to identify 16S rDNA from fungi, plants, algae, and protists from 2,000- and 4,000-year-old ice-core samples from the Hans Tausen ice cap in North Greenland; no viability tests were done in this study.

/Buford Price 2000/ has suggested a possible habitat for microorganisms in veins of fluid water located between the ice crystals in bulk ice. These veins can be formed in polycrystalline ice, since almost all solutes are excluded from the ice structure and become more concentrated in the remaining unfrozen fraction in the triple junction between the ice grains (Figure 2-5).

/Mader et al. 2006/ determined that small particles and microorganisms (i.e. *Clostridium vincentii*) were excluded from the ice grains during the crystallization process, and were found in veins of fluid water situated between the ice crystals. Small particles <2 μm in diameter were excluded to approximately 80% but larger particles were excluded to only 20%. The same study measured the increase in ion concentration by partitioning, detecting the same increase in concentration as for the small particles. As the ion concentration increases, the environment in the veins will mimic an ordinary growth medium in terms of salinity, allowing thereby microorganisms to thrive or at least survive.

It seems that the *direct* sequencing of cells present in ice yields more Gram-negative bacteria than if the cells are cultured on solid media before sequencing the 16S rRNA gene. The culturing method appears to select for Gram-positives. Retrieving DNA from Gram-positives, especially if they are present as spores, calls for a careful choice of extraction method. Many methods described are more effective in extracting DNA from Gram-negative microorganisms. If special care is not taken when extracting DNA directly from natural samples, there is a severe risk that the DNA will be biased towards Gram-negative microorganisms. This could explain the difference between the results of direct extraction and those of culturing followed by DNA extraction /Herrera and Cockell 2007/.

The englacial environment contains living microorganisms of different physiological groups, but whether and in which way these microorganisms are active *in* the ice has not been conclusively demonstrated. These microorganisms can, however, serve as inocula when they reach the subglacial environment, which contains more liquid water.

2.6 The subglacial environment

Until about ten years ago, the subglacial environment was thought to be sterile and its chemical composition dependent solely on inorganic geochemical processes. Since then, microorganisms have been demonstrated to strongly influence the signature of the chemistry of subglacial meltwater /e.g. Bottrell and Tranter 2002, Tranter et al. 2002a, 2005, Wadham et al. 2004, Skidmore et al. 2005, Wynn et al. 2006/. Such research has significantly influenced the field, causing inorganic models of rock–water interaction at the glacier bed /Raiswell 1984, Tranter et al. 1993/ to be replaced by ones that take account of microbial activity /e.g. Sharp et al. 1999, Tranter et al. 2002a, Skidmore et al. 2005/. This paradigm shift can be witnessed by comparing two interpretations of detailed geochemical datasets from a single field site: the first in 1993 presents the inorganic model /Tranter et al. 1993/, while the second, almost 10 years later, includes important microbial processes /Tranter et al. 2002a/. The first geochemical model considered only chemical reactions, with the result that the high sulphate and hydrogen carbonate values could not be explained solely by weathering by atmospheric oxygen and carbon dioxide. Not until carbon dioxide from the microbial oxidation of organic material was considered together with microbial FeS_2 oxidation, could the measured ion concentrations be explained.

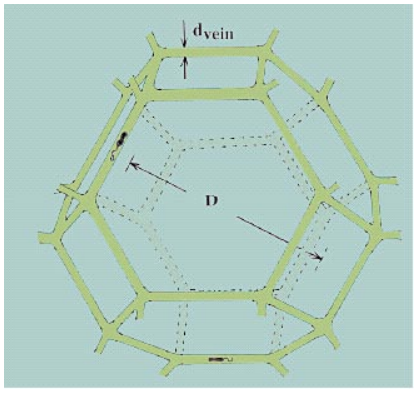


Figure 2-5. *Schematic of vein structure around an ice grain. From /Buford Price 2000/.*

There are two possible endmember types of drainage system at the glacier bed: distributed or channelized. During meltwater transport, the ion concentration increases by the dissolution of rock material. Distributed systems follow hydrological flow paths containing water under high pressure with long residence times and high rock–water contact ratios. Channelized drainage systems follow discrete flowpaths with low-pressure hydrological systems and evacuate large water volumes at high rates from the glacier. The subglacial drainage system is often dependent on the input of meltwater from the supraglacial system. High input will raise the basal water pressure, pushing a distributed drainage system to reorganise and evolve into a channelized system /Richards et al. 1996, Nienow et al. 1996/. In especially cold, polythermal glaciers, this evolution can be rapid and is coupled with glacier movement /Copland et al. 2003, Nuttal and Hodgkins 2006, Rippin et al. 2005, Bingham et al. 2006/. A distributed system means high total dissolved ion loads and low concentrations of atmospheric gases in solution.

The larger volumes and higher flow rates in channelized systems result in more dilute water and a higher concentration of atmospheric gases. Recent research has demonstrated that microbiological processes effectively consume oxygen in meltwater during transport away from crevasses, making it anoxic /Bottrell and Tranter 2002, Wadham et al. 2004, Wynn et al. 2006/. These results have created a better understanding of how alternative oxidizing agents are used by microbial metabolisms, enabling the continued acquisition of solute even after dissolved atmospheric gases have been depleted. Microbial processes were introduced to explain "anomalously" high concentrations of sulphate and bicarbonate present in the subglacial environment of some glaciers, when dissolved atmospheric oxygen and carbon dioxide were assumed to be the sole agents of oxidation and carbonation reactions, respectively, as discussed above /Bottrell and Tranter 2002, Tranter et al. 2002a, Wadham et al. 2004/.

Recent studies of the subglacial environment have included the *in situ* sampling and incubation of borehole water–sediment mixtures /e.g. Sharp et al. 1999, Tranter et al. 2002a/, the incubation of bacteria sampled at the ice margin /e.g. Sharp et al. 1999, Skidmore et al. 2000, 2005, Foght et al. 2004/, and the characterization of microorganisms entrapped at the bottom of ice cores /e.g. Priscu et al. 1999/. /Sharp et al. 1999/ studied basal ice from two glaciers: Glacier de Tsanfleoron and Haut Glacier d'Arolla, both situated in Switzerland. The total number of cells was determined by acridine orange staining and microscopic counting, while the number of growing bacteria was estimated by counting dividing cells. The cell numbers were between 9.3×10^5 and 5.9×10^7 mL^{-1} and with a mean value of 9.6×10^6 mL^{-1} in ice and sediments from Tsanfleuron glacier. In Arolla glacier meltwater, 5.3×10^4 to 1.8×10^6 cells mL^{-1} were found with a mean value of 2×10^5 cells mL^{-1}. /Skidmore et al. 2000/ inoculated meltwater from the glacial bed of the John Evans Glacier on eastern Ellesmere Island, Nunavut, Canada, to different types of microbial growth media and incubated both aerobically and anaerobically at different temperatures. The anaerobic cultures displayed the activity of sulphate- and nitrate-reducing bacteria coupled with carbon dioxide production; biogenic methane production was observed as well. The aerobic cultures also displayed positive growth, and growth experiments with radiolabelled acetate found growth at 0.3°C. /Bhatia et al. 2006/ used molecular biology methods, including restriction fragment length polymorphism (RFLP), to describe the microbial populations in basal ice, sediment, and meltwater in the supra-, sub-, and progalcial enviroments also in the John Evans Glacier. This method only reveals the distribution of the PCR band and does not identify the specific organisms present. The results indicated that the subglacial water, basal ice, and sediment microorganism populations were distinct from those detected in supraglacial meltwater and proglacial sediments.

Ice and subglacial sediments from two southern hemisphere glaciers, Fox glacier and Franz Josef Glacier in the Southern Alps of New Zealand, were studied by /Foght et al. 2004/. The sediments contained $(2–7) \times 10^6$ cells g^{-1} dry weight and 6.9×10^5 cells g^{-1} dry weight of aerobic heterotrophs could be cultured from the same sediments. Nitrate- and iron-reducing bacteria were detected in sediment from both glaciers and nitrogen-fixing bacteria were detected in sediment from the Fox Glacier. When identified, isolated microorganisms displayed similarities to organisms such as *Polaromonas vacuolata* and *Rhodoferrax antarcticus*, previously obtained from permanently cold environments.

/Wynn et al. 2006/ studied the chemical composition of snow meltwater and the runoff from the High Arctic glacier, Midtre Lovénbreen, Svalbard, Norway. They could discern a clear difference in the composition of meltwater that flowed quickly versus slowly. The slower-flowing water had

a lower oxygen concentration but higher conductivity, ammonium concentration, and hydrogen carbonate concentration than did the quickly flowing water. Stable isotope composition was studied as well, to elucidate the processes underlying the differences between the waters. There are still too many unknown processes, mostly concerning the anaerobic microbial metabolisms affecting the chemical composition of the meltwater, to be able to draw sound conclusions. Thorough investigation of the ongoing processes is clearly needed.

/Hodson et al. 2008/ speculate that most of the anaerobic groups of microorganisms presented in the introductory section of this report are present in unfrozen subglacial environments. The only group not yet confirmed is the manganese-reducing bacteria, but increase in soluble manganese has been reported /Hodson et al. 2008/. Anoxic zone development starts with oxygen depletion in microenvironments; these oxygen-depleted zones later increase in size because of the ongoing oxygen consumption by aerobic microorganisms.

Nitrogen is an important compound for all life. The environmental nitrogen budget is a complicated matter involving many types of microorganisms and both aerobic and anaerobic processes. Most nitrogen in glacial systems originates in nitrogen fixation, in both the supraglacial environment by cyanobacteria and in several parts of the ice by heterotrophic microorganisms with nitrogen-fixing abilities. In this process, atmospheric nitrogen gas is actively incorporated into cell material by reduction to ammonium. This is a very energy-consuming process and requires the input of energy and electrons from organisms. During the degradation of organic material, the mineralization process liberates ammonium from proteins and nucleic acids. This can be oxidized by ammonium-oxidizing microorganisms in one of the constituent processes of nitrification. This could be one explanation for the findings of "excess nitrate" that have been reported from Arctic, alpine, and Antarctic glacial meltwaters /Tockner et al. 2002, Hodson et al. 2005, 2006/. Another source of nitrate may be the atmosphere. Measurements of nitrate in snow and aerosols together with determination of the isotopic composition are, for example, done by The National Ice and Snow data Center at the University of Colorado at Boulder (sidads.colorado.edu). Nothing has been found in the reviewed literature about nitrate of atmospheric origin.

2.7 The proglacial environment

The proglacial environment is often young land that is seasonally flooded by glacial meltwater. The top layer of this land typically consists of till and glaciofluvial sediment. Such areas also contain proglacial lakes and rivers.

/Wadham et al. 2007/ studied FeS_2 oxidation by means of stable isotope fractionation; their main conclusion was that the oxic oxidation of FeS_2 was companioned by anoxic oxidation via microbial nitrate and Fe(III) reduction. If this is correct, it means that the proglacial environment was widely anoxic for at least part of the year. The findings of /Wadham et al. 2007/ have not been confirmed by any microbiological studies.

Available phosphorous in proglacial river sediments was investigated by /Hodson et al. 2004/ in the vicinity of the Austre Bröggerbreen and Midre Lovénbreen glaciers in Svalbard, Norway, the latter of which has subglacial drainage. Most of the phosphorous in the proglacial rivers was of rock origin and thus not available to algae; the amount that was extractable with NaOH (NaOH-P), and therefore considered available to algae, was 20–200 times lower than the total amount of phosphorous in the proglacial river sediments. NaOH-P in river sediment was 1–23 µg P g^{-1} and the total-P was 230–670 µg P g^{-1}.

/Mindl et al. 2007/ investigated the influence of carbon and phosphate on microbial productivity in supraglacial runoff to proglacial lakes in the vicinity of Midre Lovénbreen glacier, Svalbard, Norway. The average phosphate concentrations in the two lakes studied were 18.8 and 4.1 µg L^{-1}, respectively, for total phosphorous and 0.8 and 0.5 µg L^{-1}, respectively, for dissolved phosphorous. The DOC concentration in both runoff and lake water was approximately 1 mg L^{-1}. The number of bacterial cells was in the range of 1×10^4 to 12×10^4 mL^{-1}; the great size of the range could be significantly linked to higher water temperature increasing the number of cells. Experiments with added phosphorous and carbon demonstrated that the system was growth limited by phosphorous only.

Depending on the melting rate and on the ground conditions in front of a glacier, there may be proglacial lakes and rivers. In such waters, photosynthetic activity produces organic material. The melt-off will be greatest concomitant with the highest photosynthesis rate, in summer – the time of year when the most organic material will be produced. If the water in proglacial lakes and rivers is discharged before it reaches the sea, the organics will be degraded and become part of the buffer for oxygen consumption. Otherwise, most of the supraglacial runoff meltwater will finally end up in the sea.

2.8 Permafrost environment

The definition of permafrost is "ground that remains at or below 0°C for at least two years" /Gilichinsky 2002/. Permafrost is strictly a thermal phenomenon and does not depend on the composition of the ground. The seasonal variation in temperature is limited to the upper 10 m of the ground. In summer, air temperatures exceed 0°C, thawing a thin layer at the ground surface, called the active layer. Various types of structures are typically found in permafrost areas. There are ice structures such as ice wedges, connected with the atmosphere, and massive ground ice at various depths. There are also cryopegs, inclusions of high-salinity water, and taliks, unfrozen areas. The permafrost table acts as a physical and biogeochemical barrier that limits infiltration of both surface water and external environmental factors /Gilichinsky 2002/. The temperature is between –7 and –12°C in Arctic tundra and between –20 and –27°C in the Antarctic polar deserts. Permafrost reaches depths of 600–800 m in the Eurasian tundra and a depth of 1,450 m in the Antarctic deserts /Steven et al. 2006/.

Permafrost occupies more than 20% of the world's land /Panikov and Sizova 2007/. For many years, permafrost was considered a depository of ancient microbial life, but intensive winter gas fluxes of methane and carbon dioxide from tundra to the atmosphere were recently discovered, intensifying the search for organisms able to metabolize below the freezing point.

The first report of the presence of viable microorganisms in permafrost was published in 1911 by Omelyansky, and several more such reports have followed. Russian scientists have demonstrated the presence of up to 10^8 cells of different physiological groups per gram of soil in Sibirian permafrost /Shi et al. 1997, and references therein/. Table 2-2 summarizes some of the literature on microbial activity detected at temperatures from 0 to –20°C. When studying microbial life in frozen habitats, not only temperature should be taken into account, but also various biological, chemical, and physical factors at water–ice interfaces, such as salt concentration, pressure, and the physical state of the water and ice. In permafrost, 2–7% of the water persists as briny liquid films and lenses (cryopegs, see below) that form as a result of the salting out of soils as the *in situ* temperature drops and remains at about –10°C /Bakermans et al. 2003/.

/Takacs and Priscu 1998/ presented data on bacterio–plankton dynamics, including heterotrophic bacteria activity data and bacterial cell loss rates from the lakes of the McMurdo Dry Valley in Antarctica. These lakes are permanently covered with 3–5 metres of ice. The bacterial production has always been highest just below the ice cover at the beginning of the Arctic summer season. Bacterial production ranged from 0 to 0.009 µg C mL^{-1} d^{-1} and bacterial cell numbers ranged from 3.2×10^4 to 4.4×10^7 cells mL^{-1}. The bacterial loss rate (i.e. the rate of bacterial death) in the trophogenic zone and in the entire water column of these lakes in summer reached 6.4×10^{14} cells m^{-2} d^{-1} and 4.16×10^{12} cells m^{-2} d^{-1}, respectively. The authors imply that bacteria may be a source of carbon for higher trophic levels in these lakes, through grazing.

/Vorobyova et al. 1997/ summarized the research into tundra soils and permafrost in Arctic and Antarctic locations. They described how the number of cells, detected by microscopy with acridine orange staining, was approximately 10^7 cells g^{-1} dry weight in both Arctic and Antarctic samples even if no microbial growth could be detected in the samples. The number of cells that could be grown on agar plates with different amounts of nutrients varied considerably. The average for Arctic permafrost samples was 0.1–10% of the total number of cells and for Antarctic samples, 0.001–0.01%. The redox values in these systems were reducing, with Eh ranging from +40 to –250 mV.

/Gilichinsky et al. 1995/ summarized the literature on permafrost and psychromicrobiology and concluded that psychrotolerant microorganisms, both spore-forming and non-spore-forming bacteria, were relatively easily isolated from ancient (10^3–10^5 years) permafrost samples. In 2003, Bakermans et al. reported that the lowest temperatures at which microbial reproduction could occur were –5 to

–8°C, as determined by colony formation from permafrost and frozen food. The lowest temperatures for *in situ* microbial activity found at the same time were from –10 to –17°C in Siberian permafrost, South Pole snow, lichens, and cryptoendoliths.

Cryopegs are defined as a layer of unfrozen ground that is perennially cryotic (forming part of the permafrost), in which freezing is prevented by freezing-point depression due to the dissolved-solids content of the pore water. An isolated cryopeg is entirely surrounded by frozen ground /van Everdingen 1998/. The biodiversity in cryopegs in permafrost originating from the Arctic Ocean regression in Siberia were studied by /Gilichinsky et al. 2005/. From the brine in cryopegs, anaerobic and aerobic, spore-forming and non-spore forming, halotolerant and halophilic, psychrophilic and psychrotrophic bacteria, mycelial fungi, and yeast have been identified. Activities of these organisms were detected below 0°C. /Gilichinsky et al. 2005/ concluded that the microorganisms isolated showed signs of having survived for 43,000 years at –10°C.

Environmental parameters such as temperature, salinity, pressure, solar radiation, and heat from the ground differ between the permafrost areas on the Earth. These parameters affect ongoing microbial processes, and successful studies of these processes need to combine chemico–physical studies with microbiology.

It can be concluded from the literature that there are viable populations of microorganisms in permafrost (see Table 2-2). That these organisms are actively metabolizing can also be concluded from several studies (see Table 2-3). Because of the difficulties of studying microbial processes in temperatures at or below the freezing point of water, there is still a need for more research before a clear picture of the ecology and biogeochemistry of the permafrost environment can be drawn.

When the temperature rises after an ice age, the activity of permafrost microorganisms will increase concurrently with higher temperatures and access to more organic carbon from increased photosynthesis. The increased degradation of organic carbon consumes more oxygen and will thus diminish oxygen concentration in penetrating groundwater.

Table 2-2. Enumeration of microbial populations from permafrost and other environments.

Environment	Cell type	Viable cell counts (mL^{-1} or g^{-1})	Total counts (mL^{-1} or g^{-1})	References
Permafrost environments				
Antarctic permafrost	Aerobic heterotrophs	$0–10^5$	$10^5–10^6$ [a)]	/Horowitz et al. 1972, Cowan et al. 2002/
Siberian permafrost	Aerobic heterotrophs	$0–10^8$	$10^3–10^8$	/Rivikina et al. 1998, Gilichinsky 2002/
	Methanogens	$0–10^7$	–	/Rivikina et al. 1998/
	Sulphate reducers	$0–10^3$	–	/Rivikina et al. 1998/
High Canadian Arctic permafrost	Aerobic heterotrophs	$10^1–10^3$	10^7	/Steven et al. 2006/
Cryopeg water	Aerobic heterotrophs	$0–10^6$	10^7	/Gilichinsky et al. 2003/
	Sulphate reducers	$0–10^6$	–	
	Methanogens	$0–10^2$	–	
Greenland glacier ice/ permafrost	Aerobic heterotrophs	10^2	10^7	/Miteva et al. 2004/
Other cryoenvironments				
Arctic sea ice	Aerobic heterotrophs	$10^8–10^{10}$	$10^5–10^{12}$	/Monfort et al. 2000/
Arctic sediments	Sulphate reducers	10^5	NA[b)]	/Knoblauch et al. 1999a, b/
Antarctic lakes	Aerobic heterotrophs	$10^5–10^6$	NA	/Cowan and Ah Tow 2004/
High Arctic Glacial ice	Aerobic heterotrophs	$10^2–10^3$		/Skidmore et al. 2000/
Lake Vostoc accreation ice	Aerobic heterotrophs	NA	10^2	/Karl et al. 1999/
Glacier cryoconite ice	Aerobic heterotrophs	10^4	NA	/Christner et al. 2003/

[a)] Determined by ATP content per cell

[b)] Data not available

Table 2-3. Studies of microbial activity in permafrost and other naturally frozen habitats.

Technique		Habitat	Temperature (°C)	Ref
Incorporation of labelled precursors into	DNA and proteins	Glacial ice bacteria	–15	/Christner et al. 2003/
		South Pole snow	–17 to –12	/Carpenter et al. 2000/
	Proteins	Arctic sea ice	–1.3 to –1	/Ritzrau 1997/
	Lipids	Siberian permafrost	–20 to 0	/Rivkina et al. 2000/
Gas fluxes	CO_2	Barrow permafrost AK*	–40 to 0	/Panikov et al. 2006/
		Tussock tundra, AK	–12 to 0	/Mikan et al. 2002/
	CH_4	Siberian permafrost	–16.5 to 0	/Rivkina et al. 2002/
	N_2O	Alpine tundra, Colorado	–5 to 0	/Brooks et al. 1997/
	Gradient of entrapped gases	Mountain glacier, Bolivia	–40 to 0	/Campen et al. 2003/
	O_2	Antarctic peat	–1 to +1	/Wynn-Williams 1982/
	Photosynthetic $^{14}CO_2$ uptake	Alpine, Tibet	–10 to +20	/Kato et al. 2005/
		Endolithic lichen, Antarctica	–24 to +5	/Kappen and Friedmann 1983, Kappen 1993/
	Dark $^{14}CO_2$ uptake	Permafrost and tundra, AK	–80 to 0	/Panikov and Sizova 2007/
Organic matter composition	Net N mineralization and nitrification	Taiga and tundra soils, AK	–5 to +5	/Clein and Schimel 1995/
		Tundra, AK	–30 to +5	/Schimel et al. 2004/
	Plant litter weight loss	Tussock tundra, AK	–30 to 0	/Hobbie and Chapin 1996/
	Loss of K, Mg, P, phenolics, and carbohydrates	Subarctic woodland, Canada	ND	/Moore 1983/
	^{14}C-glucose oxidation	Barrow permafrost, AK	–40 to 0	/Panikov and Sizova 2007/
UV microscopy	Staining of DNA(DAPI) and respiration products (CTC)	Arctic Sea Ice	–20 to –2	/Junge et al. 2004/

*Alaska

3 Schematic model of a polythermal or temperate glacier ecosystem

To make the glacier ecosystem easily understandable, a schematic model of the ongoing microbial processes is presented. This model combines findings from the reviewed scientific reports and knowledge gained from other microbial ecosystems, such as groundwater ecosystems in the Fennoscandian Shield.

In a glacial or icesheet environment, the production of organic material starts with –photosynthesis on the ice surface and in the snow cover, mostly by cyanobacteria and green algae (Figure 3-1). At the same time, oxygen is produced. In the snow, the snow algae are responsible for the photosynthesis. The production is dependent on solar radiation and on temperature. These parts of the glacier system are described in sections 2.3 and 2.4. Concomitantly, heterotrophic degradation is occurring. In areas where access to oxygen is non-limiting, the degradation is aerobic; when the oxygen level starts to decrease, the degradation becomes anaerobic. The anaerobic microbial processes mostly comprise fermentation and/or nitrate and sulphate reduction because of the lack of other electron acceptors. Theoretically, acetogenesis and methanogenesis can occur in anaerobic environments.

Surface meltwater flows via moulins and crevasses down to the subglacial environment (see Figur 3-2), which is described in section 2.6. This environment is greatly influenced both from the surface and by its contact with fresh rock surfaces be aerobic in areas where wide channels from the surface transport large amounts of meltwater. The oxygen can be consumed by heterotrophic microorganisms that degrade any organic material present. There will probably be ongoing lithotrophic microbial processes, such as iron and sulphide oxidation, that consume oxygen; these processes remain to be demonstrated. Another group of organisms that might be active here are methane and

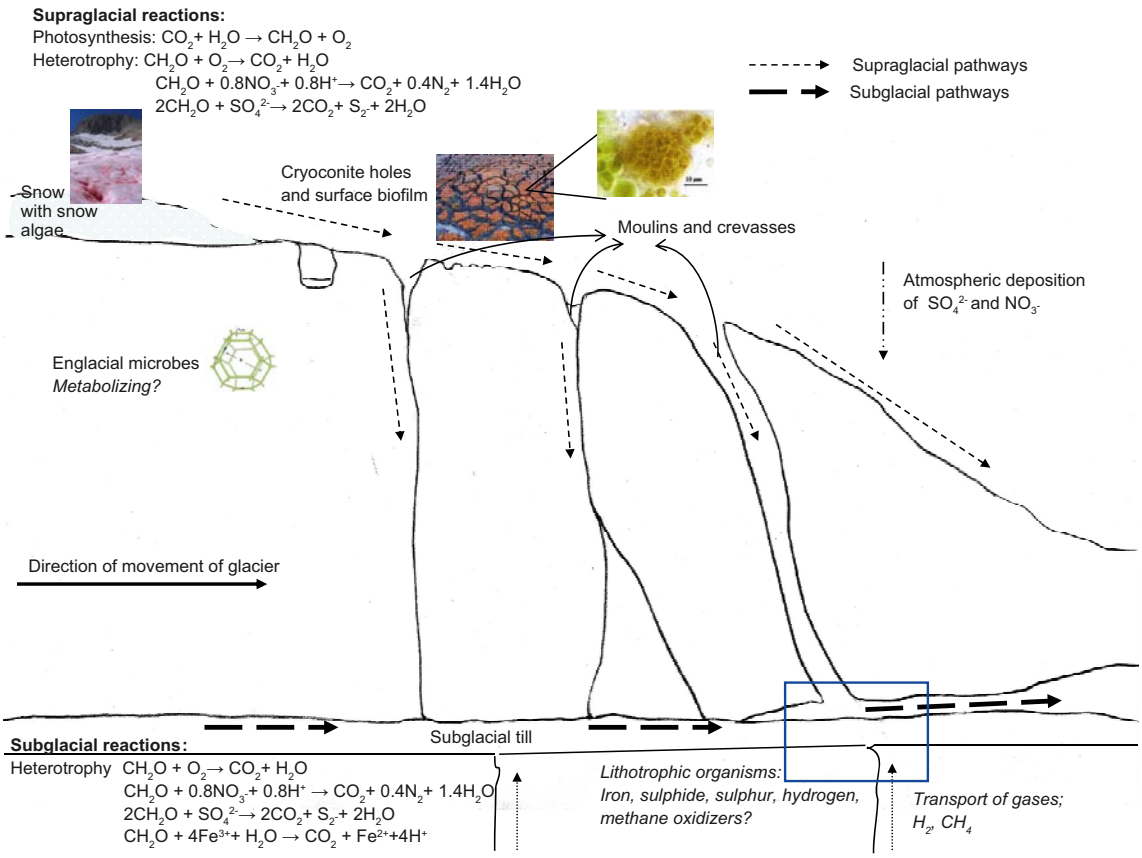


Figure 3-1. *Schematic of the ecosystem in a glacier or ice sheet. Explanations are found in the text. The area in the blue rectangle in this figure is enlarged in Figure 3−2.*

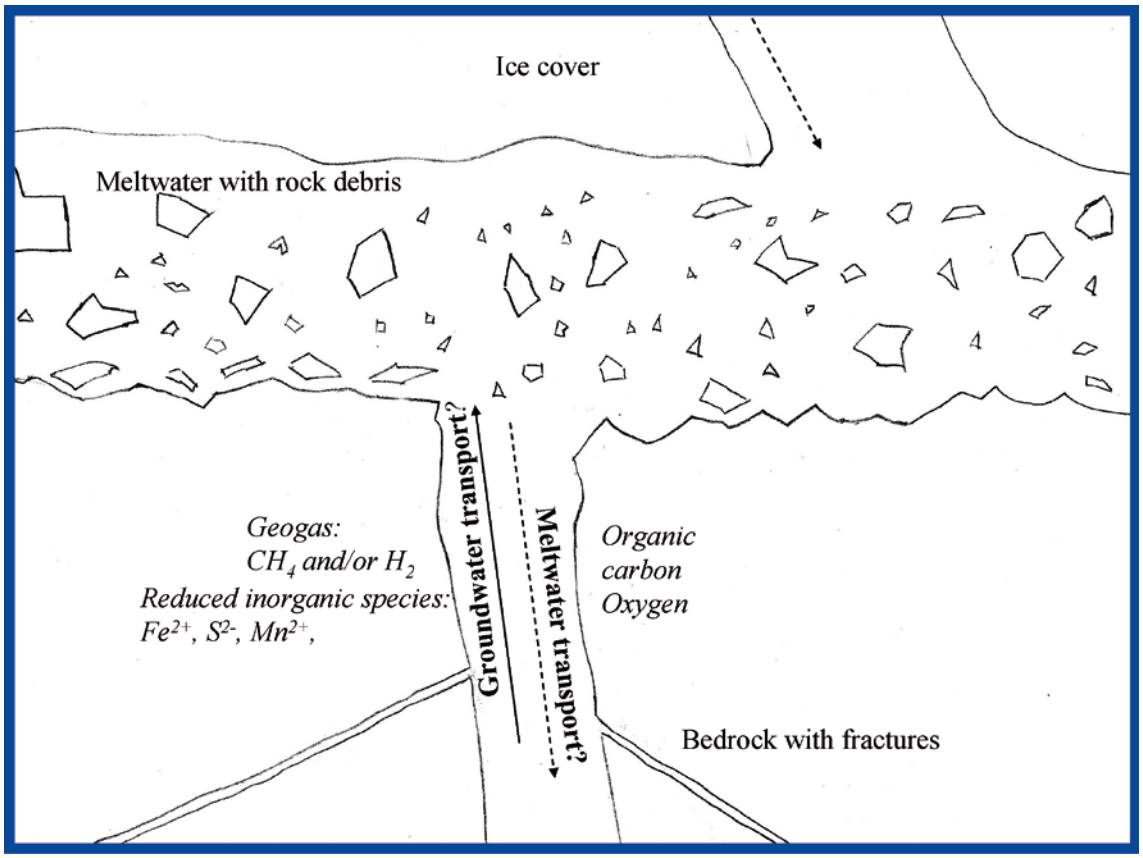


Figure 3-2. *Enlargement of the subglacial environment in a glacier or ice sheet.*

hydrogen oxidizers. Their existence depends on whether there is sufficient transport of geo-gases, such as methane and hydrogen, from deep groundwater. Neither of these groups has been studied in the subglacial environment nor has the amount or composition of transported geogas been studied.

Farther away from the channels, the environment will be oxygen deficient. In subglacial areas with narrow channels from the surface and with a distributed transport of meltwater below the ice, the environment will become anaerobic because of the limited transport of atmospheric oxygen. The anaerobic processes confirmed to take place here are nitrate, iron, and sulphate reduction.

Microbial processes in the proglacial environment depend on several parameters, as discussed in chapter 2.7. If most of the meltwater is subglacial, the possibility that it is anaerobic is greater than if it is supraglacial runoff. Supraglacial water, in contrast, could contain a large amount of organic material from photosynthetic organisms active on the ice surface. Some glaciers have developed or moved into areas with vegetation, such as forests. When such glaciers melt and retreat, the dead vegetation will be uncovered and degradation will start. Meltwater from glaciers forms proglacial rivers and lakes where photosynthesis and heterotrophic degradation occur. The runoff from these watersheds mostly ends up in the sea.

As soon as permafrost starts to melt, biological activity starts at both the macro and micro scales. The research performed so far has demonstrated that a great many microorganisms with different kinds of metabolisms are present in permafrost environments (see chapter 2.9). The research also describes how these microorganisms are more active than was anticipated, even at temperatures below the freezing point.

3.1 Nutrients in snow and ice

Studies of snowmelt in an Antarctic glacier have demonstrated that the concentration of solutes in meltwater is the highest in the early stages of the snowmelt. This phenomenon is due to the relocation of solutes, by distillation, at grain surfaces when the grains are losing mass. Under these conditions, microorganisms have an environment with a higher concentration of nutrients than average snow-melt concentrations /Hodson 2006/.

Figures for DOC in glacier ice have been measured in one polythermal glacier, the John Evans Glacier (Ellesmere Island, Nunavut, Canada), one temperate glacier, the Outre Glacier (British Columbia, Canada), and one cold-based glacier, Victoria Upper Glacier in the McMurdo Dry Valley, Antarctica; the measured quantities range from 0.06 to 46.6 ppm ($\mu g\ L^{-1}$) in all systems /Barker et al. 2006/.

There is atmospheric deposition of nitrate on glacier ice and snow. The National Snow and Ice Data Center (NSIDC) has a 25-year record of nitrate deposition in snow from the Amundsen–Scott South Pole Station. In snow sampled on one occasion, the nitrate content in the snow cover from depths of 0 and 100 cm was 422 and 181 ppb, respectively.

Sulphate is also deposited on snow and ice from the atmosphere. The main source of this sulphate is non-eruptive volcanic emissions and biogenic dimethyl sulphide of marine origin that is oxidized in the atmosphere /Legrand 1997/. Sulphate is also found in subglacial meltwater runoff. This sulphate is thought to be dissolved rock minerals /Hodson 2006/.

Phosphate is a nutrient often found in limiting amounts, and it is present in snow and ice in both Antarctic and Arctic glaciers. The source of this phosphate can be either dust from elsewhere that has travelled long distances by wind or closer sources, such as bird colonies /Hodson 2006/.

The concentrations of the various nutrients and substrates are difficult to determine because of their great variation, which depends on season, temperature, surroundings, and sampling depth.

4 Conclusions

The biogeochemistry and ecology of glaciers, ice sheets, and permafrost is still a young scientific niche, and research has been ongoing for only approximately 20 years. The most studied parts of the glacier system are the supra- and subglacial environments. The most important findings from the literature study are as follows.

- Primary production by photosynthesis is ongoing in the snow cover and ice surfaces of glaciers and ice sheets.
- The primary production is highest in summer because of the higher solar radiation and thus the higher temperature. Production, of course, differs between glaciers depending on their location. Antarctic glaciers have lower productivity, because of their lower temperatures and lower latitude, than do glaciers in the northern hemisphere.
- Heterotrophic microorganisms in the supraglacial environment consume organic material and oxygen. Anaerobic environments can be found in the cores of surface ice blocks.
- Microorganisms are present in the englacial environment, but their physiological activity is unknown.
- The subglacial environment is very active containing several kinds of microorganisms, both aerobic and anaerobic. The oxygen concentration in the subsurface varies depending on whether the surface water has been transported over a long or short residence time.
- The proglacial environment is also microbially active, containing rivers and lakes filled with glacial meltwater. There are microbial populations in these watersheds.
- Knowledge of microbial populations and activity in permafrost is limited. However, interest in what will happen in permafrost areas due to global warming has recently increased the amount of research, and our knowledge will improve in the future. The latest research has demonstrated that microorganisms are more active than expected in frozen environments.
- The groundwater beneath the ice sheet and glaciers still needs to be explored.

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