Stockholm University

Bachelor Thesis

Degree Project in
Earth Science 15 hp

Analyzing the Presence of Endospore-Forming Thermophilic Sulfate-Reducing Bacteria in Central Arctic Marine Sediment

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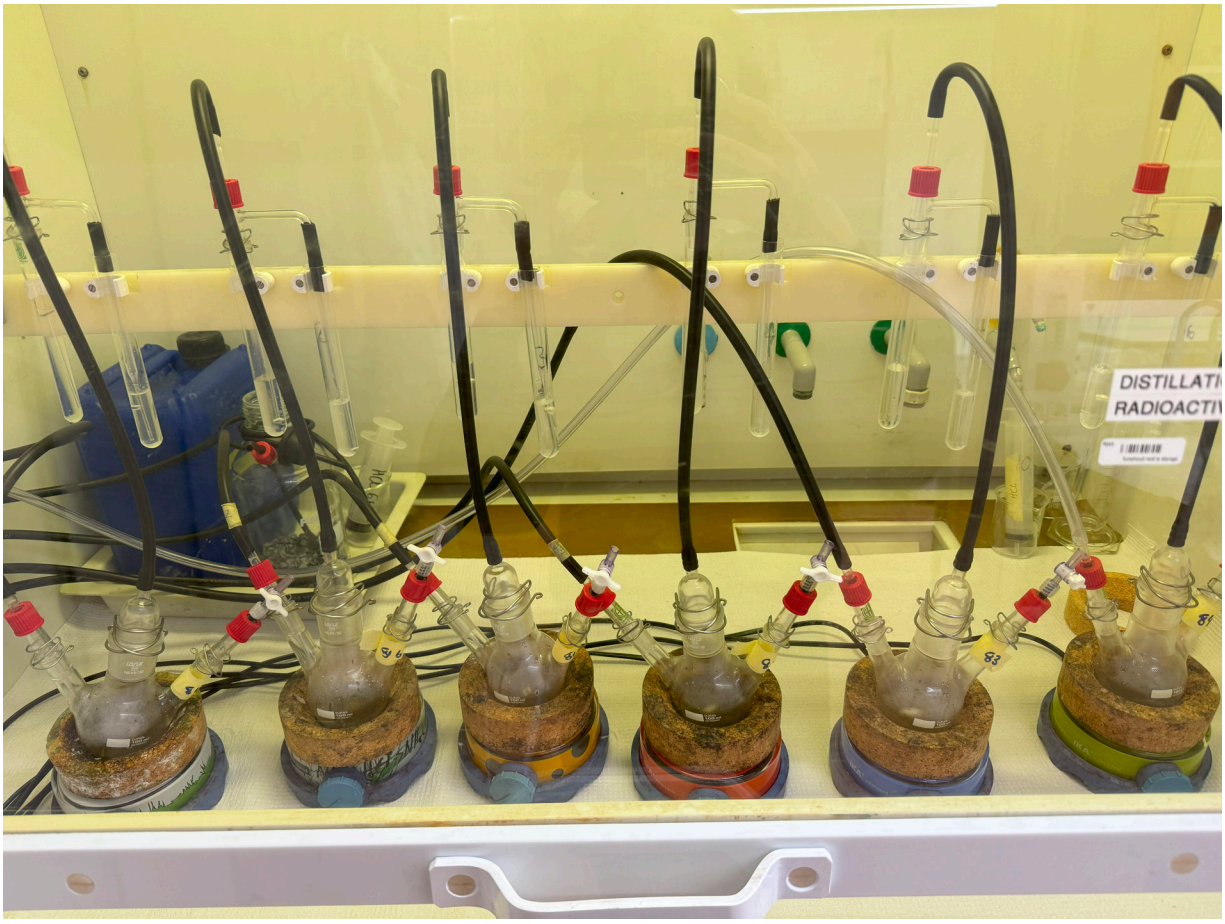

Stockholm 2024

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Abstract

Several discoveries of thermophilic sulfate reducing bacteria have been made in and around the Arctic Ocean. With the discoveries having been made at uninhabitable locations for the thermophilic bacteria they have been used to study dispersal patterns of microbial communities through its possibility to become dormant by forming endospores. Two sediment cores in the Arctic Ocean were studied to analyze presence of thermophilic sulfate reducing bacteria. The locations chosen for sampling was on the Yermak Plateau and on the Morris Jesup Rise which are affected by different currents entering and circulating the through the Arctic Ocean. 5 cm of their respective surface sediments was incubated at a temperature range from ca 2-80 ℃ and measured the rate of sulfate reduction with the radioactive ^{35}S tracer. Distillations and ion chromatographic analyses were made to obtain the results and determined the distillation method efficiency with spectrophotometric sulfide analysis which showed a low efficiency for the experiments made in this study. The sulfate reduction rate measured in the sediment core from the Yermak Plateau was above the detection limit in only one sediment sample incubated at 32 ℃. In the sediment core from the Morris Jesup Rise the samples incubated at 32-40 ℃ showed a sulfate reduction rate above the detection limit and was in one of the samples significantly higher than at the Yermak Plateau. The results showed an absence of thermophilic activity and only displayed activity corresponding to mesophilic sulfate reducing bacteria.

Table of Contents

1 Introduction

When the environment becomes uninhabitable many bacteria within the phylum *Firmicutes* can form a dormant cell structure called endospores. Physical insults, oxidants, UV etc., are almost entirely unable to affect the bacterial species in their dormant state. Endospores are metabolically inactive but can detect nutrients in their vicinity which will make them rapidly re-germinate and resume their vegetative state (Nicholson *et al.*, 2000). Endospores have been detected in different environments and are geologically widespread where wind and water contribute to the dispersal as well as other living hosts (Nicholson *et al.*, 2000). Because of the high resistance and through discoveries in deeper sediment cores, endospores have also been determined to have a great longevity (Bartholomew and Paik, 1966). Their longevity is supported by discoveries in marine sediment from Aarhus Bay where endospores were detected at a depth of 6.5 m representing 4500 year of sediment accumulation in the area (De Rezende *et al.*, 2013).

Thermophilic bacteria are characterized by growing at temperatures above 40 ℃ with an optimum activity is between 60-80 ℃ (Bajpai, 2023). However, thermophiles have a broad temperature range of growth which is the reason why they often get described as either moderate thermophiles, extreme thermophiles, or hyperthermophiles. Moderate thermophiles have a temperature optimum between 40-70 ℃ (Laemthong *et al.*, 2022) whereas some species like *Aquifex pyrophilus* have a temperature optimum of approximately 85 ℃ which makes them hyperthermophiles (Huber *et al.*, 1992). Extreme thermophiles typically grow between 55-80 ℃ and hyperthermophiles reaches their optimum growth above 80 ℃ but can grow at even higher temperatures (Berenguer, 2011). Thermophiles can therefore inhabit extremely hot environments which is possible because of special enzymes adapted to temperatures up to 120 ℃ (Angelin and Kavitha, 2022). Some of these extreme environments for example includes hydrothermal vents, thermal hot springs (Angelin and Kavitha, 2022) and submarine volcanoes (Bajpai, 2023). In 1993 the first discovery of thermophilic sulfate reducing endospores was made during experiments with sediment samples from Aarhus Bay, Denmark. The results showed growth and sulfate reduction at temperatures around 60 ℃ (specifically 62 ℃ and 60 ℃) (Isaksen, Bak and Jorgensen, 1994).

During a temperature gradient incubation experiment made with sediment samples from Smeerenburgfjorden in Svalbard, one of the temperature optimum for sulfate reduction discovered was at 56 ℃. The other temperature optimum in that experiment was at 22 ℃ (Hubert *et al.*, 2009) and in other experiments with sediment samples from Svalbard the optimum for sulfate reduction was at 27 ℃ (Sagemann, Jørgensen and Greeff, 1998). These temperature optimums are assumed to be of psychrophilic SRB which are characterized to be adapted to habitat cold environments (Isaksen and Jorgensen, 1996). The second temperature optimum from the experiment from Hubert *et al.*, (2009) at 56 ℃ is characteristic of thermophilic sulfate reducing endospores.

There was a large phylotype diversity identified in incubation experiments for the thermophilic sulfate reducing endospores discovered in Aarhus Bay. This broad diversity is hypothesized to be an indication of ongoing dispersal through passive influx from external sources which can further be supported by the detection of thermophilic endospores in the water column. The detected thermophilic endospores of *Desulfotomaculum* species at Aarhus Bay were also detected 3000 km away in sediments of Smeerenburgfjorden in Svalbard (Hubert *et al.*, 2009). This discovery is based on classification of three phylotypes that was found both in Aarhus Bay and Smeerenburgfjorden with similar 16S rRNA and dsrAB gene sequences (De Rezende *et al.*, 2013).. Global ocean circulation and connectivity to local Arctic water masses plays an important part in the dispersal and distribution of the thermophilic endospores. Baffin Bay and eastern Svalbard with a low phylotype richness seems to be connected to the partial isolation of the Artic waters from the global ocean circulation (Müller *et al.*, 2014). Endospores are suggested to be dispersed by attaching to other larger particles because when analyzing the water samples taken from Aarhus Bay the sulfate reducing thermophiles was detected in only coarser GF filters (De Rezende *et al.*, 2013) which makes the rate of deposition a dispersal limitation and could be connected to sedimentation rate (Müller *et al.*, 2014).

The focus of this project will be on the thermophilic endospore-forming bacteria in the Arctic Ocean. The study will further be limited to sulfate-reducing bacteria (SRB) which are microorganisms who utilizes sulfate to degrade organic compounds as a nutrient for cell synthesis (Muyzer and Stams, 2008). The SRB are chemoheterotrophs or specifically chemoorganotrophs since they use reduced organic compounds as energy source and carbon

source. They obtain energy through fermentation and respiration (Amils, 2011). The aim of this study was to i.) gain more knowledge of endospore-forming SRB in the Arctic Ocean and their preferred growth temperature and ii.) to gain more knowledge of the distribution and dispersal of thermophilic sulfate reducing endospores in the Arctic Ocean by using sediment samples from two different locations with different hydrographical conditions.

2 Background

2.1 Sulfate-Reducing Bacteria

The process of degradation of the organic compounds in which sulfate is used as the terminal electron acceptor is called dissimilatory sulfate reduction. The end-product of this process is then sulfide (Muyzer and Stams, 2008). Energy conservation is based on the electron donors that mostly consists of short-chain volatile fatty acids (VFA) where acetate, propionate and butyrate are important examples. Degrading and fermenting organic matter in sediments also produces electron donors for the SRB (Jørgensen and Kasten, 2006). The VFAs are oxidized through two different pathways during sulfate reduction where one of them is complete oxidation to form the end products CO_2 and sulfide. The other pathway is a noncomplete oxidation where the end products is instead acetate and sulfide (Lens, 2009).

The process of sulfate reduction is divided into assimilatory and dissimilatory sulfate reduction and is distinguished by the product sulfide. Dissimilatory sulfate reduction produces high concentrations of sulfide or mostly hydrogen sulfide (H_2S) (Rabus, Hansen and Widdel, 2013). Anaerobic SRB use the dissimilatory sulfate reduction process when sustaining energy (Simon and Kroneck, 2013).

The reaction for the dissimilatory sulfate reduction can be described in a net equation where CH_2O represents the organic material which produces the material for the bacterial cells regardless of kind and can be written in net equation 1 following (Jørgensen and Kasten, 2006):

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \qquad (1)$$

Formation of iron sulfides, mainly mackinawite (initial product of H_2S precipitation), and the stable iron sulfide pyrite occur as a result of sulfate reduction where H_2S reacts with dissolved Fe^{2+} or solid iron oxides. In the aerobic zone where there is oxygen in the pore waters there is a high abundance of iron oxides which can be seen in the brown color of the sediment. The formation of pyrite acts as a sink for marine sulfate through the SRB and H_2S formation thus making it an important component in the global sulfur cycle (Vairavamurthy et al 1995). In natural anoxic marine environments 90% of the amount of the product H_2S will reoxidize to sulfate (Canfield, 1993).

2.2 Classifying Sulfate-Reducing Bacteria

Classifying SRB is done by 16S rRNA sequence analysis where three branches of psychrophilic, mesophilic and thermophilic species can be identified (Rabus, Hansen and Widdel, 2013). In the genus *Desulfotomaculum* spp. 17 out of 30 species are thermophilic and the bacteria within the genus are anaerobic and sulfate reducing (Aüllo *et al.*, 2013).

Bacteria that get classified as thermophilic have a temperature optimum above 40 ℃. Moderate thermophiles have a temperature optimum between 40-70 ℃ (Laemthong *et al.*, 2022) and mesophiles can grow from approximately 10 ℃ to 45℃. Thermophiles are otherwise described as having optimal growth at 55-70 ℃ in general to distinguishing them from mesophiles. Temperatures between 30 and 40 ℃ is the optimal temperature range for mesophilic growth. Psychrophiles grow below 20 ℃ and could even grow below 0℃ making them more adaptable to Arctic habitats (Isaksen, Bak and Jorgensen, 1994).

2.3 Endospores

Dipiclonic acid (DPA) is a chemical and physiological feature unique for endospores (Thompson and Leadbetter, 1963) and is important for their survival by preventing spore lysis through creating a stable and water repellant material which thereby increases their longevity (Setlow, 2007). The presence of endospores can be revealed through analyzing DPA (Warth, 1979) and can be estimating the number of endospores (Fichtel *et al.*, 2007). There is a correlation between endospores with DPA and the concentration of hydrocarbon gases in cold

surface sediments. This was determined after a study of surface sediment from the Eastern Gulf of Mexico where the surface sediment had an elevated abundance of endospores at conduit sites releasing hydrocarbons (Rattray *et al.*, 2022).

However, there is still a possibility of high endospore abundance even if there is an absence of hydrocarbon gases which have several different explanations. One explanation could be that the location is in proximity to an active hydrocarbon seep and through lateral dispersion from the active hydrocarbon seep could result in the high abundance of endospores (Rattray *et al.*, 2023). If a hydrocarbon seep becomes inactive the endospores that have been moving up towards the surface of the seabed with hydrothermal fluids will become suspended and therefore not be dispersed. Furthermore, paleo-hydrocarbon sites could then also be an explanation for high endospore abundance with an absent of detected hydrocarbons (Rattray *et al.*, 2023).

2.3.1 Hydrothermal Vent Systems

Hydrothermal vent systems associated with mid-ocean spreading is an example of a source habitat for endospore-forming SRB because of the high temperatures and the mixture of the hydrothermal fluids with cold seawater which is rich in SO_4^{2-} (Dahle *et al.*, 2015). Hydrothermal fluids have low density and because of that will form a hydrothermal plume when it gets mixed and diluted with the seawater and then advect from the source horizontally when the density becomes equal to the seawater. The ocean currents will then carry the hydrothermal fluids away from the source (Dahle *et al.*, 2015).

Two active vent fields were discovered at Mohns Ridge at the southwestern part of the ridge and consists of multiple white smoker chimneys which contain venting fluids up to 270 ℃. Mohns ridge also passes into another ridge called the Knipovich Ridge and at the place where they pass Loki's Castle Vent Field is located. This hydrothermal vent field consists of four (305-317 ℃) hot black smoker chimneys where anaerobic SRB had a relative abundance less then 7% and they were most abundant at the increasing temperature (Dahle *et al.*, 2015). The mid-ocean ridge called Gakkel ridge, located in the Eurasian Basin of the Arctic Ocean, contains hydrothermal vents known as the Aurora vent field. The vent plume above the field have given evidence for an enrichment in methane and possibly hydrogen in the venting fluids which create good conditions for increasing microbial activity through chemosynthesis

(Ramirez-Llodra *et al.*, 2023). The Aurora vent field consist of both active and inactive hydrothermal vents where the active vents are located at a depth of approximately 3800 m.

2.4 Arctic Ocean Circulation Patterns

The Arctic Ocean is linked with the Atlantic Ocean through the 2600 m deep Fram Strait, carrying water from the Norwegian Atlantic Current. The Atlantic Ocean also flows through Barents Sea which is another Norwegian Atlantic branch entering the Arctic Ocean (Rudels and Carmack, 2022). Generalized circulation patterns for Atlantic and Pacific water entering the Arctic Ocean can be seen in figure 1.

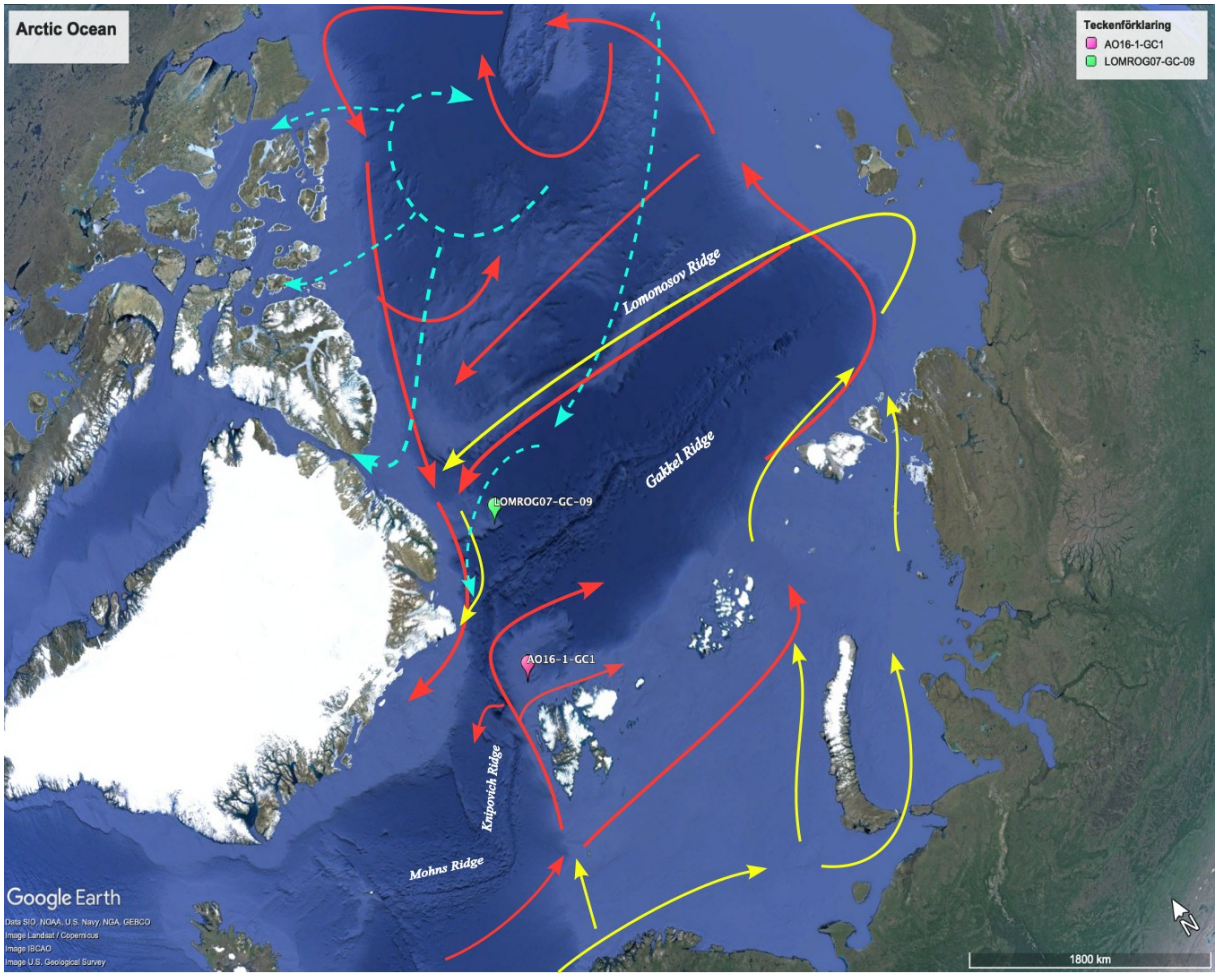

Figure 1. Generalized main circulation patterns from intermediate Atlantic water (red arrows), surface Atlantic water (yellow arrows) and surface Pacific water (blue dotted arrows) in the Arctic Ocean (modified from Raimondi, Wefing and Casacuberta, 2024; Rebesco *et al.*, 2013) together with named ocean ridges. The sediment core locations for this study are also mapped (AO16-1-GC1 and LOMROG07-GC-09).

A large part of Atlantic water flowing through the Nordic Seas takes a path following the slope of Mohns Ridge and along the Knipovich Ridge before entering Fram Strait (Orvik and Niiler, 2002). The West Spitsbergen current is the branch entering through Fram Strait where some of the Atlantic water will recirculate and the rest will split into two streams and enter the Arctic Ocean. The strongest of the two streams flows north of Svalbard while the other stream flows at the western and northern parts of the Yermak Plateau. From the Fram Strait, north of Svalbard, a deeper boundary current starts and flows along the Eurasian continental slope eastward and is starting to flow as Fram Strait Branch (Rudels, 2001). Half of the boundary current will enter Amundsen Basin at the Lomonosov ridge while the rest will enter the Circulation Basin (Beaufort Gyre). After getting split a couple of times more, the boundary current will return to the Eurasian basin by the north of Greenland and then continue back to Fram Strait while getting mixed with other streams and together exit the Eurasian Basin in the East Greenland Current (Rudels, 2001).

The Transpolar Drift and Beaufort Gyre account for the largest part of surface circulation which commonly distribute sediments from the continental shelves through transporting sea ice throughout the Arctic Ocean. Bering Strait links the Pacific Ocean with Artic Ocean and together with freshwater from rivers in Siberia and North America they are called Polar Surface Water (Woodgate and Aagaard, 2005). At water depth 250-1700 m intermediate waters circulate with Atlantic water and shelf water in the Eurasian Basin, the Makarov Basin and the Canada Basin (Björk *et al.*, 2007).

3 Methodology

The method in this study largely follows the method made by Røy *et al.*, (2014) but adapted to our circumstances.

3.1 Site and Sediment Description

Two marine sediment cores (AO16-1-GC1 and LOMROG07-GC-09) retrieved during two earlier expeditions to the Arctic Ocean were used. The sediment cores stored in the cold storage room at Stockholm University.

3.1.1 AO16-1-GC1

The samples for the first experiment were taken from a core drilled by a gravity corer during an expedition to the Arctic Ocean in 2016. 2 cm at the top of the core seemed to be missing so the samples used were approximately from 2-7 cm depth. The location of the core is at the Yermak Plateau (80.5532 °N, 8.052 °E) outside of Svalbard which can be seen in figure 1.

The sedimentation rate varies significantly at different sites on the Yermak Plateau but the average rate have been calculated to be 4.8 cm/ka (Sellén, O'Regan and Jakobsson, 2010) and is speculated to be affected by ice erosion in the shallow parts, a varied amount of ice rafted debris (IRD) from the continental shelf of Svalbard and proximity to Atlantic water inflow.

3.1.2 LOMROG07-GC-09

The second core was retrieved during the LOMROG expedition with icebreaker Oden in 2007. The sediment sample was taken from 0-5 cm from the top of the core. According to the description made by Leonid Polyak (Martin Jakobsson, Christian Marcussen & LOMROG Scientific Party, 2008) the first 0.5-3.5 cm contains dark brown mud with a small amount of sand. There was also a Scaphopod near 3.5 cm that was picked out some time before our sediment samples were taken. From 3.5-5.5 cm the sediment becomes a paler brown and the sediment type is described as lumpy sandy mud (Martin Jakobsson, Christian Marcussen & LOMROG Scientific Party, 2008).

Morris Jesup Rise is the location of the sediment sample acquired from the sediment core (85.2945 °N, -14.8922 °E), seen in figure 1, which was taken in a glacial scour which happened during previous ice ages at a water depth of 1016 m. It is situated at a continental margin 100-200 km north of the shelf edge of North Greenland (Martin Jakobsson, Christian Marcussen & LOMROG Scientific Party, 2008). Average sedimentation rate have been analyzed to be 1.4 cm/ka (Sellén, O'Regan and Jakobsson, 2010).

The sediments accumulated on Morris Jesup Rise is said to be deposited from melting sea ice, IRD (Sellén, O'Regan and Jakobsson, 2010). IRD mainly comes from the Siberian ice shelves (Stein, Grobe and Wahsner, 1994) where the Laptev sea is the major area producing the ice shelves (Sellén, O'Regan and Jakobsson, 2010) and gets transported with the Transpolar Drift throughout the central Arctic Ocean (Stein, Grobe and Wahsner, 1994). Suspended sediments from the waters of the Amundsen Basin and Amerasian basin could also be deposited on the Morris Jesup Rise when the water from the basin flows across the rise (Sellén, O'Regan and Jakobsson, 2010).

3.2 Preparation of Sediment Samples

Exetainers was used for storing the sediment slurries during incubation and before adding the sediments the empty exetainers were weighed. The same was done to the centrifuge tubes before centrifugation of the sediment samples after incubation. The exetainers including their caps were autoclaved in CertoClav steam sterilizer before usage at slightly above 125 °C and 5 bar for 20 min.

3.2.1 Sediment Slurries

The sediment taken from the chosen core was mixed in a bag and then added to a Duran bottle and mixed with brackish artificial seawater in a 1:2 ratio (w:w) was made made by adding the following (Widdel and Bak, 1992):

- Milli-Q: 1.0 l
- NaCl: 7.0 g
- $MgCl_2$ x $6H_2O$: 1.2 g
- $CaCl_2$ x $2H_2O$: 0.1 g
- Na_2SO_4: 4.0 g
- NH_4Cl: 0.25 g
- KH_2PO_4: 0.2 g
- KCl: 0.5 g

To get the desirable environment for the potential sulfate-reducing thermophiles in the sediment, volatile fatty acids (VFA) were added as nutrients to the bottle with the sediment creating a sediment slurry.
Following types and amounts of VFA were added to the sediment slurry:

- Acetate (2 M): 0.36 ml was added to the slurry to get the desired concentration (2 mM).
- Lactate (2 M): 0.72 ml was added to the slurry to get the desired concentration (4 mM).
- Formate (2 M): 1.44 ml was added to the slurry to get the desired concentration (8 mM).
- Propionate (2 M): 0.54 ml was added to the slurry to get the desired concentration (4 mM).
- Butyrate (1 M): 0.72 ml was added to the slurry to get the desired concentration (4 mM).

Additionally, 0.9g of Spirulina was added to get the concentration 2.5 g/L. The sediment slurry was then mixed on the magnetic stirrer for about 20 minutes while flushing with N_2. Thereafter, 1.8 ml of 1 mM Na_2S was added to help drive out all the oxygen to create an anaerobic environment for the microbes.

Approximately 6 ml sediment slurry was transferred from the Duran bottle with N_2-flushed syringes into exetainers. These exetainers were then pasteurized at 80 ℃ to terminate any vegetative cells in the sediment before incubation.

3.2.2 Incubations

An aluminum temperature gradient block ranging between 4-80 ℃ were used for the incubations. Twenty samples were placed throughout the temperature gradient as seen in figure 2. In addition, seven replicates were incubated parallel the low, middle, and high-temperature end. The temperature, which was controlled at the beginning and at the end of the incubation, was found to be fluctuating ±1 ℃ at the cold end of the block. The samples were incubated for four days.

Figure 2. Aluminum temperature gradient block ranging from low temperature at the left side, to high temperature on the right side.

After one day of incubation a ^{35}S-radiotracer was added. The starting concentration of the stock solution of the radiotracer was 370 MBq/ml where the reference date is 23/2-2024. Since it was added a month after the reference date, the radiotracer had decayed which needed to be considered when calculating the volume of radiotracer added to the sediment samples with Equation 2:

$$A(t) = A_0 \times e^{-\lambda t} \qquad (2)$$

The radioactivity in equation 2 is A, t is time, and the decay constant is λ. The known half-life of ^{5}S is 87.51 days. Furthermore, the radiotracer needed to be diluted x10 to be manageable to work with and to get a desired concentration of 120 kBq/sample, it resulted in 4 µl/sample was added for experiment 1, and 5 µl/sample for experiment 2. While adding the radiotracer, N_2 was added through a hose to flush out any oxygen that get into the exetainer.

Another 72 h after the radiotracer was added the incubations were terminated. To the separate exetainers 12 ml ZnAc 20% was added and transferred to centrifuge tubes after shaking the exetainer to mix the sediment slurry with the ZnAc. When all sediment samples had been transferred to the centrifuge tubes they were put in a freezer until they were going distillated.

The samples were then centrifuged for 10 min with the settings; 6000 RPM and 5 °C. This process separated the solid sediment from the liquid sulfate called the supernatant. The supernatant was extracted from the centrifuge tubes and put into smaller tubes, 1.5 ml of

supernatant in each tube. The rest of the supernatant was then thrown away in a can as radioactive waste and saved the solid sediment for the distillations.

3.3 Chromium Chloride-Hydrochloric Acid Distillation

The chromium-hydrochloric acid solution was prepared by mixing 150 g of $CrCl_3$ with 96 ml HCl and 516 g milli-Q. The solution was used for separating TRIS back to H_2S so the sulfide could be transferred and precipitated to a tube of Zinc acetate during the distillation process. A solution of 5% Zinc acetate was also prepared.

Zinc pellets was put in a Duran bottle and connected to a hose with nitrogen gas (N_2). The pellets needed to be cleaned with 2M HCl before the chromium chloride solution was added to be reduced. The reduction process could be observed when the oxidized chromium with a green color turned to a blue color which indicates a reduced product as Cr^{2+}. The mixture was then transferred to 60 ml syringes after approximately 20 minutes of bubbling with N_2 and placed in the fridge (at around 5 °C) until the distillations were made.

For the distillations, three neck distillation bottles were used. In them 1-2 drops of silicone-based antifoam were added together with 1 ml ZnS (0.8 mM) and a magnet. The sediment pellet that was left in the centrifuge tubes after the supernatant was decanted was mixed with the help of a vortex and 10 ml of N-Dimethylformamide (C_3H_7NO). Then each sediment mixture from the centrifuge tubes was put in separate distillation bottles. A couple of drops of milli-Q was used to wash down any spillage at the top of the distillation bottles down to the rest of the mixture. Then they were assembled with Pasteur pipettes and hoses connected to N_2 and to the aerosol trap with 0.1 M citrate buffer, which is the left tube in figure 3 which in turn is connected to 7 ml 5% Zinc acetate in the right tube which is the H_2S trap. Each distillation bottle was placed on a magnetic stirrer and there was room for a total of six bottles in the fume hood during one round of distillation. The N_2-flow was adjusted so that each aerosol and H_2S-trap had visibly around the same number of bubbles at the same speed.

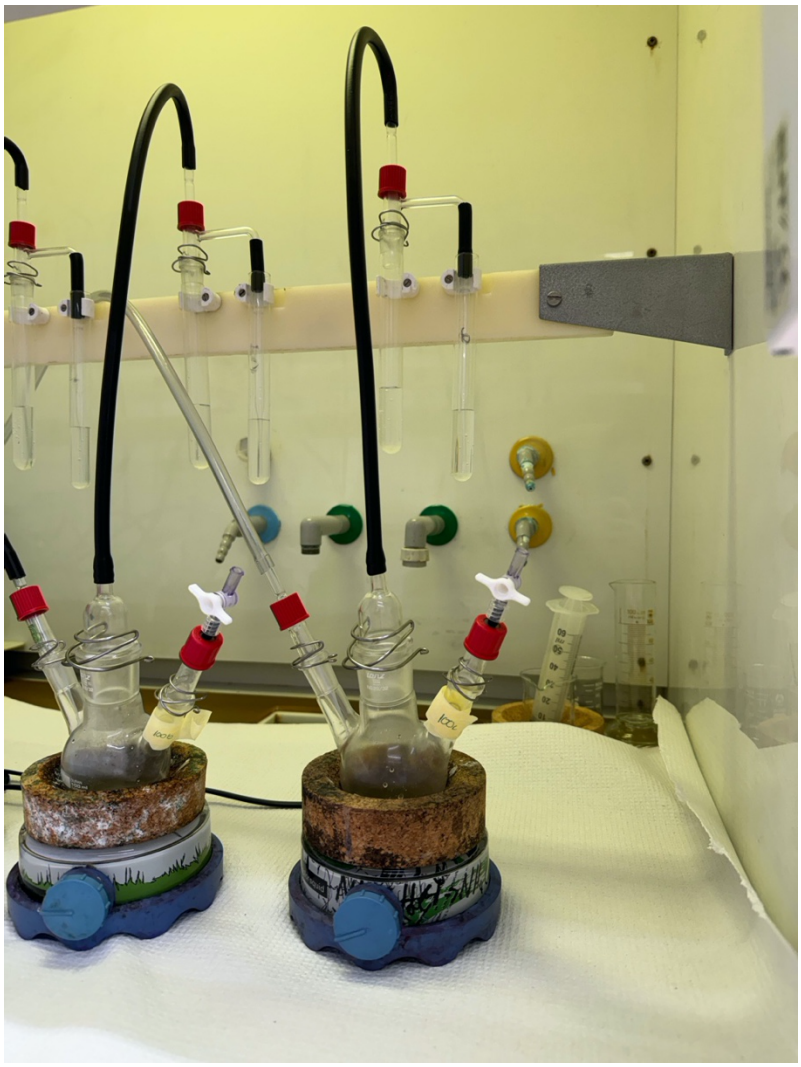

Figure 3. shows the three-neck distillation bottle. The left neck is connected to N_2 via a transparent tube. The middle neck is connected to the aerosol trap in the left test tube which is connected to the H_2S trap in the right tube. The right neck is connected to a valve where HCl and $CrCl_3$ is injected.

Approximately 20 minutes was allowed to flush out the oxygen with N_2, after which 8 ml of 6M HCl and 16 ml of $CrCl_3$ was added to the distillation bottles to convert the TRIS to H_2S.

After 1 hour and 40 minutes of distillation, the pressure of the nitrogen gas was increased and then after another 20 minutes the distillate was taken down and saved for the beta scintillation counting while the bottles were disassembled and washed.

One control sample distillation was made the same way as the other samples but without the sediment slurry. This was then used to analyzed to determine sulfide concentration and then calculate the method efficiency which is described further below.

3.3.1 Beta Scintillation Counting

100 µl supernatant was transferred to scintillation vials and mixed with 2 ml Ultima Gold XR and 900 µl milli-Q. After wiping the vials with ethanol to minimize contamination they were placed in a liquid scintillation counter Tri-Carb 2910 TR. To determine background activity, two blank samples were used where 1 ml milli-Q was mixed with 2 ml Ultima Gold XR.

The H_2S traps with ZnS from the distillations were mixed with a vortex for about one minute each and then added to larger scintillation vials together with 14 ml of Ultima Gold XR. Two blank samples were also made where 7 ml of 5% ZnS was mixed with the 14 ml of Ultima Gold XR. All the vials were mixed before they were also placed in the liquid scintillation counter. The beta scintillation counting measure the radioactive decay in counts per minute (CPM) from the solutions in the small and large vials.

3.4 Sulfate and Sulfide Analysis

3.4.1 Ion Chromatography

Sulfate concentration analyses were done through ion chromatography (IC), specifically anions, with the Shimadzu IC where some of the supernatant samples from our experiments were chosen to be analyzed. Each sample was diluted x400 (39.9 ml milli-Q + 0.01 ml supernatant) and transferred to 1.5 ml vials. An anion eluent containing 10.08 g $NaCHO_3$ and 0.64 g Na_2CO_2 and diluted x10 was used in the process.

The data was processed by a computer system for Shimadzu IC and got generated into a chromatogram from where the sulfate concentrations could be extracted. The results from the ion chromatography could then be used to calculate the SRR and highest detection limit with equation 3.

3.4.2 Spectrophotometry

Sulfide concentrations in control experiments were determined by spectrophotometry as described by Cline, 1969 using an Evolution 260 Bio-UV Visible spectrophotometer from

ThermoFisher Scientific and the associated software INSIGHT2 v.2.1.133. A calibration curve was constructed before analyzing the control samples by using a dilution series of a ZnS standard stock solution (0.8 mM) with ZnS concentrations ranging from approximately 0 to 40 µM. 0.8 ml diamine mixed reagent was mixed with 10 ml of each concentration and immediately capped to prevent volatilization of H_2S. After being placed for 30 min in the dark, 1 ml of the concoction was transferred to 1 ml cuvettes and measured at 670 nm in the spectrophotometer.

The calibration curve was plotted with the absorption as the x-axis and respective sulfide concentrations on the y-axis. By fitting a linear function to the data points, an absorption coefficient could be obtained which subsequently could be used to calculate the concentrations from the absorbance of the measured samples. Which were prepared the same way as the standard as just described.

3.5 Calculating Sulfate-Reduction Rate

Calculations of the sulfate-reduction rate (SRR) can be made with equation 3 following (Kallmeyer *et al.*, 2004):

$$SRR = [SO_4^{2-}] \times P_{SED} \times \frac{a_{TRIS}}{a_{TOT}} \times \frac{1}{t} \times 1.06 \times 1000 \qquad (3)$$

which includes the sulfate concentration $[SO_4^{2-}]$, radioactivity of reduced sulfur (TRIS) which is aTRIS in the equation and radioactivity of total amount of sulfate used (aTOT). The porosity (P_{SED}) of the sediment slurry and incubation time (t) is also considered. The isotope fractionation factor is the value 1.06 in equation 3 and used to make a correction of the slower turnover from $^{35}SO_4^{2-}$ by using the relation to the lighter isotope $^{32}SO_4^{2-}$.

4 Results

4.1 AO16-1-GC1

Only one sample from the core AO16-1-GC1 showed any evidence of microbial activity above the detection limit. This occurred at 32 °C with a SRR of 0.02 nmol/cm^3/h, seen in figure 4.

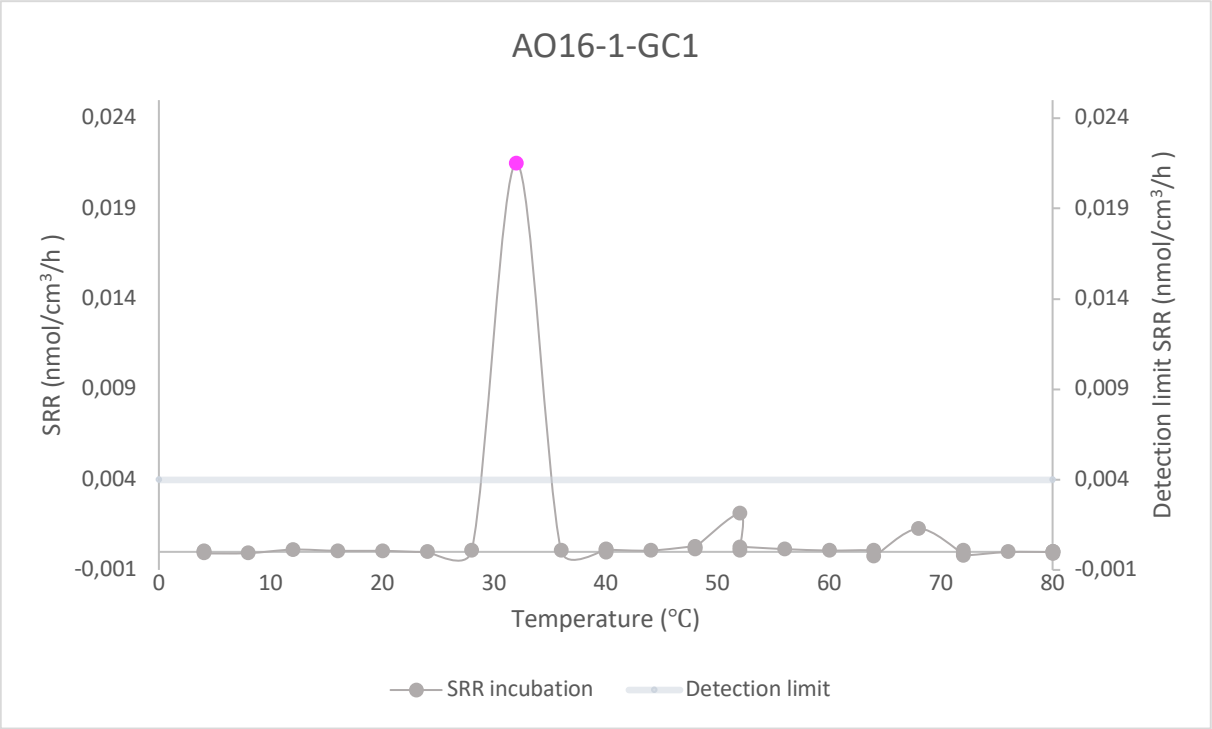

Figure 4. SRR vs temperature of the first experiment using sediment from core AO16-1-GC1. Grey dots indicate SRR below detection limit. The dot highlighted in pink is the sample with a SRR above the detection limit.

The highest detection limit for sulfate reduction was calculated by using equation 3 which resulted in the following:

$$SRR = 28.7 \times 1 \times \frac{41}{4351331} \times \frac{1}{72} \times 1.06 \times 1000 = 0.00398 \text{ nmol/cm}^3\text{/h}$$

Which used the highest a_{TRIS} (41 CPM) which was from the control distillation sample and the lowest a_{TOT} from all the samples (4351331 CPM). The sulfate concentration $[SO_4^{2-}]$ was from the analyzed reference sample (i.e., a sample that was not incubated) and was 28.7 mM.

4.2 LOMROG07-GC-09

Visible black precipitation, seen in figure 5, of possibly FeS_2/FeS due to microbial activity in the exetainer incubated at temperature 32-40 °C which could be observed after the end of the incubations. This indicates that sulfate has been reduced to sulfide and has either reacted with iron oxides in the sediment or from the artificially made brackish water.

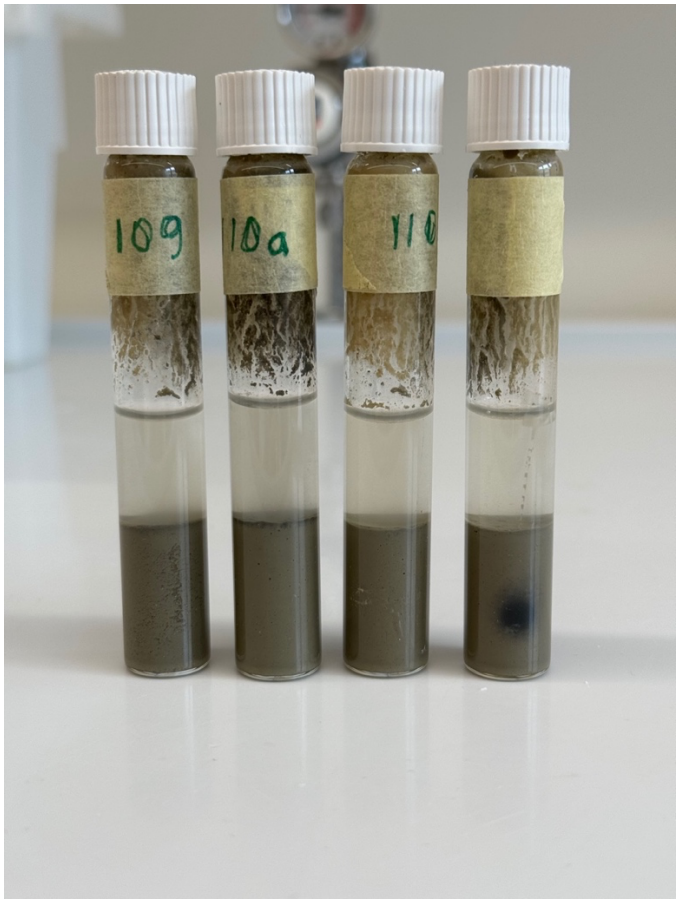

Figure 5. Black precipitation (FeS) visible in sample from left to right 109, 110a, 110b and 110c incubated at temperature 36-40 °C.

The highest detection limit for the sulfate reduction was calculated with equation 3 and was:

$$SRR = 28.7 \times 1 \times \frac{41}{5231812} \times \frac{1}{72} \times 1.06 \times 1000 = 0.003 \text{ nmol/cm3/h}$$

which was put into a scatter plot together with the results from the other samples and the results from the sulfate concentration analysis in figure 6.

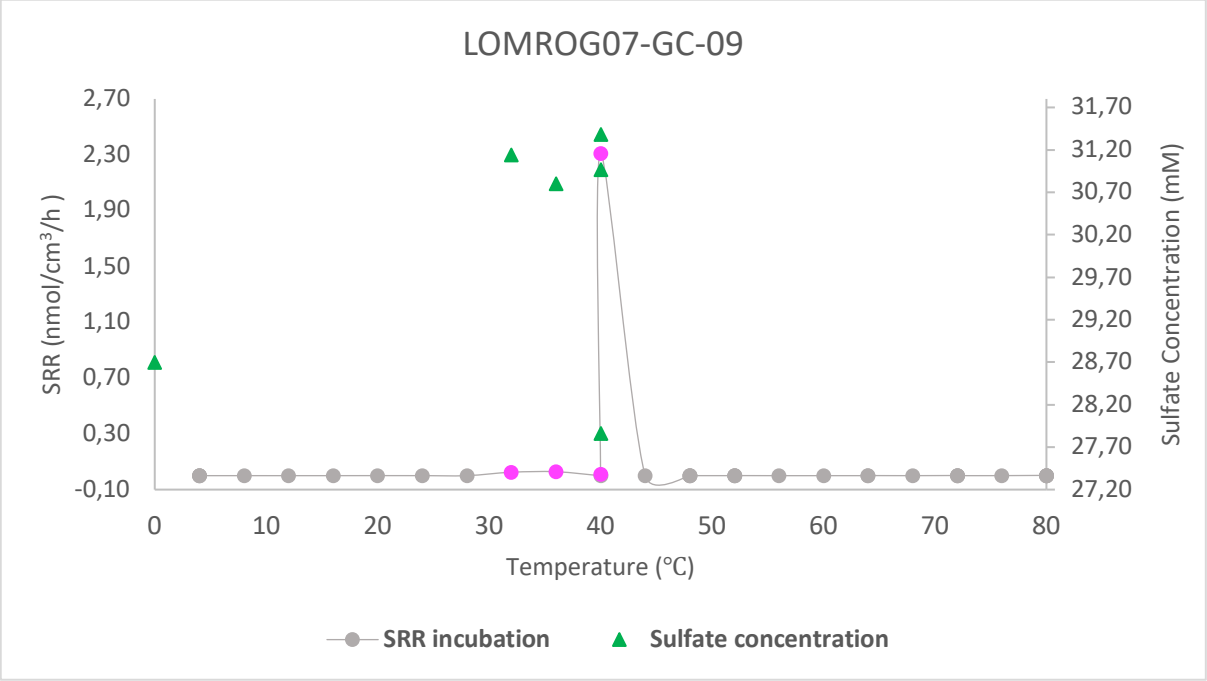

Figure 6. SRR vs temperature and sulfate concentration from the ion chromatography. The grey dots represent the samples that had a SRR under the detection limit and the pink dots are the samples with a SRR above the detection limit. Five samples with the most activity was analyzed to get their respective sulfate concentration which are displayed as green triangles. The green triangle at 0 °C represents the sulfate concentration for the reference sample.

4.3 Sulfide Analysis

With the calibration curve, figure 7, obtained after spectrophotometric analysis and the absorbance results from the control samples, a concentration could be calculated for each of the samples (Table 1).

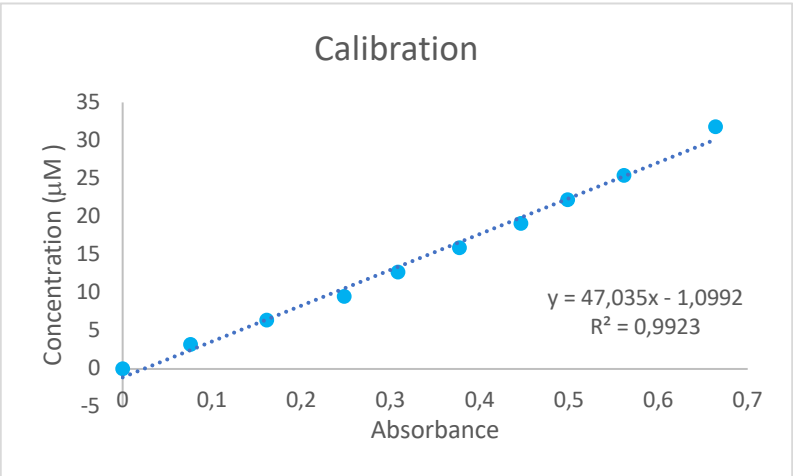

Figure 7. Calibration Curve for the spectrophotometric analysis.

The concentrations for the control samples were then divided by the concentrations from the calibration and multiplied by 100 which resulted in different and very low distillation efficiencies.

Table 1. The results from the Spectrophotometric analysis of six control samples from different experiments and a calculated mean efficiency with a calculated standard deviation for all of the six control samples. The sixth control sample is done for this study.

Control Sample	**Absorbance (670 nm)**	**Concentration (μM)**	**Distillation efficiency (%)**
CTRL 1	0.52	23.2	73
CTRL 2	0.499	22.3	70
CTRL 3	0.388	17.3	55
CTRL 4	0.338	15.1	48
CTRL 5	0.549	24.5	77
CTRL 6	0.405	18.1	57
Mean efficiency			63 ± 12

CTRL 6 is for the experiments done in this project. The other control samples were taken during earlier experiments but analyzed at the same time as CTRL 6 and were used to calculate a mean efficiency for the distillations.

5 Discussion

5.1 Presence of Endospores in Arctic Marine Sediments

LOMROG07-GC-09 showed evidence for higher microbial activity among the two sediment cores used for this study. However, no thermophilic activity can be seen in neither of the cores since the activity could only be found at 32, 36 and 40 ℃ as seen in figure 4 and 6. The highest SRR was at 40 ℃ in the core LOMROG07-GC-09 and was 1.97 nmol/cm^3/h. This activity would be classified as mesophilic since their growth range is between 10-45 ℃ and the optimum growth is 30-40 ℃. It could be argued to also resemble some moderate thermophilic activity since thermophiles start to grow at approximately 40 ℃ but their

optimum growth is at 55-70 ℃ (Isaksen, Bak and Jorgensen, 1994) which makes it highly unlikely for this experiment to have thermophilic activity. Additionally, the samples were pasteurized before incubation which should have killed the psychrophilic and mesophilic vegetative cells (Müller *et al.*, 2014) meaning that the activity found in the samples after incubation must be in the form of endospores.

Two of three replicates of samples incubated at 40 ℃ from the core LOMROG07-GC-09 had a SRR above the highest detection limit which is a little troubling. Since there are three replicates, meaning that they are incubated at the same temperature (40 ℃) they should have relatively similar activities.

Moreover, the sulfate concentration showed that two of three replicates had a sulfate concentration higher than the reference sample while the third had a lower sulfate concentration than the reference sample. A lower sulfate concentration can be seen as evidence for microbial activity since SRB reduce sulfate to sulfide (Muyzer and Stams, 2008) and thus lowering the concentration of sulfate in the water. The, in comparison to the reference sample, high sulfate concentrations from the other two replicates could be because of reoxidation of sulfide back to sulfate during incubation or that other microorganisms utilized sulfide and oxidized it back to sulfate.

5.2 Dispersal Vectors and Possible Endospore Source Habitats

The average sedimentation rate for Morris Jesup Rise was as mentioned 1.4 cm/ka (Sellén, O'Regan and Jakobsson, 2010) which in our experiment with 5 cm surface sediment represents sediment deposited for the last approximately 3600 years rounded up. On the Yermak Plateau with average sedimentation rate is said to be 4.8 cm/ka (Sellén, O'Regan and Jakobsson, 2010) the sediment samples used in the first experiment reflects sedimentation from the last approximately 1000 years. In Smeerenburgfjorden where thermophilic SRB endospores were discovered the sediment in that area accumulated at a rate of 0.19 cm/year (Hubert *et al.*, 2009) which is over 100 times more than in Morris Jesup Rise. The water depth by Smeerenburgfjorden is between 0-50 m and seems to mainly affected by the East Spitsbergen Current (Knies, Brookes and Schubert, 2007). However, there are studies that

show that there is an influence of the West Spitsbergen current deep in fjords of West Svalbard.

Baffin Bay had a low phylotype richness of thermophilic endospores in comparison with West Svalbard (Müller *et al.*, 2014). The currents affecting Baffin Bay are the relatively warm West Greenland current, because of the influence from Atlantic water, and the cold Baffin current, which is outflow of the Arctic Ocean water (Bourke and Paquette, 1991). There was also lower phylotype richness in East Svalbard when comparing to West Svalbard (Müller *et al.*, 2014). The sediments sampled from Baffin Bay was more influenced by water from the Arctic Ocean and when compared with West Svalbard, it was discussed that the reason behind this could be a limitation of sources dispersing thermophiles in the Arctic Ocean together with low connectivity to world oceans with a higher abundance of thermophilic sources (Müller *et al.*, 2014)

The average sedimentation rate in Baffin Bay is around 6.5 cm/ka (Simon, St-Onge and Hillaire-Marcel, 2012) which is similar to the Yermak Plateau. However, unlike Baffin Bay, the studied sediment core from the Yermak Plateau had an absence of thermophilic activity and is also closer connected to world oceans through its proximity to the West Spitsbergen Current. Since there is no discovered evidence of thermophilic activity at the location we studied, it could be because of stronger currents possibly making endospore deposition and accumulation more difficult.

A potential dispersal source of thermophiles is the Aurora vent field at the Gakkel Ridge due to its habitable conditions. Mesophilic SRB have previously also been discovered in deep-sea hydrothermal vents where samples from hydrothermal water with in situ temperatures ranging from 100-400 ℃ showed evidence of mesophilic SRB (Elsgaard *et al.*, 1995). The mesophilic activity in this study could because of this, possibly be linked to the Aurora Vent Field by the Gakkel ridge through the advection from the hydrothermal plumes (Dahle *et al.*, 2015) and currents that flows between the Lomonosov ridge and Gakkel ridge flowing across the Morris Jesup Rise (Sellén, O'Regan and Jakobsson, 2010).

The Gakkel Ridge as mentioned is also a habitable environment for thermophilic bacteria meaning that even though there is no current evidence for thermophilic activity at Morris

Jesup Rise there could still be dispersal of thermophilic endospores along with the mesophilic endospores. There might be a higher abundance of the mesophilic endospores than thermophilic which means that there might be a higher probability for a couple of the mesophilic endospores to accumulate at Morris Jesup Rise since the average sedimentation rate was low. More experiments must be conducted in the area since the sediment core only represent a couple of cm in diameter of the seabed.

5.3 Uncertainties and Potential Errors with the Experiments

This is a sensitive experiment since it is conducted on living SRB which means that any type of contamination will affect this microbial community's metabolism and therefore give inaccurate results. Previously tested experiments with the same method showed a margin of error of less than 10% during separation and quantifying radioactive TRIS (Røy *et al.*, 2014).. An important and large part of the method is keeping the environment anoxic with N_2 since SRB are anaerobic which then would be perturbed by oxygen contamination. Since it is difficult to always keep the environment purely anoxic during the experiment there is a high risk that the samples have been contaminated.

Sulfate reduction to H_2S have a rapid turn-over rate and is small in pool size. H_2S does not often stay in its original form because some of it will reoxidize to sulfate and some will precipitate to iron sulfides or elemental sulfide (S^0). Because of this process that can happen during the incubation of the sediment samples, the sulfate reduction is underestimated. In a similar experiment with marine sediment from Aarhus Bay, a calculation of the asymptotic net rate of sulfate reduction in oxidized 0-1.5 cm of the sediment core was 1/5 of the gross rate of the actual sulfate reduction. The rest had been reoxidized to sulfate. (Moeslundi, Thamdrup and Barker Jørgensen, 1994). These differences are hypothesized to be a result from the oxic conditions in the surface sediment layer which can then be argued to be an error source in our experiment in the case that they were contaminated by oxygen.

The sediments used for the samples were taken from the surface sediment layer from the respective cores which corresponds to the oxic-suboxic zone. However, the oxygen should have been flushed out by N_2, if done correctly, while transferring the sediment slurries to the exetainers before the incubations and when adding Na_2S to the sediment slurries. When

opening the lids of the exetainers, erratic errors can occur despite trying to flush out all the oxygen during every step.

Another possible source of error is that sulfide could have gotten stuck in the distillation equipment meaning that all the precipitated sulfides did not end of in the H_2S-trap. In the method made by (Røy *et al.*, 2014) they used equipment almost entirely made of glass whereas we used tubes made of butyl which sulfide should not stick to but since the tubes have been washed by hand and reused it raises a question if the tubes get worn and raise the risk of sulfide getting stuck and therefore not reach the H_2S trap. Røy *et al.*, (2014) continues to mention that they wrapped the Pasteur pipette in cotton and drilled a hole in the lid of a scintillation vial (H_2S-trap) and inserted the Pasteur pipette through there which was to avoid losing aerosols. Our H_2S-trap was a test tube made of glass and there was no lid on it which means that there could have been a loss of aerosols.

The contents from the H_2S trap were then stirred with a vortex and transferred to a scintillation vial and since it is difficult to transfer every drop to the scintillation vial it also makes this step a source of potential loss of sulfide since it was not analyzed in the beta scintillation. Moreover Røy *et al.*, (2014), unlike us, also broke of the tip of the Pasteur pipette into the scintillation vial which got included in the beta scintillation counting. It was visible that something got stuck in the tip of our Pasteur pipettes, however how much of that was sulfide could not be determined. We tapped on the pipettes to get as much as possible out but should have broken off the tips to increase the certainty.

In the control experiments testing the distillation efficiency, the sulfide analysis gave very low results (57%) in comparison to earlier experiments where the results were above 80% and the exact same method was used. This raises suspicion of an error with the laboratory equipment used since the sulfide analysis determines the distillation efficiency. The purpose of the H_2S trap is to catch the entire amount of precipitated sulfide during the distillation which should result in an efficiency of 100%. However, this result could explain the absence of sulfate reduction in one of our replicates, if a large amount of H_2S had been lost at one or several steps during the distillation even though there initially was an indication of sulfate reduction in the form of FeS.

Taking the potential sources of error and loss of sulfide during the distillation into consideration, it is difficult to say how much it would have affected the SRR results if they were minimized as much as possible. There was only one sample in the AO16-1-GC1 experiment that went above the detection limit and five samples in LOMROG07-GC-09. The SRR for those samples maybe could have been higher if all error sources were eliminated but it would still represent mesophilic activity. It could have raised the SRR of some of the thermophilic temperatures but to a very small degree and it is uncertain if the SRR would even then be above the detection limit.

6 Conclusion

The incubation experiments displayed an activity equivalent to mesophilic sulfate reducing bacteria and was in the form of endospores since pasteurization of the samples occurred before incubation eliminating vegetative cells. The endospores found in the sediments in Morris Jesup Rise must therefore have been dispersed from a distant source during the last 3600 years. Aurora vent field located at the Gakkel Ridge is a potential dispersal source for these endospores.

Exact sulfate reduction rates for this study are not known since the results are unreliable due to the low method efficiency displayed in the sulfide analysis meaning there has been a loss of sulfide during the distillation of the samples. The sulfate reduction rate is therefore suspected to be higher. It is unknown whether a method efficiency of 100% would reveal thermophilic activity hence why, for this study, the only certainty is mesophilic activity.

More thermophilic endospore studies need to be made in the Arctic Ocean to see a clearer dispersal pattern and determine potential dispersal sources. Up until now only assumptions of dispersal patterns have been made suggesting that the Arctic Ocean water contain lower endospore abundance in comparison to water heavily influenced by the Atlantic Ocean (Müller *et al.*, 2014).

Acknowledgements

I would like to thank Emelie Ståhl and Volker Brüchert for the opportunity to be a part of this project and for all the support and interesting discussions. I would also like to thank Julia Steinbach for the kind help and patience with the IC analyses.

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