The interplay between Neoarchean oceans and Cyanobacteria. Oxygen production and the oxidation of Fe(II)

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Titlepage shows a 2767-2717 Ma old Algoma type Banded Iron Formation from the 11b Outcrop in the Temagami Greenstone Belt, Ontario, Canada (Ginley 2016). Photo taken by Achim J. Herrmann on 25.08.2018 on the SPP1883 Field Workshop Canada.

I. Declaration of Originality

Hiermit versichere ich, dass die vorliegende Dissertation von mir in allen Teilen selbstständig angefertigt wurde und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt wurden.

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Achim Jan Herrmann

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III. Abbreviations

BIF	Banded iron formation
Ga	Giga annum = billion years
GOE	Great oxygenation event
GR	Green rust
MOR	Mid-oceanic ridge
NOE	Neoproterozoic oxygenation event
PAL	Present atmospheric levels
ppm	Parts per million
ppmv	Parts per million as volume fraction
RW	Riverine water

1. Introduction

Life on Earth evolved under atmospheric conditions very different to those existing today. For over 2 billion years, the atmosphere contained no free oxygen, with only faint, localized "oxygen whiffs" being detected from 3 billion years onward. The permanent oxygenation of Earth's atmosphere around 2.4 billion years ago is largely ascribed to Cyanobacteria, which were releasing large amounts of O_2 into the atmosphere through the process of oxygenic photosynthesis. This dissertation focuses on possible mechanisms for the delay between the first detected "oxygen whiffs" and the large-scale oxygenation of the Archean atmosphere. Therefore, I would first like to introduce the reader to the conditions in the Archean eon, before focusing on the conditions in the Neoarchean era from 2.5 to 2.8 Ga .

1.1 The Archean eon

The Archean eon is the second of the four geological eons of Earth. It started after the Hadean eon 4.0 Ga ago, and was superseded by the Proterozoic eon approximately 2.5 Ga ago (International Commission on Stratigraphy 2020). The Archean-Hadean boundary is dated to the formation of a stable crust at around 4.0 Ga ago by the oldest known rock formations of the Acasta Gneiss Complex in Nuvvuagittuq Greenstone Belt, Canada (Whitehouse *et al.* 2001). However, evidence for a crust and oceans exist prior to the Archean eon in the form of 4.4 Ga old detrital zircons, which show signs of low temperature interaction with a liquid hydrosphere (Wilde *et al.* 2001). Other 4.1 Ga old zircons even hint at the first signs of biological life in the form of carbon inclusions, which show a similar isotopic fractionation to biogenic carbon (Bell *et al.* 2015). Nevertheless, so far zircons older than 4 Ga were only found embedded in younger host rock, suggesting that the original Hadean crust was at least partially remelted. This is largely attributed to a hypothetical intense period of asteroid impacts, called the Late Heavy Bombardment (Kasting 2019). Traces of life in the Archean were found in the form of

isotopically light carbon micro particles at two geological unrelated sites in the 3.8 Ga old sedimentary rocks in the Isua supracrustal belt in West Greenland (Mojzsis *et al.* 1996; Rosing 1999). Direct signs of ancient life are present in the 3.45 Ga old carbonate rocks in the Pilbara Craton, West Australia, in the form of stromatolites (van Kranendonk *et al.* 2003). The early ancient life during the Archean consisted most likely of anaerobic prokaryotic organisms, which used the various reactive substances released by strong volcanic activities and/or anoxygenic photosynthesis to generate energy and reduction equivalents for their metabolism (Kasting 2019; Lepot 2020). Before delving further into the causes, which led to the demise of this anaerobic paradise and the end of the Archean eon at 2.5 Ga, I first would like to introduce the conditions of the Neoarchean (2.8-2.5 Ga) environment in order to better understand the scope of the fundamental changes that were about to happen.

1.2 The (Neo)-Archean environment

1.2.1 A ferruginous ocean

Although the subject of intense study efforts, the composition of the Archean ocean and its properties are not well understood. Not only is the available sample material limited, as most of the Archean crust was reworked by plate tectonics, but the remaining material experienced extensive alteration during the eons (Kasting 2019). The sample material is also limited by deposition mechanisms, as very soluble components of the ocean, like sodium chloride, will only leave indirect evidence, as they are either washed out by fluid intrusions or will not deposit at all under normal ocean conditions (Holland 1984). Despite these difficulties, some of the Archean oceans most important properties could be approximated from the rock record.

As geological evidence for the salinity of the Archean ocean are scarce, geochemical mass balance equations were used to determine the halogen flux from the mantle to the ocean and the ocean crust (Holland 1984). If one assumes that all halite evaporates were once in the

oceans, the salinity of Archean oceans would be 1.2 times the average modern day salinity of around 35 g × kg^{-1} (Holland 1984; Millero *et al.* 2008). Later models also included brines from evaporating saltwater in the mass calculations and derived a salinity of 1.5 to 2 times higher than modern oceans (Knauth 2005). Novel experimental approaches measured the fluid inclusions inside of hydrothermal quartz crystals from the Dresser formation in the Pilbara Craton, NW Australia and the Barberton Greenstone Belt, South Africa to derive the salinity from the $K/^{36}Ar$ to $Cl/^{36}Ar$ ratios and suggest a salinity in the range of today's oceans (Marty *et al.* 2018). The ratio of approximate 50 times more Cl^- to K^+ ions is also close to today's ratio of 53.5 in the ocean (Millero *et al.* 2008; Marty *et al.* 2018). With these values, and an expected Archean ocean salinity equal to the modern ocean, the theoretical composition of Archean seawater can be modelled if the concentration of the other major contributors to the ocean salinity, besides Na^+ and Cl^-, are known. Extrapolating from today's ocean composition, these ions would be Mg^{2+}, Ca^{2+} and SO_4^{2-}, which currently have an average concentrations of 54.74 mM, 10.66 mM and 29.27 mM, respectively (Millero *et al.* 2008). The concentrations of Mg^{2+} and Ca^{2+} ions in the Archean ocean can be modelled by comparing the mid-oceanic ridge (MOR) hydrothermal weathering with riverine water (RW) flux rates (Wilkinson and Algeo 1989). By calculating the variations in the MOR/RW flux over time and testing the model on Precambrian carbonate precipitates of pseudomorphs based on aragonite ($CaCO_3$), the concentrations of Mg^{2+} and Ca^{2+} ions at a given time in Earth's history can be estimated (Hardie 2003). This infers for the onset of the GOE at 2.5 Ga ago, a calcite sea composition with nearly equal amounts of Mg^{2+} (~45 mM) and Ca^{2+} (~55 mM)-ions (Hardie 2003). More recent models with additional variables for continental volume and the effects of long-term hydrothermal activity, lowered this value for Mg^{2+} to ~ 25 mM and ~ 40 mM Ca^{2+} (Jones *et al.* 2015).

In contrast to the theoretical modelled values for Mg^{2+} and Ca^{2+} concentrations in the Archean ocean, SO_4^{2-} concentrations can be calculated from the isotope fractionation of abundant

Archean bulk pyrite (Habicht *et al.* 2002). Unlike the modern day large sulfate fractionation, Archean sediments show almost no mass dependent sulfur isotope fractionation, which would infer a sulfate concentration of less than 200 µM (Habicht *et al.* 2002). Archean sulfate concentrations might have been even lower as a study comparing the sulfur fractionation of Lake Matano (Indonesia), an extreme low sulfur environment (Crowe *et al.* 2008), with Archean sulfur mass fractionation found the upper limit of Archean sulfate concentrations to be no more than 15 µM (Crowe *et al.* 2014).

Iron, although only a trace element of the modern ocean due to its poor solubility in its oxidised state (Millero *et al.* 2008), was one defining feature of the anoxic Archean ocean (Holland 1973). Under anoxic conditions, Fe(II) is highly soluble in water and was most likely washed into the sulfate depleted Archean oceans by anoxic continental weathering and hydrothermal fluids (Holland 1973; Derry and Jacobsen 1990; Kump and Seyfried 2005). The concentration of Fe(II) in the Archean ocean was estimated on the basis that the molar ratio of dissolved Fe(II) to Ca^{2+} is equivalent to the ratio of the solubility product of siderite ($FeCO_3$) to calcite ($CaCO_3$) (Canfield 2005). If the Archean ocean was at its saturation point with both mineral phases, this results in an Fe(II) concentration between 40 to 120 µM (Canfield 2005). The concentration of Ca^{2+} used for this calculation was estimated to be between 10 to 30 mM (Horita *et al.* 2002). Newer models predict a Ca^{2+} concentration of around 40 mM, which would infer a maximum Fe(II) concentration of approximately 160 µM.

As the Archean ocean concentration of Ca^{2+} strongly depends on the model used for hydrothermal fluid interactions, and the concentration values for Fe(II) from Canfield (2005) are widely accepted, the upper limit of Canfield's calculation of 120 µM Fe(II) was used in this study. Otherwise, the composition of the sea water media was not changed, as the salinity of the Archean ocean seems to be in the range of the modern ocean, while the concentrations of Mg^{2+} and Ca^{2+} vary depending on the hydrothermal activity but do not stray away too far from

the composition of the modern ocean. The sulfate levels of the media were also not changed as the photosynthetic production of O_2 by Cyanobacteria would have most likely mobilized sulfate from the host rock or pyrite precipitates, raising the local concentrations of sulfate to unknown levels.

1.2.2 Temperature, carbonate chemistry and pH

The pH and especially temperature of the Archean oceans is highly debated as it is crucial for understanding the early evolution of life. Predictions for temperature constrain the possible composition of an Archean atmosphere, primarily the estimated amount of the important greenhouse gases methane (CH_4) and carbon dioxide (CO_2) in the atmosphere and ocean (Mitchell 1989). Increased amounts of greenhouse gases were especially important as the primordial sun only had 70% of today's sun radiation intensity, while geological records still show signs of liquid water, coining the term "faint young Sun paradox" (Sagan and Mullen 1972; Newman and Rood 1977). Various methods have been used to infer the Archean ocean temperatures such as $^{18}O/^{16}O$ fractionation of 3.5 Ga cherts from Swaziland, South Africa, which indicate an ocean temperature of 55 – 85°C (Knauth and Lowe 2003). However, following studies suggest that overall Archean ocean water composition of ^{18}O was ~ 10‰ lighter then today because of larger, low temperature hydrothermal zones and shallower ocean water penetration in MOR hydrothermal systems (Kasting *et al.* 2006). More recent studies using deuterium or phosphate associated ^{18}O fractionation, in addition to chert ^{18}O isotope fractionation, suggest lower ocean temperatures of around 40°C or 26-35°C respectively (Hren *et al.* 2009; Blake *et al.* 2010). These temperatures were most likely maintained by greenhouse gases such as CH_4 and CO_2 (Kasting *et al.* 1983; Owen *et al.* 1979). The amount of heating by CH_4 and CO_2 is limited by the formation of climate cooling organic hazes if the CH_4/CO_2 ratio exceeds more than 0.2 (Trainer *et al.* 2004). At a proposed methane concentration of 1000 ppmv to maintain early Earths temperatures prior to the GOE, the maximum CO_2 concentration before

fogging occurs would have been 5000 ppmv (Pavlov *et al.* 2000). Modelling of a methane atmosphere suggests that pCO_2 levels would have been in the range of ≤0.03 bar (Haqq-Misra *et al.* 2008), a value which is in the constrains of studies of a 2.2 Ga year old paleosol, which suggests a maximum concentration of CO_2 in the range of <0.039 bar (Rye *et al.* 1995). The amount of CO_2 in the atmosphere also acts as an important temperature buffer, for the release of CO_2 leads to a subsequent increase in temperature associated with increased CO_2 burial by stronger weathering, thereby cooling down the Earth again (Walker *et al.* 1981). Early models employing carbon cycling during the Archean therefore suggest a mean temperature of ~0°C (Sleep and Zahnle 2001). The newest models, which include the effect of CO_2 on ocean pH and seafloor weathering, suggest a climate closer to today with a temperature of $19\frac{+26}{-12}$°C, a more acidic ocean of pH $7.0\frac{+0.7}{-0.5}$ and CO_2 concentrations of $0.012\frac{+0.12}{-0.01}$ bar at 2.5 Ga (Krissansen-Totton *et al.* 2018). The large uncertainty of CO_2 concentrations leads to a bigger range of possible inorganic carbon concentrations in the Archean ocean of between 5 and 30 mM, with a Calcium/Carbonate alkalinity (HCO_3^- + 2×CO_3^{2-}) ratio of less than 0.75 (Blättler *et al.* 2017).

During this work, the CO_2 concentration of the Archean simulation experiments was set at the lower Archean limit of CO_2 at 0.002 bar (2000 ppm), while the media pH was buffered to a neutral pH of 7, which is in contrast to the modern day values for CO_2 and sea water pH of around 440 ppm and 8.1, respectively (Millero *et al.* 2008; Herrmann and Gehringer 2019). The incubation temperature was maintained at room temperature of 22°C, which is well within the expected temperature range of an Archean environment.

1.3 A history of Earth's atmospheric oxygen

The present, lower Earth atmosphere consists to over 99% of mostly inert dinitrogen gas (N_2, 78.08%) and very reactive dioxygen gas (O_2, 20.98%) (Farmer and Cook 2013). The atmospheric O_2 gas is constantly being removed during many biological and geological processes, like cell respiration or weathering of rock surfaces. Therefore, O_2 must be replenished perpetually by the activity of oxygenic photosynthetic organisms to maintain the present levels of atmospheric O_2.

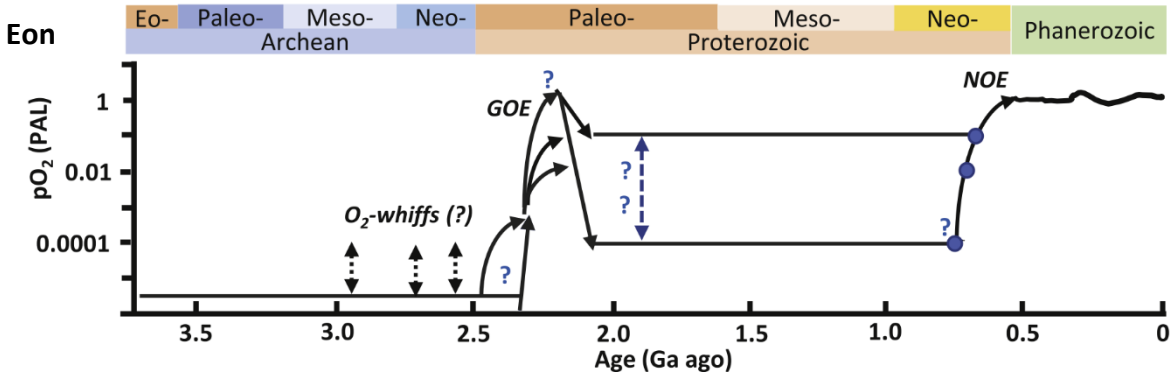

Figure. 1: O_2 partial pressure relative to the present atmospheric levels (PAL) over time during the Eons. Indicated are signs of O_2 whiffs in the rock record as well ass the Great Oxygenation Event (GOE) and the Neoproterozoic Oxygenation Event (NEO). From Lepot (2020), which was adapted from Lyons *et al.* (2014).

The high level of atmospheric O_2 is, relative to Earth's complete history, a modern phenomenon and developed during the Neoproterozoic Oxygenation Event (NOE) around 0.85 to 0.54 Ga ago (Figure. 1, Holland 2006; Och and Shields-Zhou 2012). The NOE had the atmospheric oxygen concentration go up from < 1 % O_2 to O_2 levels comparable to today, and was the second big change in the atmospheric oxygen concentration of Earth (Holland 2006; Planavsky *et al.* 2018). Possible causes for the NOE are the evolution of complex life and bioturbation accompanied by a greater availability of elemental nutrients via oxidative

weathering which in turn led to an increase in net carbon burial, as well as a higher primary productivity (Holland 2006; Och and Shields-Zhou 2012).

Preceding the NOE by 1 billion years, the Great Oxygenation Event (GOE) was the first big shift in the redox environment of Earth and marked the advent of the permanent oxygenation of Earth's atmosphere (Holland 2002). The start of the GOE, which is defined by the transition of mass-independent fractionation of sulfur isotopes to a mass-dependent fractionation and global glaciation events (Holland 2002), lies between 2.5 and 2.426 Ga ago (Gumsley *et al.* 2017; Warke *et al.* 2020) and lasted until the end of the Lomagundi-Jatuli isotope excursion ~2.06 Ga (Karhu and Holland 1996).

The causes for the GOE seems to be more obvious then the NOE as it was most likely caused by the massive activity of oxygenic photosynthesis by Cyanobacteria, as only oxygenic photosynthesis has a high enough primary productivity to change the atmospheric composition of early Earth permanently (Hohmann-Marriott and Blankenship 2011). Much more interesting is the timing of the GOE, as evidence for free oxygen and Cyanobacteria can be found much earlier in the rock record than the start of the GOE at 2.41 Ga ago (Holland 2002). Undisputed signs of oxygenic photosynthesis date back to 300 million years prior to the GOE in the form of stromatolites and tufted microbial mat like structures in 2.7 Ga old Tumbiana Formation in West Australia (Buick 1992; Flannery and Walter 2012). Indirect evidence for the presence of oxygen, presumably released by oxygenic photosynthesis, can be found in even older rock strata of ~2.8 Ga shallow marine limestone at Steep Rock Lake, Canada, where rare earth element analysis showed a large cerium anomaly, which is indicative of a removal of cerium by the oxidation to its insoluble oxides (Riding *et al.* 2014), although later alteration of the samples was possible (Planavsky *et al.* 2020). Also, the formation of limestone itself is an indication of oxygenation, as the dissolved Fe(II) of the ferruginous Archean ocean would have suppressed the formation of $CaCO_3$ in favour of $FeCO_3$, requiring the removal of Fe(II) by oxidation to its

insoluble Fe(III) oxidation state (Riding *et al.* 2014). Other dating systems put the existence of oxygenic photosynthesis at least 500 million years before the GOE, such as molybdenum fractionation in the 2.95 Ga old near shore rock in the Sinqeni Formation, South Africa, (Planavsky *et al.* 2014) or Fe fractionation in combination with Uranium-Thorium-Lead isotopic data in 3.2 Ga old Manzimnyama Banded Iron Formation (BIF) in South Africa (Kendall *et al.* 2015). Fossilised biological material in the rock record of a shallow tidal zone in the 3.2 Ga old Barberton Greenstone Belt in South Africa also shows structures which closely match the resemblance of modern day phototrophic mats (Heubeck *et al.* 2016).

With indications for the evolution of oxygenic photosynthesis at least 500 Ma before the GOE and evidence of tidal communities of oxygenic phototrophic mats at least 200 Ma before the GOE, it is still unclear why oxygen production predates the accumulation of atmospheric oxygen by this large margin. Different theories give possible solutions to this conundrum and most likely, the delay of oxygen accumulation is a combination of multiple factors, some of which are presented in detail in the following two sections.

1.3.1 Buffering of oxygen by reduced elements

The anoxic oceans of the Archean contained large amounts of reduced elements, most notably Fe(II) in concentrations between 40 – 120 µM (Canfield 2005). Dissolved Fe(II) is highly susceptible to oxidation by O_2, converting it to mostly insoluble Fe(III) hydroxide species. The large reservoirs of Fe(II) in the Archean ocean would thereby have acted as a buffer to the release of oxygen (Kasting 2013). The oxidation of large amounts of Fe(II) would have also contributed to the formation of Banded Iron Formations (BIF) (Posth *et al.* 2013), which consist of layered sedimentary formations of oxidised iron minerals interwoven with chert sheets (James 1954) and were deposited from the Archean till the Proterozoic (3.4 till 1.85 Ga) (Isley and Abbott 1999). Most BIFs are deposited before the onset of the GOE from 3 – 2.5 Ga with a peak around 2.7 Ga (Figure. 2, Isley and Abbott 1999) which coincides with evidence for Cyanobacterial mat systems (Flannery and Walter 2012).

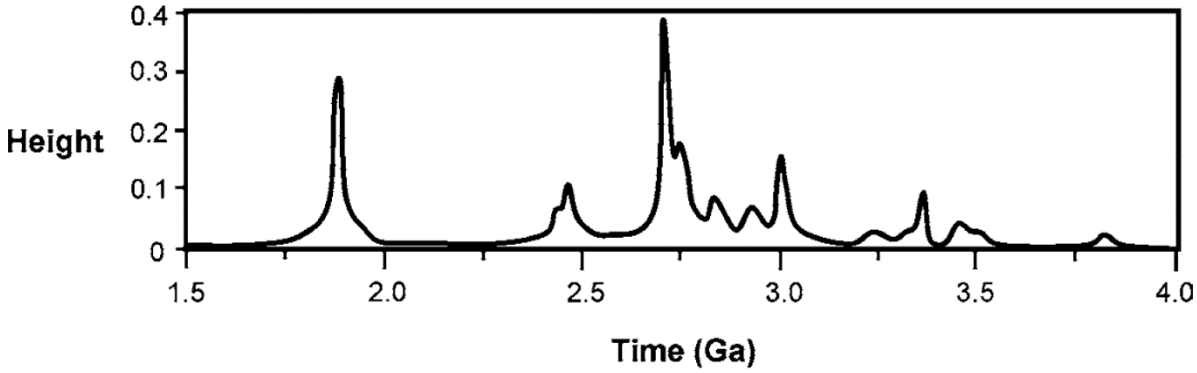

Figure. 2: Relative abundance of Banded Iron Formations over time. From Canfield (2005), which was adapted from Isley & Abbott (1999).

Shallow coastal regions of high photosynthetic activity could have formed localised "oxygen oases" in the otherwise still anoxic environment of the Archean (Fischer 1965; Cloud 1965). Periodic upwelling of Fe(II) rich anoxic water into the oxic ocean top layers or coastal oxygen

oases mediated by hot-spot activity cycles or storm-mixing would be one explanation for the alternating layers of different minerals found in BIFs (Cloud 1973). Additionally, annual weather cycles could have influenced the amount of Fe(II) oxidation caused by varying photic activity as well as silica deposition by increased evaporation or cooling of the silica saturated Archean ocean (Morris 1993; Maliva *et al.* 2005). The lack of BIF deposition after the start of the GOE till 1.8 Ga ago also suggests that at least the top layers of the Proterozoic ocean were Fe(II) depleted when oxygen started to accumulate in the atmosphere (Figure. 2, Canfield 2005).

Other important scavengers of O_2 are the emission of reducing gases, like hydrogen (H_2) and methane (CH_4), emitted by volcanism and methanogens (Catling and Kasting 2017). CH_4 and H_2 can form in the crust and upper mantel by the interaction of carbon bearing rocks with hydrothermal fluids under high pressure and temperature (McCollom 2013). Large amounts of H_2 can also be produced by the oxidation of Fe(II) in ultramafic rocks by hydrothermal fluids, a process known as serpentinization (Charlou *et al.* 2010). One possible reason the GOE was delayed could be the diminishing emission of H_2 over time as the crust became increasingly oxidised with each ongoing reworking cycle, while the released H_2 escaped into space resulting in a net oxidation of Earth (Zahnle *et al.* 2013). The decreased release of H_2 over time could also have influenced the production of biogenic methane by methanogens (Konhauser *et al.* 2009). Additionally, the activity of methanogens could have been declining before the GOE as the amount of dissolved Nickel, an important part of the enzyme reaction centres involved in methanogenesis (Ellermann *et al.* 1989) was decreasing, as evidenced by analysing the Ni/Fe ratio in BIFs (Konhauser *et al.* 2009). The resulting drop in atmospheric methane concentration before the GOE, could have enabled the accumulation of oxygen, the ending of mass-independent fractionation of sulfur isotopes and caused the glaciation of Earth (Zahnle *et al.* 2006).

1.3.2 Delayed expansion of Archean Cyanobacteria

For the Archean Earth's atmosphere to become permanently oxidised, the active release of O_2 by oxygenic phototrophs must have been greater than the chemical sinks described in 1.3.1. As phototrophs are dependent on the influx of solar radiation, their total activity correlates with the overgrown surface area exposed to radiation rather than the total volume, as organisms beneath the top layer get shaded by the organisms on top. Therefore, Cyanobacteria would have needed to cover large areas of the Archean Earth to reach a high enough activity to cause the GOE. Molecular clock analysis however, suggests that Cyanobacteria could have evolved in fresh water environments, as the most primitive recent Cyanobacteria are almost exclusively not adapted to high salinity environments (Uyeda *et al.* 2016; Sánchez-Baracaldo *et al.* 2017). Today, liquid fresh water habitats (lakes and rivers) cover only 4.28% of earths non-glaciated continental surface, which is only 1.1% of Earths total surface area (Allen and Pavlov 2018; Verpoorter *et al.* 2014). The extent of the Archean land mass(es) is not known, but assuming modern day distribution of land vs sea, Cyanobacteria would have had to expand into the saline environment of the Archean ocean to cover large enough areas in order to bring about the GOE. This would have contributed to delaying the GOE, as Cyanobacteria would have had to evolve mechanisms to maintain the cell homeostasis in a more saline environment, while concomitantly being exposed to the high Fe(II) concentrations in the anoxic Archean ocean. Previous studies have suggested that the Fe(II) levels in the Archean ocean were toxic to Cyanobacteria, thereby restricting their growth to shallow marine environments, the so called oxygen oases (Farquhar *et al.* 2011; Swanner *et al.* 2015). The localised high photosynthetic activity of Cyanobacterial mats would have created an oxygenated environment, thereby preventing the influx of Fe(II) by oxidising it to its almost insoluble Fe(III) hydroxides (Swanner *et al.* 2015). Only after the removal of Fe(II) in the surface water of the Archean ocean would Cyanobacteria have been able to expand into the open ocean, which, in turn, would have vastly increased their potential growth area and hence the total amount of O_2 released into

the atmosphere (Swanner *et al.* 2015). Evidence of oxygen oases are found in the rock record in the form of oxic weathering of surface areas thought to have been caused by the release of O_2 in nearby oxygen oases (Anbar *et al.* 2007). The dissolved oxygen concentrations in these oxygen oases is modelled to have reached between 1- 10 µM (Olson *et al.* 2013), higher than the Pasteur point of oxygen (0.3% vol ≙ 3.2 µM dissolved O_2 in sea water), where the switch from fermentation to aerobic respiration happens in modern day facultative anaerobes (Engelhardt 1974).

1.4 Basal Cyanobacteria

In order to approach the conditions of the Archean as close as possible the Cyanobacterial strains chosen for this study belong to the basal clade of Cyanobacteria at the base of phylogenetic Cyanobacterial tree (Sánchez-Baracaldo *et al.* 2017; Sánchez-Baracaldo *et al.* 2014; Schirrmeister *et al.* 2015).

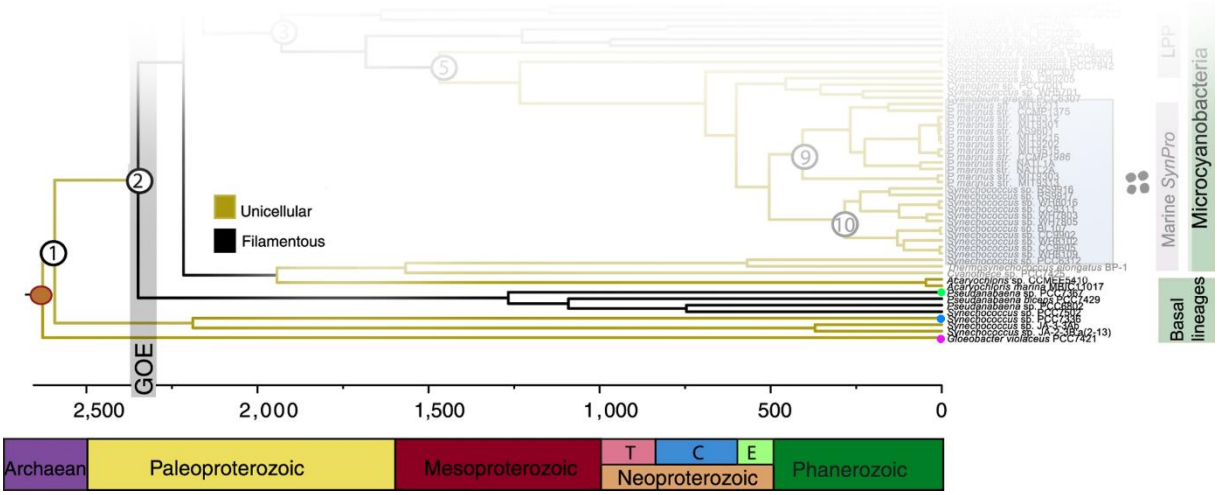

Figure 3: Polygenetic tree of Cyanobacteria diversification as inferred from geologic time. The brown dot marks the calibration point for the first biomatter of Cyanobacterial origin. 1 marks the divergence of majore clades, while 2 markes the first evolution of filamentous strains. The strains utilized in this work are highlighted with a purple (*Gloeobacter violaceus* PCC7421), blue (*Synechococcus* sp. PCC 7336) and green dot (*Pseudanabaena* sp. PCC 7367) and belong to the basal clade of Cyanobacteria. Modified from (Sánchez-Baracaldo 2015).

One of the organisms used during the salt tolerance experiments (2.1) was in fact the most primitive known Cyanobacterium *Gloeobacter violaceus* PCC 7421, which is used as phylogenetic root Cyanobacterium (Seo and Yokota 2003). This unicellular freshwater Cyanobacterium was isolated from limestone rock in a Swiss forest and lacks thylakoid membranes, a feature commonly found in Cyanobacteria (Rippka *et al.* 1974). An additional morphological characteristic is its distinctive barrel shaped phycobilisome structure (Guglielmi *et al.* 1981).

The second strain used in the salt tolerance experiments (2.1) was the halotolerant Cyanobacterium *Chroococcidiopsis thermalis* PCC7203. *C. thermalis* PCC7203 is a

unicellular, non-heterocystous Cyanobacterium, which was isolated from a soil sample near Greifswald, Germany (Komarek 1972; Waterbury and Stanier 1978). Although not a member of the basal clade of Cyanobacteria, fossils of the genus *Chroococcidiopsis* could be identified with little morphological difference in 400 Ma old Rhynie cherts, Scotland (Taylor *et al.* 1995), and genetic analysis propose that the genus *Chroococcidiopsis* as the closest extant relative before the evolution of heterocystous Cyanobacteria (Fewer *et al.* 2002). *C. thermalis* PCC7203 is also suggested as a model organism for terrestrial life on early Earth without an ozone layer as its cryptoendolithic lifestyle, in combination with a thick exopolymeric substances sheet and cell wall, protect it from harsh ionising radiation (Büdel *et al.* 2004; Billi *et al.* 2011). As *Chroococcidiopsis* species are commonly found in arid and desert environments they are not only resistant to high radiation but also osmotic stress during dry phases (Cumbers and Rothschild 2014). *C. thermalis* PCC7203 was therefore chosen as a comparison strain to *G. violaceus* PCC 7421 because this resistance to osmotic stress also enables the freshwater strains of *Chroococcidiopsis* to tolerate high media salinities (Cumbers and Rothschild 2014).

To test for the potential toxicity of Fe(II) in the Archean ocean saltwater strains had to be used. As of now there are only two saltwater strains known in the basal clade, *Pseudanabaena* sp. PCC 7367 and *Synechoccocus* sp. PCC 7336 (Sánchez-Baracaldo 2015). *Pseudanabaena* sp. PCC 7367 was isolated from a snail shell in the intertidal zone in Puerto Penasco, Mexico (Stanier *et al.* 1979), while *Synechoccocus* sp. PCC 7336 was isolated from a seawater tank at Berkeley University, USA (CRBIP-Catalogue 1971; Coutinho *et al.* 2016). Both strains possess relatively large genomes compared to more modern strains, with *Synechoccocus* sp. PCC 7336 having the second largest genome (5.07 Mbp) of all fully sequenced *Synechoccocus* species as of 2013 (Coutinho *et al.* 2016). A coincidental benefit of choosing these strains is that *Pseudanabaena* sp. PCC 7367 is a multicellular, filamentous strain in contrast to the unicellular *Synechoccocus* sp. PCC 7336. The emergence of multicellular growth was dated by

Schirrmeister *et al.* at the same time as the GOE and they proposed filamentous growth as a potential fitness factor leading to GOE (Schirrmeister *et al.* 2013).

1.5 Aims of this thesis

As of now it is still unknown what caused the delay between the evidence for localised oxygen availability, presumably through oxygenic photosynthesis, and the onset of the GOE. Many different hypotheses exist which are largely derived from theoretic modelling of possible scenarios within an Archean environment and their influence on the primordial Cyanobacteria. As models reduce the complex real-world interactions of living systems to very few key aspects, it is important to verify these assumptions in live experiments to test for unaccounted variables during the model's creation. Therefore, I conducted laboratory experiments with Cyanobacteria from the basal clade of the Cyanobacterial lineage to verify if the hypotheses, inferred from theoretical modelling, are plausible in a real-life scenario. I also conduct a thorough investigation into the influence of different growth systems on the outcome of Archean simulation experiments with Cyanobacteria. Taken in totality, I have been able to establish a robust framework for further experimentation in this field, whereby I highlight the importance of strain selection as well as culture conditions in generating realistic data on oxygenic photosynthesis in the Archean.

The first paper of my cumulative dissertation (2.1) explores the implications of a freshwater origin of Cyanobacteria and their later expansion into the saline environment of the ocean, which could have delayed the GOE, as described in 1.3.2. Therefore, the effects of increased media salinity under a Neoarchean atmosphere were investigated on the basal, freshwater Cyanobacterium *Gloeobacter violaceus*. PCC 7421 and compared to the more modern, halotolerant Cyanobacterium, *Chroococcidiopsis thermalis* PCC 7203.

In my second paper (2.2) the toxicity of Fe(II) in the ferruginous Archean ocean on Cyanobacteria is explored, which would have contributed to the delay in the expansion of Cyanobacteria into the open ocean, as described in 1.3.1. The experiments also explore the principal concept of the proposed oxygen oasis and the scavenging of oxygen by Fe(II), as mentioned in 1.3.1. Therefore, the two known saltwater strains of the basal clade, *Pseudanabaena* sp. PCC 7367 and *Synechococcus* sp. PCC 7336, were subjected to different Fe(II) concentrations during either a single dose or repeated exposures, and their growth and the interaction of the released oxygen with the added Fe(II) measured.

The third paper (3.1) describes the construction of a custom-built anaerobic chamber, used during the experiments in 2.2 that enables investigators with limited assets to conduct experiments under a self-regulating anoxic atmosphere.

2. Published Manuscripts

2.1 An investigation into the effects of increasing salinity on photosynthesis in freshwater unicellular Cyanobacteria during the late Archaean

Figure 4: Cultures of basal Cyanobacteria after incubation under anoxic conditions (Cover image, Geobiology Volume 17, Issue 4, July 2019).
Cultures are, from left to right, top to bottom: Top row: *Croococcidiopsis thermalis* PCC 7203 (used in this study) & *Calothrix* sp. PCC 7507: Middle row: *Gloeobacter violaceus* PCC 7421 (used in this study); *Pseudanabaena* sp. PCC 7429; *Pseudanabaena* sp. PCC 7367; abiotic control: Bottom row: *Nostoc* sp. PCC 7524 ; *Cyanothece* sp. PCC 7425. Photo taken by Achim J. Herrmann.

An investigation into the effects of increasing salinity on photosynthesis in freshwater unicellular Cyanobacteria during the late Archaean

As published in Geobiology, Volume 17, Issue 4, 343-359, July 2019

https://doi.org/10.1111/gbi.12339

Achim J. Herrmann & Michelle M. Gehringer

This paper investigates the effects of increased medium salinity under Neoarchean like atmospheric composition on the basal, freshwater Cyanobacterium *Gloeobacter violaceus* PCC 7421. As reference the freshwater *Chroococcidiopsis thermalis* PCC 7203 is used, which is known to be extremely resilient to harsh environmental conditions such as high osmotic stress. Both organisms are exposed to media with brackish and saltwater like salinities under either an atmosphere of present-day composition or with a possible Neoarchean composition of 2000 ppm CO_2 and 600 ppm O_2 in pure N_2. The biological response is assessed by measuring growth parameters of biomass, cellular Chlorophyll *a*, protein and glycogen, as well as metabolic activity measurements of the oxygen production and carbon uptake.

In the perspective of this thesis, this paper expands on the hypothesis that one reason for the delay between the evidence for oxygenic photosynthesis and the onset of the GOE could be the evolution of Cyanobacteria in freshwater and the time they needed to adapt to more saline environments (Sánchez-Baracaldo *et al.* 2017). The conducted experiments show that the most primitive, extant Cyanobacterium *Gloeobacter violaceus* PCC 7421 could have tolerated the brackish environment of a possible Neoarchean river delta and would thus have had time to adapt to more saline environments necessary for eventual ocean expansion.

A.J. Herrmann conducted all experiments, evaluated the data and prepared the graphics and pictures. Material and Methods, as well as Results are written in full by A.J Herrmann and he was a cowriter of the Discussion.

2.2 Diurnal Fe(II)/Fe(III) cycling and enhanced O_2 production in a simulated Archean marine oxygen oasis

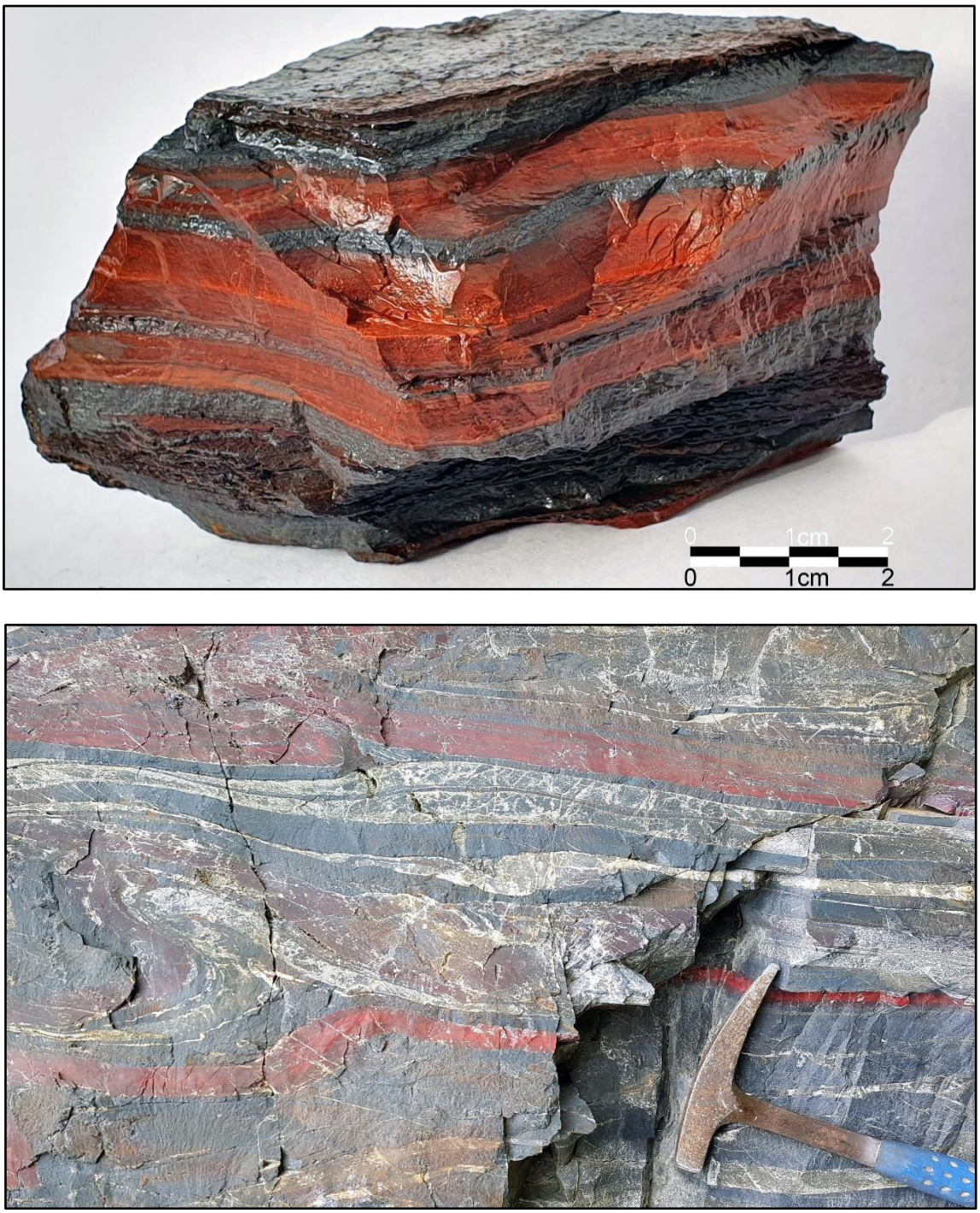

Figure 5: Banded iron ore around the world.

Banded iron formations were deposited as sedimentary rocks and consist of alternating fine layers of gray, metalic iron oxides with white to redish cherts. Microbial processes could have been an important factor in the depositional history of certain banded iron formation types by oxidising soluble Fe(II) to insoluble Fe(III). Top: Sample from the Daitar greenstone belt, Tomaka Formation (3-3.5 Ga old, Jodder, unpubl.). Sampled in the Odisha Mining Company strip mine near Daitari, India by Achim J. Herrmann in February 2020 on the SPP 1883 Field Workshop India. Bottom: Picture of the 11b Outcrop in the Temagami Greenstone Belt, Ontario, Canada (2.717-2.767 Ga old(Ginley 2016)). Photo taken by Achim J. Herrmann on 25.08.2018 on the SPP1883 Field Workshop Canada.

Diurnal Fe(II)/Fe(III) cycling and enhanced O_2 production in a simulated Archean marine oxygen oasis

As published in Nature Communications Volume 12, Nr. 2069, April 2021.

https://doi.org/10.1038/s41467-021-22258-1

Achim J. Herrmann, James Sorwat, James M. Byrne, Nicole Frankenberg-Dinkel & Michelle M. Gehringer

This paper investigates the proposed toxic effects of Fe(II) in the ferruginous Archean oceans on Cyanobacterial growth, by subjecting two basal saltwater Cyanobacteria strains, *Pseudanabaena* sp. PCC 7367 and *Synechococcus* sp. PCC 7336, to different Fe(II) concentrations under anoxic conditions. Not only were the effects of single and repeated exposures to Fe(II) tested, but also the effect of different growth systems in order to highlight the influence of experimental design on the results. Also, the effects of an anoxic atmosphere on oxygen production, and the toxicity of green rust, an oxidation product of Fe(II) under hypoxic conditions, were assessed.

In the perspective of this thesis, the data presented in this paper expands the hypothesis that Fe(II) toxicity delayed the GOE by restricting early Cyanobacteria to oxygen oases and limiting their spread into the ferruginous Archean ocean (Swanner *et al.* 2015). It could be shown that, instead of Fe(II) in the concentrations of the Archean ocean, the formation of green rust had a strong inhibitory effect on Cyanobacterial growth. Also, under anoxic conditions *Pseudanabaena* sp. PCC 7367 exhibited a significantly higher net oxygen production rate then under a current oxic atmosphere, which is significant for modelling the Archean atmosphere.

A.J. Herrmann conducted and planned all experiments, evaluated, and interpreted the data and prepared the graphics and pictures. The paper is written in full by A.J. Herrmann, except for the part regarding ^{57}Fe-Mössbauer spectroscopy. It was revised and edited with Michelle. M Gehringer and N. Frankenberg-Dinkel.

3. Unpublished Manuscripts

3.1 A low-cost automized anaerobic chamber for long-term growth experiments and sample handling

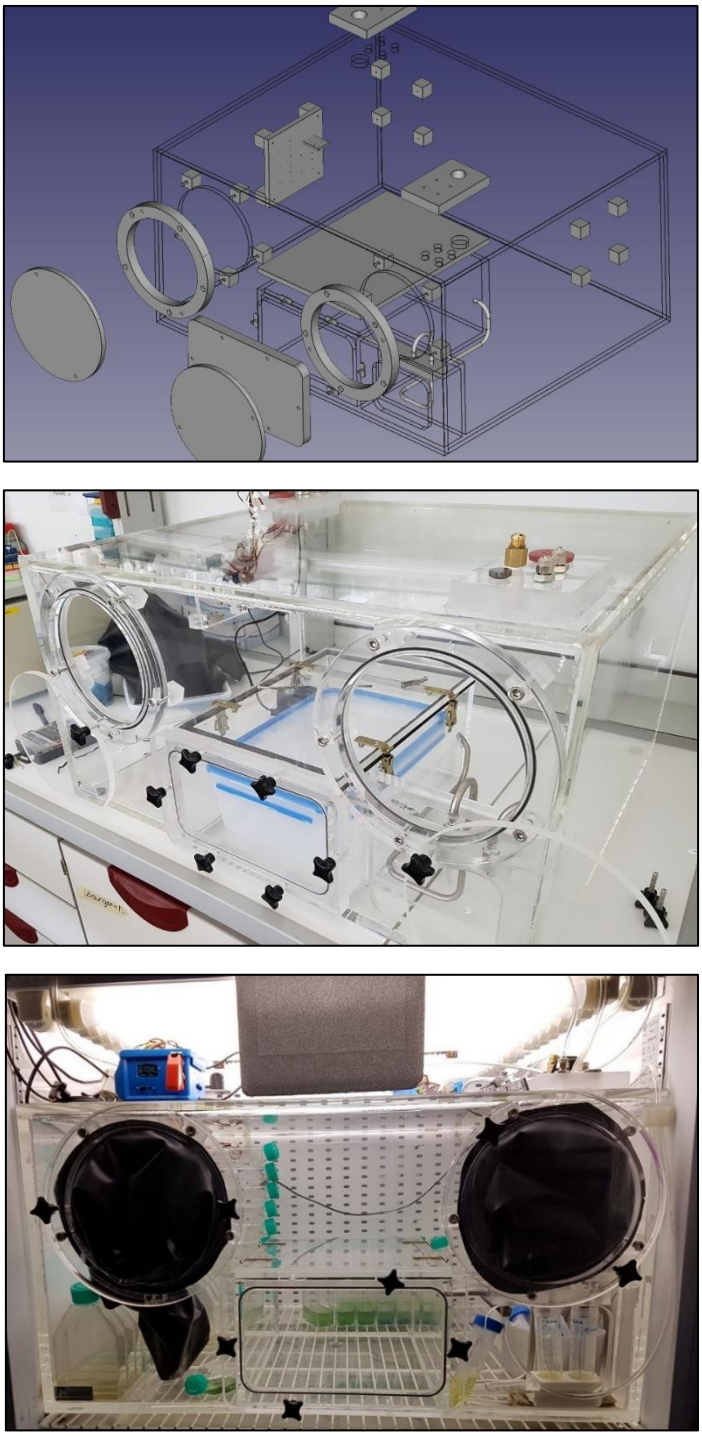

Figure 6: Different stages in the development and assembly of an anaerobic box.

Top: Technical plans for the body of the anaerobic box (AutoCad). Middle: Fully assembled body of the anaerobic box. Bottom: Fully operational anaerobic box inside growth chamber with flasks of growing Cyanobacteria.

A low-cost automated anaerobic chamber for long-term growth experiments and sample handling

Under review, available as preprint

bioRxiv, Nr. 423238, December 2020

https://doi.org/10.1101/2020.12.17.423238

Achim J. Herrmann & Michelle M. Gehringer

This paper describes the construction of a low-cost anaerobic chamber for anoxic sample handling and cultivation of microbial cultures with advanced features only found in high-cost commercial solutions. The anaerobic chamber can maintain an anoxic atmosphere over extended periods by automatically regulating the humidity as well as the H_2 content of the atmosphere. It also logs these parameters, as well as oxygen concentration, temperature and light intensity, to later verify the integrity of long-term experiments. The anaerobic chamber is based on an Arduino controller and can easily be expanded or adapted to control and measure additional parameters, according to the user and experimental requirements.

In the perspective of this thesis, this paper describes the construction of the anaerobic chamber used during the experiments in 2.2 and enables other scientists without the budget to obtain a ~ 60.000 € anaerobic workstation to conduct advanced anaerobic experiments and contribute to the scientific discourse.

The paper is written in full by A.J. Herrmann. A.J. Herrmann created the technical drawings, designed the circuit diagram and wrote the software. After the main body was assembled by the TUK metal workshops, A.J. Herrmann installed and wired all components.

Title: A low-cost automized anaerobic chamber for long-term growth experiments and sample handling

Authors: Herrmann, Achim J.; Gehringer, Michelle M.

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1 Abstract

The handling of oxygen sensitive samples and growth of obligate anaerobic organisms requires the stringent exclusion of oxygen, which is omnipresent in our normal atmospheric environment. Anaerobic workstations (aka. Glove boxes) enable the handling of oxygen sensitive samples during complex procedures, or the long-term incubation of anaerobic organisms. Depending on the application requirements, commercial workstations can cost up to 60.000 €. Here we present the complete build instructions for a highly adaptive, Arduino based, anaerobic workstation for microbial cultivation and sample handling, with features normally found only in high cost commercial solutions. This build can automatically regulate humidity, H_2 levels (as oxygen reductant), log the environmental data and purge the airlock. It is built as compact as possible to allow it to fit into regular growth chambers for full environmental control. In our experiments, oxygen levels during the continuous growth of oxygen producing cyanobacteria, stayed under 0.03 % for 21 days without needing user intervention. The modular Arduino controller allows for the easy incorporation of additional regulation parameters, such as CO_2 concentration or air pressure. This paper provides researchers with a low cost, entry level workstation for anaerobic sample handling with the flexibility to match their specific experimental needs.

Keywords: Anaerobic workstation; Glove box; anaerobic; anoxic; culturing system

Specifications table *[please fill in right-hand column of the table below]*

Hardware name	*Anaerobic chamber*
Subject area	• Biological Sciences (e.g. Microbiology and Biochemistry) • General
Hardware type	• Biological sample handling and preparation
Open Source License	MIT License
Cost of Hardware	~2000 €
Source File Repository	10.17605/OSF.IO/J84WD

2 Hardware in context

Handling oxygen sensitive samples can provide a challenge in modern earth's atmosphere, as oxygen is the second most abundant gas and even trace amounts can lead to the rapid and irreversible oxidation of a sample. Handling oxygen sensitive samples can be further complicated if the sample is alive, like obligate anaerobic organisms, where oxygen and its radical forms acts as a potent toxin, oxidizing important proteins and catalytic metal ligands (Hentges 1996). Cheap solutions like sealed glass tubes or flasks with rubber lids used in combination with syringes, produce a lot of waste and their use is limited if complex sample handling is required. This hardware offers flexibility in manual sample processing, in an anaerobic environment, in an affordable, programmable anaerobic workstation.

3 Hardware description.

This paper describes the construction of a low-cost alternative to commercial anaerobic chamber with advanced features usually only found in high cost, automated anaerobic chambers. Barebone anaerobic workstations with glove ports can easily cost more than 10.000 €, even without "comfort" functions like an automated airlock or automatic maintenance of an anaerobic atmosphere. Advanced features like humidity or CO_2 control quickly add 5,000 to 10,000 € to the cost, depending on the feature and company in question. Here we describe the complete build instructions to a cheap, Arduino base, automated anaerobic workstation, which can be used in the cultivation of bacteria or for general sample handling. In contrast to other DIY solutions, the automated control allows for long term experiments or storage of samples without user input. Additional features like CO_2 regulation or pressure control can be added as needed per experimental requirement, even after the final installation.

Advantages of this design:

- automatic regulation of humidity and H_2 (as oxygen reductant) concentration
- Logger for monitoring environmental conditions during the experiments
- Automatic air-lock purging
- Easily expandable for individual requirements like controlling the CO_2 concentration
- Compact design allows for the integration into growth chambers/cell incubators and/or minimal impact on available lab space

Possible use cases:

- Growth of anaerobic organisms
- Growth of oxygen producers like cyanobacteria under anaerobic conditions
- Handling and storage of oxygen sensitive samples
- Radioactive labeling experiments under anaerobic conditions

4 Design files

Design Files Summary

Design file name	File type	Open source license	Location of the file
Mainbody	CAD .fcstd	MIT	10.17605/OSF.IO/J84WD
FrontDoor	CAD .fcstd	MIT	
GloveGasket	CAD .fcstd	MIT	
GloveLid	CAD .fcstd	MIT	
AirlockTop	CAD .fcstd	MIT	
GasPortAirlock	CAD .fcstd	MIT	
GasPortTop	CAD .fcstd	MIT	
FanHolder	CAD .fcstd	MIT	
HolderSensor	CAD .fcstd	MIT	
LSensorHolder	CAD .fcstd	MIT	
ScrewBacking25	CAD .fcstd	MIT	
ScrewBackingFlat	CAD .fcstd	MIT	
ChamberPipeInlet	CAD .fcstd	MIT	
ChamberPipeOutlet	CAD .fcstd	MIT	
OverpressurePipe	CAD .fcstd	MIT	
CompleteAssembly	CAD .fcstd	MIT	
ArduinoCad	Electronic .dsn	MIT	
SS3_eng	Software .ino	MIT	
SS3_eng_setup	Software .ino	MIT	

File description:

The files named “Mainbody” through to “ChamberPipeOutlet” describe the parts needed in the assembly of the PMMA body of the anaerobic workstation and are shown assembled in the file “CompleteAssembly”. All CAD files were created using FreeCAD 0.18, build 4.

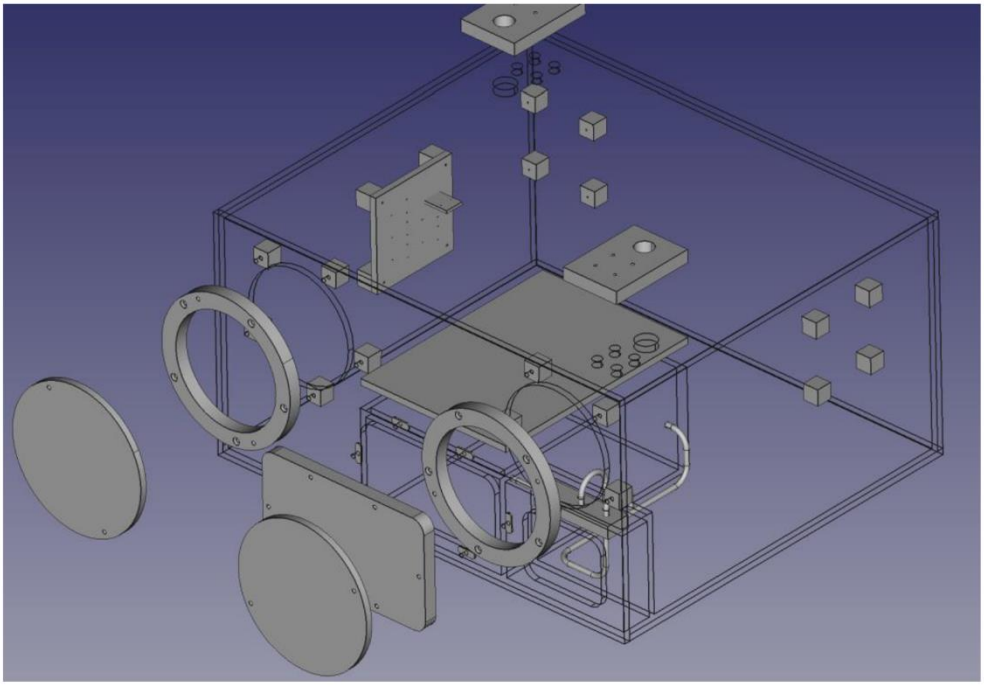

Fig. 1: Overview of all the parts assembled from the file "CompleteAssembly".

"Mainbody" describes the body onto which all parts are assembled. It was assembled from multiple slabs of PMMA, which were cut and drilled, before being glued together. In order to reduce costs, the bottom, sides and back plate could be made from a cheaper material, as only the front and top part needs to be translucent in order to see inside.

"FrontDoor" describes the front door of the airlock, which seals the box hermetically. The groove on the inside should be fitted with the round cord to ensure an airtight seal.

"GloveGasket" describes the gasket to secure the gloves in place during operation and ensure an airtight seal. The groove on the inside should be fitted with the round cord to ensure an airtight seal. For an easier installation of the gloves inside the gaskets, clear sticky tape can be used to hold the gloves in place.

"GloveLid" describes a cover for the glove holes, if the workstation is not in use, to prevent the gloves from sticking out due to the internal over pressure.

"AirlockTop" describes the top cover of the airlock. Holes for the toggle fastener are not described, as they are part dependent, and have to be adjusted for a tight fit with the seal strip.

"GasPortAirlock" describes a block with screw fittings for the airlock gas in- and outlet, as well as the main chamber gas outlet. It needs to be glued in the Gas outlet bay (Fig. 4).

"GasPortTop" describes a block with screw fittings for the gas inlets and cable port. The biggest hole is for a Rubber stopper diaphragm, if the drawing of gas with a syringe is needed.

“FanHolder” describes single blocks for attachment of the pc fans and the sensor holder. These need to be glued in sets of four, with the required spacing inside the main body, to ensure a constant removal of oxygen.

“HolderSensor” describes the mounting plate for up to five Grove sensor shields and the “LSensorHolder” for an upright mounting of the light sensor. Needs to be mounted on one set of “FanHolder”.

“ScrewBacking25/-Flat” describe pieces of PMMA which cover the screw holes in the main body for an airtight compartment.

“ChamberPipeInlet/-Outlet” describe the pipes needed to connect the airlock in and outlet to the “GasPortAirlock”.

“OverpressurePipe” describes the pipe connected to the “GasPortAirlock”, where pressure from the main box is vented. It is needed to prevent the gloves from accidentally obstructing the gas outlet.

“CompleteAssembly” shows the complete assembly of all the parts so far described (Fig. 1).

“ArduinoCad” shows the layout of the electric connections of the sensors to the Arduino controller, as well as the power supply and relay cabling. It was created using TinyCad version 3.00.02.

“SS3_eng” is the main software running on the Arduino controller during operation of the anaerobic workstation.

“SS3_eng_setup” is used during the initial sensor and timing setup and for verification of sensors functionality (see 6).

5 Bill of Materials

The bill of materials is uploaded to the open science framework at 10.17605/OSF.IO/J84WD .

6 Build Instructions

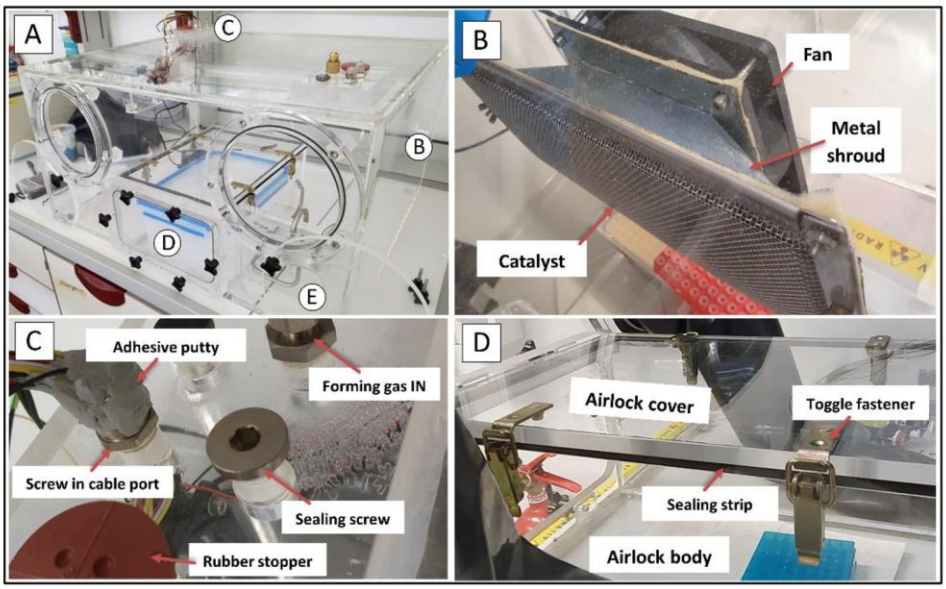

Fig. 2: A: Completely assembled PMMA body of the anaerobic chamber with round nominators for in detail pictures. B: Final catalyst assembly with a metal shroud connecting it to one of the fans screwed to the fan holders. C: Cable entry port in detail. D: Airlock assembly with cover, sealing strip and closed toggle fastener.

Assemble the PMMA body of the anaerobic box (Fig. 2A) as per construction file (CompleteAssembly). Be sure to install all internal parts before sealing the cover plate, especially the airlock top plate (AirlockTop), as it is too big to be inserted through the airlock afterwards. The airlock cover plate must be connected to the airlock main body with four toggle fasteners (Fig. 2D). The boreholes for the toggle fasteners are not specified in the blueprints and should be adjusted to ensure a tight fit with the underlying sealing strip if the toggle fasteners are engaged. The sealing strip is attached to the top of the airlock walls.

Mount the fans on the fan holders and attach the catalyst (StakPak) in front of one of the fans (Fig. 2B). In our case, this was achieved by a folded metal shroud made of 2 mm steel. As a cost saving measure, a catalyst can be easily made from scratch: 10 to 20 g of Palladium on Carbon (5%) pellets (2-3mm) should be encased between two layers of fine stainless-steel wire mesh. The sides can then be sealed by folding them and stapling them shut. Do not use flammable substances in the construction as the catalyst can become hot during operation and could melt plastic or char cardboard.

Fan power and connection cables are passed through a screw-in cable port and the port sealed airtight with either adhesive putty or epoxy resin (Fig. 2C). Connect all sensor cables to the Arduino as described in the “Arduino CAD” file.

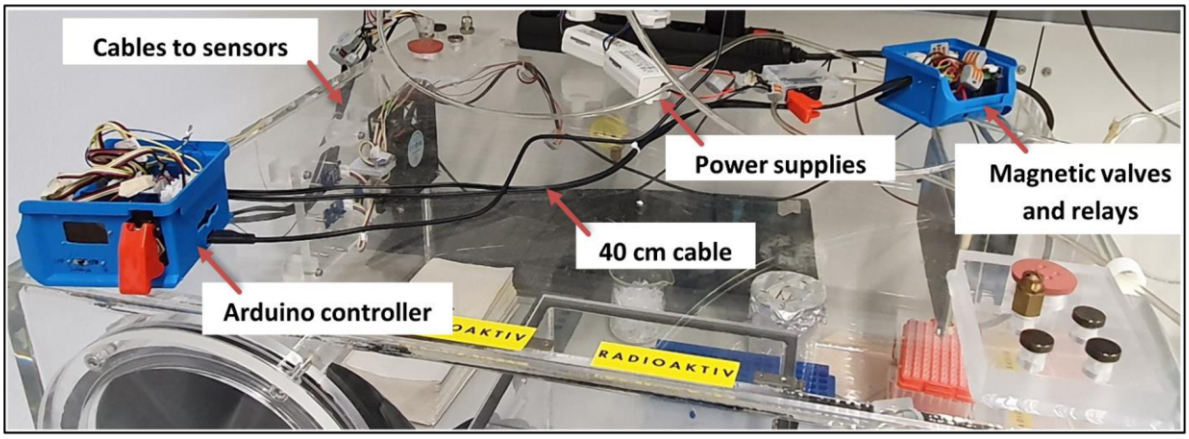

Fig. 3: Spatial separation between the Arduino controller and the magnetic gas valves and power supply units to prevent interference.

The Arduino controller must be electronically shielded or spatially separated from the magnetic gas valves and their power source, as their operation can create strong magnetic fields and hence system instability. In our case, this was achieved by placing the Arduino in one case made from a plastic storage box, while the gas valves with their relays were placed in a second plastic box and connected with 40 cm of wiring (Fig. 3).

The gas sensors for H_2 need to be calibrated using the Arduino Program "SS3_eng_setup". Open the serial monitor and note the R_0 value of the H_2 sensor in ambient air. This value needs to be adjusted in the main program "SS3_eng". Additionally, the other sensors should be checked for their functionality in the setup. The setup file also sets the Real Time Chip. Adjust the files in void setup in SS3_eng_setup" according to your current date and time:

```
void setup() {
  …;
    clock.begin();                    // fill in the correct date and time to initialize clock  [3]
  clock.fillByYMD(2017,1,19);         //Jan 19,2017
  clock.fillByHMS(15,28,30);          //15:28 30"
  clock.fillDayOfWeek(SAT);           //Saturday, arguments [MON, TUE, WED, THU, FRI, SAT, SUN]
  clock.setTime();                    //write time to the RTC chip
}
```

The above process sets the time to the specified values every time the code is run and should be deleted after the initial setup or the displayed time may be inaccurate.

After measuring the R_0 value, adjust the lines in the main program "SS3_eng":

```
//H2 SetUp
const float R0 = 6.5;        // resting voltage of H2 Sensors; Determine by using "H2 setup"
```

Further information on the function, principal and application of the utilized sensors can be found on: https://wiki.seeedstudio.com

Safety instructions:
The anaerobic workstation uses non-toxic, asphyxiating gases and should not be operated in confined spaces without ventilation. If forming gas with ≤ 5% H_2 is used, there is no explosive risk, as the minimal flammability limit cannot be reached by mixing forming gas with ambient air. The risk of electric shock is minimal as the maximal voltage used is only 24V.

7 Operation Instructions

The ends of the chamber/airlock gas outlet tubes must be submerged in water or oil to prevent the inflow of ambient air. This can be achieved either by placing the end of a short section of stiff tubing inside a liquid filled reaction tube or similarly sized vessel inside the outlet bay or running the tubing to an external gas washing flask or similar device. Care should be taken to not completely fill the vessels, as the immersion depth of the tubing correlates to the maximum internal overpressure during gassing operations. High internal pressure can make the gloves rather stiff and unpleasant to handle while also posing the risk of an outward spillage of oil/water during a rapid insertion of the gloves. Likewise, a rapid retraction of the gloves poses the risk of sucking in liquid and ambient air contaminating the internal atmosphere/ surroundings. Therefore, care should be taken while inserting and removing one's arms and, if necessary, gas can be manually inserted by activating the manual gas injection switch to alleviate an eventual negative pressure. Tip: If the liquid level in the container, which houses the outlet from the airlock, is lower than in the chamber outlet, less ambient air will enter the main chamber during an airlock cycle.

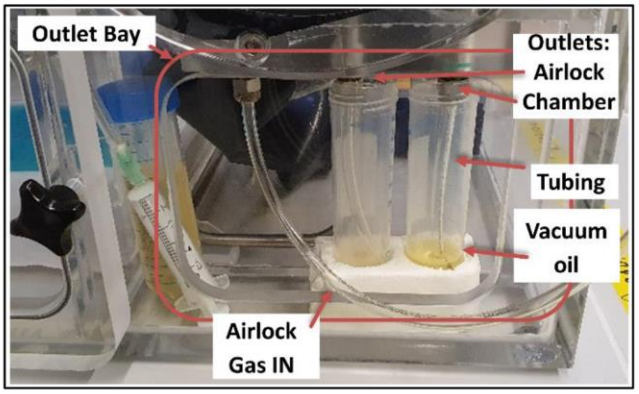

Fig. 4: Gas outlet bay with 50 ml test tubes filled with vacuum oil filed just above the tube opening.

N_2 can be used in certain steps as a cost saving measure. All steps describing the usage of N_2 gas can also be performed with forming gas if no N_2 is available.

7.1 Initial Setup

1. Make sure all tubes are connected, all unused screw ports are covered by sealing screws and all internal sensors are working. Fill up the outlet vessels with oil/water to slightly cover the tube endings (~5 mm).
2. All necessary equipment like pipettes, test tube holders e.g. should be inserted before making the chamber anaerobic, as this can save time otherwise spent on unnecessary airlock cycles.
3. (Optional)Flush the box with N_2 gas for 20 min to bring down the O_2 levels and save forming gas.
4. Switch on the controller with the connected forming gas. Forming gas injection will start to remove the remaining O_2. Humidity should rise as O_2 reacts with H_2 to form H_2O. This exothermic reaction can heat up the catalyst and damage delicate equipment if they are in direct contact.
5. The controller should auto adjust the injections of forming gas and N_2 until an atmosphere is established within the parameters set for humidity and enough H_2 gas to remove all O_2 present in the anaerobic chamber. As the frequency of gas injections during setup is most likely over the safe operating threshold during normal operation, the gas override switch should be switched to ON (7.2).

7.2 User interface

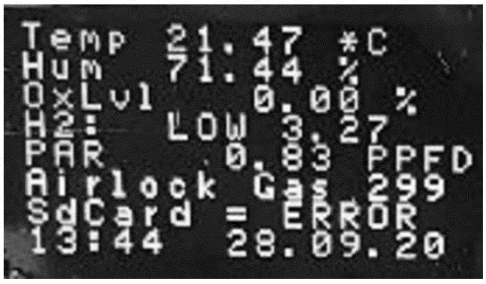

Fig. 5: User interface on OLED display.

The OLED display (Fig. 5) shows all relevant environmental conditions during the operation:

1. Temperature in °C
2. Relative Humidity in % saturation at the temperature in line 1
3. Oxygen levels in % with an error of 0.03%
4. Indication of the H_2 level in the chamber with:
    a. NONE ~ H2 < 1%
    b. LOW ~ H2 < 3%
    c. NORM ~ H2 >3%

    The number is displays the Rs (Reading Sensor)/ R_0 (Reading Zero) ratio, NOT H_2 in % (Fig. 6), and is a rough indication of the presence of hydrogen in the atmosphere. As the sensor can also detect alcohols, alcohol-based disinfectants should not be used inside of the glove box, otherwise the auto injection of forming gas will not work.

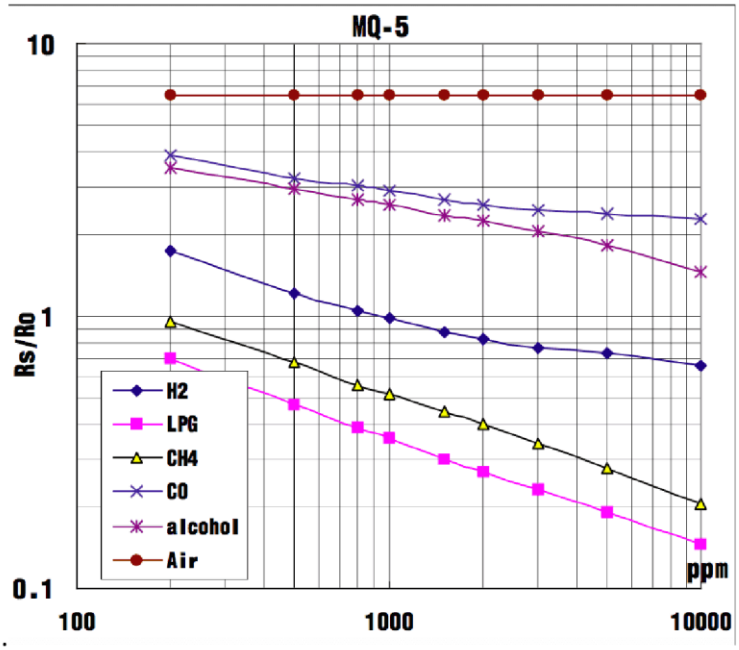

Fig. 6: R_S (Reading Sensor)/ R_0 (Reading Zero) value gives an indication of the gas levels in the atmosphere. Note that the sensor will react to other gases in the atmosphere, which should be taken into account during the operation of the automated gassing system (https://wiki.seeedstudio.com/Grove-Gas_Sensor-MQ5/; 13.10.2020)

5. PAR in PPFD. Only works if the light sensor is calibrated, otherwise just an indicator of the current day-night / light-dark cycle
6. Status of the automatic gas injection
    a. GASSING ERROR: The auto injection was disabled because gas was injected too often in a short interval, hinting at a leak or sensor malfunction. To reset this message and enable auto injection flip the OVERRIDE switch to ON and return to OFF.
    b. GAS OVERRIDE: Displays the status of the OVERRIDE switch as ON.
    c. AIRLOCK GAS = n: Automatic gassing of the airlock with n seconds remaining
7. Status of the SDCard of the automated saving of data
    a. ERROR = SD Card is not mounted, either no SD Card is inserted or it could not be mounted
    b. OK = SD Card is mounted and data gets saved every minute
8. Time and date

Fig. 7: Operator control interface with the Killswitch (1) and Slide Switch (2) disengaged (left) and engaged (right). Note the change in the OLED display line 6.

The controller has two operational modes, which can be controlled by switching the Killswitch (1) and Sliding Switch (2) to the corresponding positions.

1. Killswitch (1) is OFF:
    a. Slide Switch (2) is OFF:
    Emergency Stop Function is active: the controller checks if 5 consecutive gas injections were triggered within 10 seconds of each other (indication of a leakage or an empty gas bottle) and stops further gas injection without user input.
    b. Slide Switch (2) is ON:
    Manually injects forming gas. Useful to prevent negative pressure while operating the gloves.
2. Killswitch (1) is ON:
    a. Slide Switch (2) is OFF:
    Display shows GAS OVERRIDE. Resets the emergency Stop Function and disables it while the Killswitch (1) is engaged.
    b. Slide Switch (2) is ON:
    Floods the airlock chamber with N_2 for 300 seconds (can be adjusted in program file) and displays the remaining time on the display. Gassing can be stopped by disengaging any off the input switches (1 or 2) to OFF. In order to minimize the inflow of ambient air from the airlock chamber into the main body, a tight fit of the inner airlock top cover must be ensured. During the first minute of gassing, the airlock front door can also be slightly opened for venting in order to prevent any gas from leaking into the chamber.

7.3 Cleaning and maintenance

Avoid using solvents during the cleaning and disinfection of the anaerobic chamber as the MQ5 sensor detects not only H_2 but also hydrogen in hydrocarbons. It may give a false indication of H_2 levels, if volatile solvents like ethanol or isopropanol are present in the anaerobic chambers atmosphere. Solvents can also

lead to cracking or cloudiness of the PMMA material and adsorb onto the activated charcoal catalyst, reducing its activity. In order to remove adsorbed solvents from the catalyst, it can be baked overnight at 200°C (Caution: depending on the quantities adsorbed on the catalyst this can pose an explosion hazard!!!). Therefore, only use water and mild detergents during cleaning operations if possible.

Care should be taken during the incubation of organisms that produce or require SO_2/H_2S or CO as these gases can lead to a poisoning of the palladium catalyst (Albers et al. 2001).

The oxygen sensor detects oxygen by slowly reacting with O_2, producing a current, and lasts for ~ 2 years under ambient oxygen levels. In order to maximize the sensor lifetime, it should be stored in an anaerobic environment, if the anaerobic chamber is not currently in use.

8 Validation and Characterization

The anaerobic chamber described in this paper was successfully used in studying the effects of Fe(II) on cyanobacteria. To do so, the anaerobic workstation was inserted inside a Percival culture chamber (E-22L), which controlled lighting, temperature and CO_2 levels via an external sensor. In order to test for the effect of photooxidation of Fe(II), or the buildup of oxygen inside the anaerobic chamber, control cultures without cyanobacteria were set up and Fe(II) measured throughout the whole experiment by means of the colorimetric ferrozine assay. The whole experiment was then repeated inside discrete, hermetically

sealed, anaerobic bottles (which were also set up inside the anaerobic chamber) to test for the effects of O_2 buildup on cyanobacterial growth.

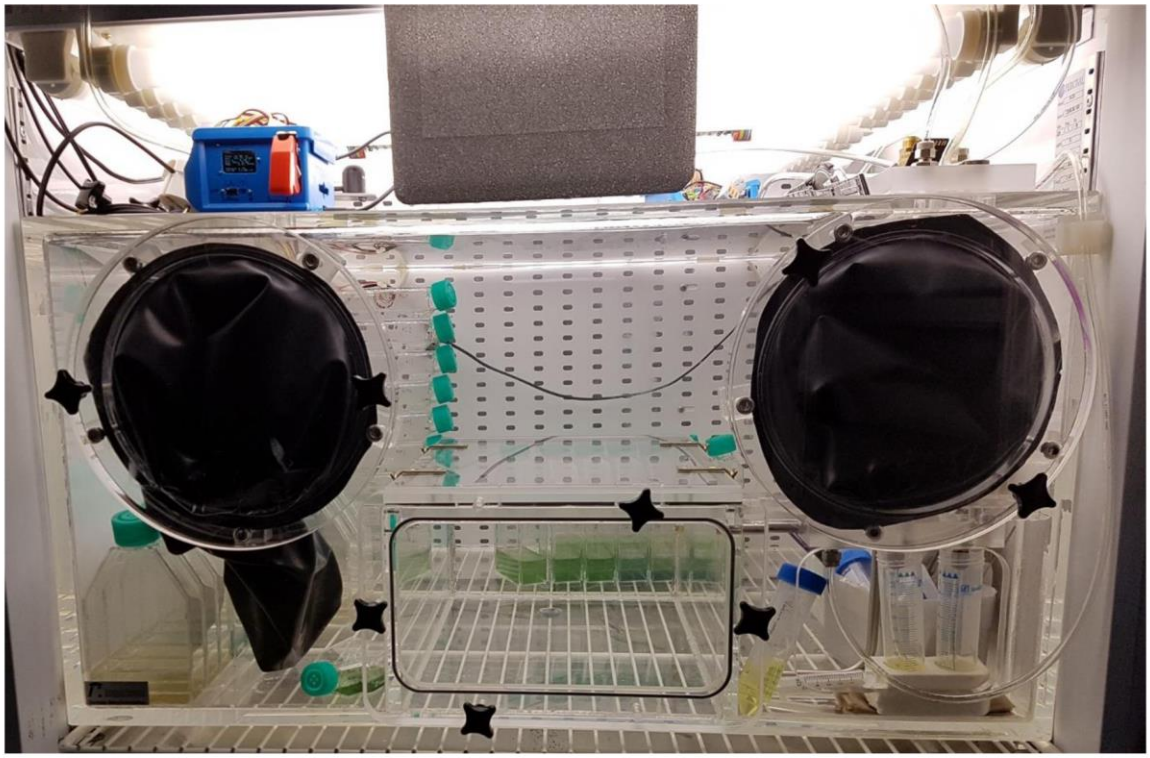

Fig. 8: Anaerobic chamber fully functional and wired inside a culture chamber with growing cyanobacterial cultures in ventilated culture flasks.

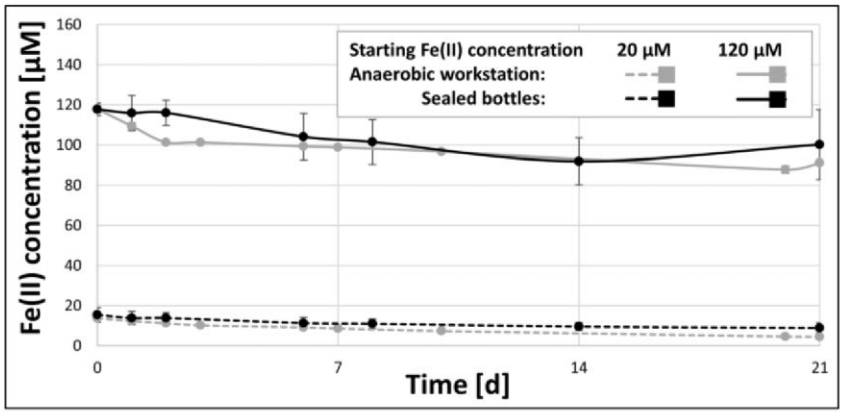

Fig. 9: The Fe(II) concentrations of growth media over the course of 21 days, if either incubated inside the here described anaerobic chamber (grey), or in sealed anaerobic bottles (black). The starting Fe(II) concentrations were either set at 20 µM (dotted lines) or 120 µM (solid lines).

The Fe(II) measurements of the controls showed that the anaerobic box maintained an anerobic atmosphere throughout the whole experiment just as well as the individually sealed bottles. Under aerobic conditions, the complete oxidation of Fe(II) would have been observed within 20 minutes. The

slight drop in Fe(II) concentration over the whole length of the experiments is most likely the result of photo-oxidation, as the media was constantly exposed to the light needed for the growth of the cyanobacteria. This result clearly demonstrates the efficiency of the anaerobic workstation, as the control cultures in ventilated flasks were incubated at the same time as the cyanobacterial cultures inside the anaerobic workstation. Despite the cultures producing ~ 170 ml of pure O_2 per day near the end of the experiment, the Fe(II) in the control flasks was not oxidized. Without the automated injection of H_2 and the regulation of humidity, this high influx of oxygen would have required manual user intervention at least every second day. An open bottle placed inside the anaerobic chamber during the experiments with media containing resazurin (20 mg × l^{-1}), a commonly used indicator for anaerobic conditions, also showed no signs of oxygen in measurable quantities.

The anaerobic box was also used for the study of phosphorylation of redox sensitive proteins using radioactive ^{32}P, hence the radioactive stickers in Fig. 3. The thick PMMA body of the anaerobic chamber offers better protection against beta-radiation then the thin plastic used in anaerobic tents, while the compact interior also allows for an easier clean-up after the end of the labeling experiments.

9 Acknowledgements

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10 Declaration of interest

Declarations of interest: none

11 Human and animal rights

No human or animal studies were conducted in this work.

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4. Closing discussion

This thesis explores the viability of different proposed hypotheses to explain the delay of at least 300 Ma between the first signs of oxygenic photosynthesis in the rock record and the onset of the GOE. Chapter 2.1 examines the hypothesis, that Cyanobacteria evolved in a freshwater environment and had to adapt to a higher salinity in sea water before expanding to the large area of the Archean oceans, thereby delaying the onset of the GOE. Chapter 2.2 investigates how, once Cyanobacteria had adapted to higher salinities, the toxicity of Fe(II) in the ferruginous Archean ocean could have hindered Cyanobacterial expansion. Chapter 3.1 describes the construction of an automated low-cost anaerobic box, which enables work groups with a lower budget to conduct anoxic experiments and further the understanding of the processes at the time of the GOE, as demonstrated by the experiments conducted in chapter 2.2.

4.1 Saltwater barrier

Although it is not clear whether the salinity of the Archean ocean was higher than today, it was at least as high as the modern ocean (Marty *et al.* 2018). This would have delayed the expansion of freshwater Cyanobacteria in the ocean until mechanisms to overcome the higher osmotic stress were acquired (Sánchez-Baracaldo *et al.* 2017). During the experiments in Chapter 2.1 the basal root Cyanobacterium, *Gloeobacter violaceus* PCC 7421, showed no biological activity in saltwater and almost no growth in brackish water. The experiments exposed the organisms to a constant salinity, whereas, in a natural tidal environment, the organisms would have been exposed to varying levels of salinity. In such a scenario, we suggest that *Gloeobacter violaceus* PCC 7421 might have been able to grow during times of low salinity and endure the increased salinities during high tide. Over time, this would have enabled better adapted Cyanobacteria to migrate down the salinity gradient to the open ocean, as observed for *C. thermalis* PCC 7203.

Also, other microorganisms already residing in the higher salt environment of the ocean could have provided salt tolerance genes by lateral gene transfer, thereby enabling their adaption to a saltwater environment rather more quickly, if measured on a geological scale. Additional structural changes might have been necessary in order to migrate into the ocean, as the root Cyanobacterium, *Gloeobacter violaceus* PCC 7421, does not possess thylakoids. Its photosynthetic machinery is located on the inner plasma membrane, which not only restricts productivity by the limited surface area of a single membrane in comparison to the folded thylakoids, but also subjects the photosynthetic machinery directly to the osmotic stress of a high salt environment. Therefore, the evolution of the thylakoid might not only have benefited the overall amount of photosynthesis a single cell is capable of, but also have protected the photosynthetic machinery from the increased salinity and other environmental factors. During the experiments, *Gloeobacter violaceus* PCC 7421 cultured in the high salt conditions also most likely lost a lot of energy to the active Na^+-efflux pumps. In contrast, the more modern *Chroococcidiopsis thermalis* PCC 7203 has genes coding for the synthesis of compatible solutes. Compatible solutes raise the osmotic potential inside the cell without disturbing the cytoplasmic ion balance, thereby reducing the influx of large amounts of charged Na^+ and Cl^- ions. Taken together, this data offers an explanation as to why the expansion of Cyanobacteria was hindered as they reached the saline ocean water. It also supports the freshwater origin of Cyanobacteria hypothesis and explains why there were large mat systems in an alkaline freshwater lake 300 Ma prior to the GOE (Flannery and Walter 2012). Nonetheless, it is also possible that Cyanobacteria could have evolved in the saline ocean and that the conclusions from the polygenetic mapping of the Cyanobacterial clade are skewed by survivorship bias. During Earth's history there are many periods of cooling with an associated glaciation and shrinkage of the open ocean area not covered with ice. This would have restricted the area available for photosynthetic organisms to the top layers of the ice, comparable to recent Cyanobacteria living in snow on high mountains and the Arctic regions of today (Vincent *et al.*

2000). Saltwater Cyanobacteria strains which were unable to adapt to these freshwater niches with almost no salinity would have become extinct (Kirschvink *et al.* 2000), thereby shifting the make-up of the current extant basal clade of Cyanobacteria in the direction of fresh-water strains. For example, during the GOE there were many periods of global glaciation most likely caused by the removal of methane in the then oxidising atmosphere and the uptake and burial of massive amounts of CO_2 by Cyanobacteria (Kirschvink *et al.* 2000). More "recently", around 720 Ma to 635 Ma ago during the Cryogenian, a glaciation event took place so massive, that it might have covered all continents including the tropics, with an ice shell between 500 and 1500 m thick (Kirschvink *et al.* 2000; Hoffman *et al.* 1998).

Concluding from this it is entirely possible, that the adaptation of fresh-water Cyanobacteria to more saline environments could have delayed the GOE from the first observations of free oxygen and Cyanobacterium-like fossils in the rock record. Estimating the duration of this adaptation is difficult, as there is no direct evidence to date the acquisition of salt tolerance genes in Cyanobacteria owing to a lack of preserved biological material. One cannot even be sure how many more basal clade Cyanobacteria are still not discovered, how the composition of the basal clade shifted over time and if the very distant calibration points for dating branch splits are accurate. Therefore, it might be interesting to investigate the acquisition of salt tolerance genes in the two basal clade salt-water strains *Pseudanabaena* sp. PCC 7367 and *Synechococcus* sp. PCC 7336 and look at similarities to salt tolerance genes in other organisms.

4.2 The toxicity of the Archean ocean

Assuming that Cyanobacteria evolved in fresh-water and gradually acquired the necessary tolerance mechanisms to migrate up the salinity gradient to the ocean, they would also have had to overcome another hurdle before colonising the oceans. The anoxic oceans contained large reservoirs of dissolved redox sensitive elements, like Fe(II) and Mn(II), which had accumulated during the last billion years by weathering and hydrothermal fluids (Holland 1973). Previous

studies explored the effects of Fe(II) on Cyanobacteria and how the oxygen they released would react with oceanic Fe(II), and concluded that Fe(II) is toxic and would have retarded the expansion of Cyanobacteria into the ocean (Swanner *et al.* 2015). Also, all Fe(II) would have been oxidised to Fe(III), while only minimal amounts of O_2 would have escaped (Swanner *et al.* 2015). In another study, this toxic effect was not observed and most Fe(II) remained in solution while the oxygen escaped into the atmosphere (Rantamäki *et al.* 2016). Those studies are hard to compare to each other in order to obtain a clear picture regarding Fe(II) toxicity in the Archean for several reasons. Firstly, different growth systems with extremely variable amounts of CO_2 were used without determining whether these systems influenced the growth rates of the strains under investigation. Secondly, no acclimation periods were included to allow the micro-organisms to adjust to the vastly different growth conditions investigated. Thirdly, the strains investigated are not closely related, with Swanner et al.(2015) using a modern marine Cyanobacterium, *Synechococcus* sp. PCC 7002, and Rantamäki et al.(2016) using different toxic/ non-toxic strains of *Nodularia spumigena* and *Microcystis aeruginosa*. None of these strains fall into the basal clade of Cyanobacteria (Sánchez-Baracaldo 2015). Even though those microorganisms fall within the phylum Cyanobacteria, their responses to Fe(II) can be expected to be vastly different, thereby making comparative studies difficult. For these reasons, this study aimed to standardise the approaches used to simulate the interaction of Cyanobacteria and their Archean environment to address the concern of Fe(II) toxicity in the Archean ocean.

The most promising strains to utilise in this study were the, so far only known, two basal saltwater strains *Pseudanabaena* sp. PCC 7367 and *Synechococcus* sp. PCC 7336. The choice of these strains is justified as they are the closest living relative species to the Cyanobacteria proposed to have evolved during the Archean. As discussed in section 3.2, one cannot exclude that these species are just a small fraction of the total number of species prevalent during the Archean. However, by investigating the most primitive extant Cyanobacteria, one can obtain

greater insight into Cyanobacterial growth on early Earth. The experiments conducted with these strains did not only show that the open-culture system supported far better growth then the closed culture system traditionally used to simulate the Archean, but also that strain selection has an important influence on the outcome and interpretation of the experiments. *Synechococcus* sp. PCC 7336 was unable to grow under the 10% CO_2 atmosphere used in our closed-culture system and previous studies of Swanner *et al.*, whereas *Pseudanabaena* sp. PCC 7367 could, however with signs of Fe(II) toxicity. If these experiments were only conducted with *Pseudanabaena* sp. PCC 7367 in the closed-setup, it would have replicated and reinforced the conclusions of Fe(II) toxicity as presented in Swanner *et al.* 2015. However, by utilising a culture system that allowed for the constant replenishment of CO_2, a more realistic Archean CO_2 concentration of 0.2% could be maintained throughout the whole experiment. In this system we could show that a single exposure to Fe(II) in concentrations of the Archean ocean had only a minimal toxic effect on both strains tested, while unlike the study of Rantamäki et al.(2016), all Fe(II) was rapidly oxidised. Also, the high CO_2 concentration in the closed-culture system had supressed the formation of green rust (GR), which was only observed under an Archean-like atmosphere with lower CO_2 levels. The formation of GR, a mixed Fe(II)/Fe(III)-hydroxide, could be an important contribution to explain the oxidation state of the sediments that formed banded iron formations, as these were not deposited as pure Fe(III), but also contained Fe(II) (Halevy *et al.* 2017). This study not only demonstrated the formation of GR by the partial oxidation of Fe(II) by Cyanobacteria, but also that its formation can have a strong negative effect on the growth of the Cyanobacterial species under investigation. The mechanism of GR toxicity was not fully explored, but it could be caused by an encrustation of the cell with GR or Fe-hydroxides. During oxygenic photosynthesis, the immediate surroundings of the cell change from anoxic to micro-anoxic which facilitates the oxidation of Fe(II) to Fe(III). The just produced Fe(III) in turn reacts with remaining Fe(II) to form GR, which starts to encrust the cell, as depicted in Figure 7. Further oxidation of the GR to Fe-hydroxide leads to an increase

in volume and could exert a mechanical stress on the cell. On the other hand, if the O_2 production is high enough, Fe(II) would get oxidised further away from the cell or would be completely converted to Fe-hydroxide without the intermediate GR step (Halevy *et al.* 2017).

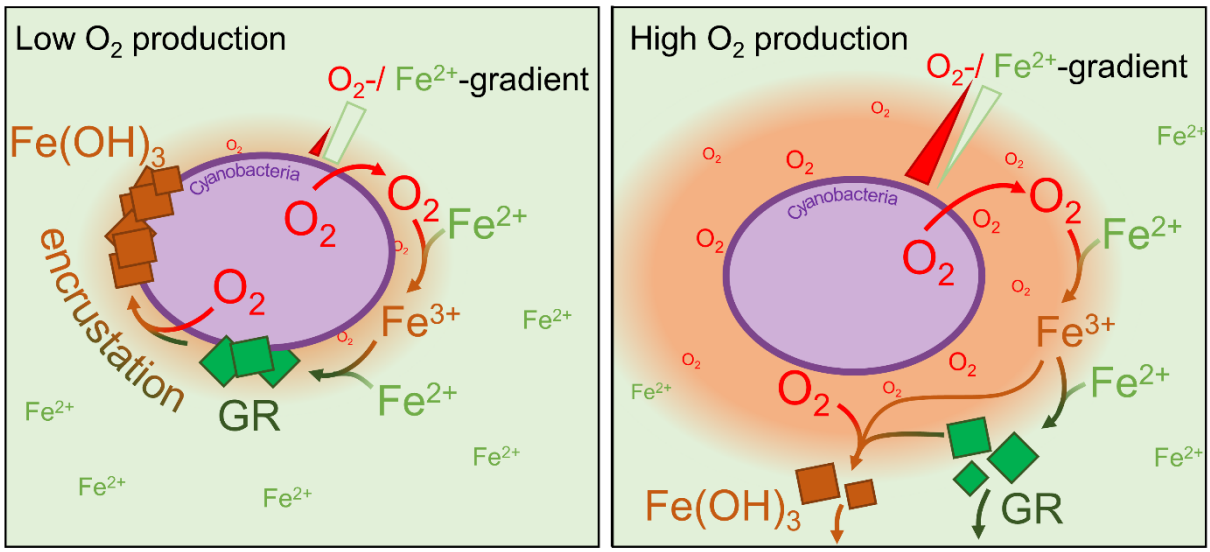

Figure 7: Proposed mechanisms for green rust formation and toxicity under different levels of O_2 release.

Left side: Low oxygen production leads to an oxidation of Fe(II) to Fe(III) in close proximity to the cell wall which reacts with Fe(II) to insoluble green rust before enough Fe(III) is formed to precipitate as $FeOH_3$. As green rust is further oxidised to $FeOH_3$ its volume expands and could possible mechanical restrict or damage the encrusted cell. Right side: High oxygen production leads to a strong O_2/Fe(II) gradient and Fe(II) is oxidised further away from the cell, either complettly to $FeOH_3$ or partially to green rust (Halevy *et al.* 2017), which precipitate out of the solution without encrusting the cell.

The fitness advantage of high, local oxygen production to prevent Fe encrustation could also tie in with the hypothesis, that the emergence of multicellular strains started the GOE (Schirrmeister *et al.* 2013). The concentrated photosynthetic activity of tightly packed individual cells in a multicellular strain could lead to the fast establishment of a strong oxygen gradient, which could have prevented the encrustation by Fe (Figure 7, right). Single celled Cyanobacteria would take far longer to oxygenate their immediate surroundings and would therefore be more susceptible to encrustation by GR and Fe (Figure 7, left). This is especially important in the open (Archean) ocean, where the low concentration of cells cannot generate an oxygen oasis effect, as proposed for shallow coastal regions. Our data supports this thought process in that the multicellular strain *Pseudanabaena* sp. PCC 7367 exhibited stronger

resilience to the toxic effects of constant nightly Fe(II) exposure then the single celled *Synechococcus* sp. PCC 7336.

In conclusion, these investigations indicate that Fe(II) in the Archean ocean could have prevented the migration of Cyanobacteria in the open ocean by the formation of green rust, while having no to little effect on the colonisation of shallow water environments, where the localised oxygenic photosynthesis would have created oxygen oases. Also, the formation of green rust by the incomplete oxidation of Fe(II) provides further insights and avenues of investigation into the genesis of banded iron formations.

5. Outlook

Even though the experiments in this study verified the possibility of a "freshwater-origin" and "Fe(II)-toxicity" hypothesis there are still many open questions which could give further credence or refute these hypotheses. Foremost the isolation and identification of more basal Cyanobacterial species would give a broader insight into the distribution of fresh- vs saltwater Cyanobacteria and the possible shared properties of ancient Cyanobacteria in general. These data sets could be used to compare the salinity resistance genes in the basal Cyanobacterial clade with other organisms which are thought to have existed in similar niches in the Archean ocean, such as photoferrothrops or other anoxic photosynthesisers. This could give more insight into the possibility of lateral gene transfer between these species, which would most likely have reduced the time to adapt to a high salt environment significantly. Also of interest could be, if the acquisition of compatible solute genes was, on its own, sufficient for growth in high salinity environments or whether the reorganisation of the photosystem into thylakoids was not only essential for increasing the total photosynthetic capacity but also for protecting the photosystem from interference by the inflow of Na^+ and Cl^- across the outer membrane.

To follow up on the Fe(II)-toxicity study it would be of great interest to further examine the properties and formation of green rust, especially in a media composition which resembles the Archean ocean composition as regards silica content. By replacing the $NaHCO_3$ buffer with silica the type of green rust formed may change from carbonate green rust to chloride green rust, however silica is also known to adsorb to green rust which might alter the processes identified in this investigation (Halevy *et al.* 2017). Iron-silica-aggregates could be an important factor in the formation of banded iron formations, which often consist of alternating layers of iron oxides and silica rich rocks. Future studies could make use of laboratory generated pseudo mats of Cyanobacteria, like in the "freshwater-origin" experiments, exposed to a daily inflow of ferruginous Archean seawater and analyse if iron(hydro)oxides are retained in the matrix of

the pseudo mat or are washed out into the open ocean. This process would also tie in with the formation of iron-silica-aggregates, as these can act as natural sunscreen against the high flux of UV radiation in the Archean caused by the absence of an ozone layer (Mloszewska *et al.* 2018). As Fe(II) toxicity would not have prevented the colonisation of shallow oxygen oases, it would be interesting to explore the possibility of Fe(II) toxicity during the expansion into the open Archean ocean. Another point of interest would be to explore if multicellular, filamentous strains of marine planktonic Cyanobacteria can resist the toxic effect of Fe-encrustation better then unicellular Cyanobacteria strains if incubated at the faint cell density found in natural ocean water.

Taken together all these suggestions represent only a fraction of potential small niche investigations into how our life evolved on early Earth, and how the atmosphere became oxygenated to support life as we know it today. Evidence in the rock record are highly fragmented and scarce, given the small amount of well preserved rocks from the Archean and early Proterozoic Eras available to study. Only the combination of the historical rock record with modern day simulations can give us a better understanding of how abiotic and biotic processes shape the evolution on a geological scale not only on Earth but also possibly on other planets.

6. Summary

About 2.4 Ga ago the Great Oxygenation Event (GOE) started the permanent oxygenation of Earth's anoxic atmosphere. The oxygen was most likely produced by oxygenic photosynthesis in Cyanobacteria. However, hints for local occurrences of Cyanobacterial life and free oxygen exists for at least 300 Ma prior to the GOE. Different hypotheses were proposed to explain this delay between the evolution of oxygen producers and the start of the GOE. For this thesis, theoretic predictions made by two of those hypotheses were tested in laboratory experiments using ancestral, basal clade Cyanobacteria grown under simulated Archean like conditions.

Cyanobacteria might have evolved in freshwater environments and subsequently had to adapt to the higher salinity of the Archean ocean. In turn, this would have delayed their global expansion required for the GOE. Experiments with the most primitive freshwater Cyanobacterium *Gloeobacter violaceus* PCC 7421, showed its ability to tolerate and slowly grow in brackish water, thereby providing a route for the evolution of open ocean dwelling, salt tolerant species. The Archean ocean may have presented another hurdle to Cyanobacterial expansion as it contained large amounts of Fe(II), which is presumed to be toxic to Cyanobacteria. This thesis shows that the localised activity of Cyanobacteria could have formed marine oxygen oases in shallow coastal regions. This would have negated the toxicity of Fe(II) and could have produced more net O_2 then modern oxic systems. Additionally, the formation of green rust was observed, which seemed to have a toxic effect on Cyanobacterial growth and could be an important factor for the genesis of banded iron formations.

In conclusion, this thesis could show the viability of both, the "freshwater-origin" and "Fe(II)-toxicity", hypothesis. Nevertheless, how long it took for Cyanobacteria to overcome the restrictions described above to expand into the open ocean is uncertain and needs to be further studied.

7. Zusammenfassung

Die permanente Anreicherung der Erdatmosphäre mit Sauerstoff begann vor 2.4 Ga und wird gemeinhin als die Große Sauerstoffkatastrophe (GOE) bezeichnet. Der hierfür benötigte Sauerstoff wurde wahrscheinlich durch oxygene Photosynthese von Cyanobakterien produziert. Hinweise auf lokale Vorkommen von cyanobakteriellem Leben und freiem Sauerstoff existieren jedoch bereits seit 300 Ma vor dem GOE. Zur Erklärung, warum die Evolution von Sauerstoffproduzenten und der Start des GOE zeitlich entkoppelt sind, existieren zahlreiche Hypothesen. Diese Arbeit testet theoretische Vorhersagen von zwei dieser Hypothesen in realen Experimenten an basalen Cyanobakterien unter Bedingungen des Archaikums.

Cyanobakterien könnten in Süßwasser entstanden sein und hätten sich vor ihrer globalen Expansion erst an den höheren Salzgehalt des Archäischen Ozeans anpassen müssen. Experimente mit dem primitivsten Süßwassercyanobakterum *Gloeobacter violaceus* PCC 7421 zeigten dessen Fähigkeit, Brackwasser nicht nur zu tolerieren, sondern auch langsam darin zu wachsen. Dies könnte einen Weg zur graduellen Anpassung an Salzwasser ermöglicht haben. Nicht nur der Salzgehalt des Archäischen Ozeans, sondern auch sein anoxisches, Fe(II) reiches Wasser könnte eine Wachstumsbarriere für frühe Cyanobakterien gebildet haben, da angenommen wird, dass Fe(II) toxisch für Cyanobakterien ist. Diese Dissertation konnte jedoch zeigen, dass die lokale Aktivität von Cyanobakterien zumindest in flachen Küstenregionen eine marine Sauerstoffoase gebildet haben könnte, welche die Toxizität von Fe (II) negiert und mehr Netto-O_2 als oxische Systeme produziert. Ein toxischer Effekt wurde hingegen bei der Bildung von grünem Rost beobachtet, der zusätzlich ein wichtiger Faktor in der Genese von Bändereisenerz spielen könnte.

Zusammenfassend konnte diese Dissertation sowohl die Hypothese des „Süßwasserursprungs“ als auch der „Fe(II)-Toxizität“ bekräftigen. Wie lange dies die Expansion von Cyanobakterien verzögert haben könnte, ist jedoch noch ungewiss und bedarf weiterer Untersuchungen.

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9. Curriculum Vitae

Personal Data:

Name:	Achim Jan Herrmann
Date of Birth:	personal information
Place of Birth:	personal information
Citizenship:	personal information

Education:

M.Sc., Microbial and Plant Biotechnology, University of Kaiserslautern, **Nov. 2016**
Focus: Microbiology, Cloning and fluorescence microscopy
Thesis: Localization studies of the histidine kinase CiaH in *Streptococcus pneumoniae*.

B.Sc., Life science, University of Kaiserslautern, **Oct. 2013**
Focus: Microbiology, Cloning
Thesis: Evaluation of a reporter system in *Streptococcus pneumoniae* Hu15.

Graduation from high school, Hohenstaufen Gymnasium Kaiserslautern, **Mar. 2009**

Employment History:

May 2017 – Present
Ph.D. student, University of Kaiserslautern, Dept. Microbiology
Focus: Geobiology, Oxygen production in Archean environments
Title of thesis: The interplay between Neoarchean oceans and Cyanobacteria. Oxygen production and the oxidation of Fe(II)

Dec. 2016 – Mar. 2017
Research assistant, University of Kaiserslautern, Dept. Plant Ecology and Systematics Tasks: Cultivation of Cyanobacterial pseudo mats under Neoarchean conditions, microsensor O2 measurements.
Course supervisor

Student assistant:	Basic Microbiology Techniques	**Jun. 2014**
	Advanced Microbiology Techniques	**Oct.- Dec. 2013, 2014, 2015, 2016**

Publications, posters, talks:

2018

Poster: Herrmann, Achim J.; Gehringer, Michelle M. (**2018**): Could cyanobacteria have made the salinity transition during the late Archean? In: German Microbiology Association (VAAM), Wolfsburg, Germany

Poster: Herrmann, Achim J.; Gehringer, Michelle M. (**2018**): Could cyanobacteria have made the salinity transition during the late Archean? In: SPP1883, Göttingen, Germany

Poster: Herrmann, Achim J.; Gehringer, Michelle M. (**2018**): Could cyanobacteria have made the salinity transition during the late Archean? In: GeoBonn, Bonn, Germany

2019

Publication: Herrmann, Achim J.; Gehringer, Michelle M. (**2019**): An investigation into the effects of increasing salinity on photosynthesis in freshwater unicellular cyanobacteria during the late Archaean. In: *Geobiology* 17 (4), S. 343–359. DOI: 10.1111/gbi.12339.

Poster: Herrmann, Achim. J., Enzingmüller-Bleyl, T. C. and Gehringer, Michelle. M. (**2019**): Surviving the ferruginous Archean ocean. The potential toxicity of Fe^{2+} on basal Cyanobacteria. In: German Microbiology Association (VAAM), Mainz, Germany

Poster: Herrmann, Achim J.; Gehringer, Michelle M. (**2019**): Surviving the ferruginous Archean ocean. The potential toxicity of Fe^{2+} on basal Cyanobacteria In: Cyano2019, Tübingen, Germany

Poster: Herrmann, Achim J.; Gehringer, Michelle M. (**2019**): Surviving the ferruginous Archean ocean. The potential toxicity of Fe^{2+} on basal Cyanobacteria In: GeoMünster, Münster, Germany

Co-organiser, Talk: Herrmann, Achim J. (**2019**): Rust world. How to simulate the Archean. In: Geomicrobiology Workshop TUK, Kaiserslautern, Germany

Talk: Herrmann, Achim J. (**2019**): Rust world - The effects of Fe^{2+} on cyanobacteria. In: SPP1883, Köln, Germany

Talk: Herrmann, Achim J. (**2019**): Rust world - Surviving the Ferruginous Archean Ocean. In: Fachbereichsseminar Biologie, Thallichtenberg, Germany

2020

Poster: Herrmann, Achim J.; Gehringer, Michelle M. (**2020**): Surviving the ferruginous Archean ocean. The potential toxicity of Fe^{2+} on basal Cyanobacteria In: Gordon Research Conference, Gordon, Texas, USA

Talk: Herrmann, Achim J. (**2020**): Rust world - Surviving the Ferruginous Archean Ocean. In: Gordon Research Seminar, Gordon, Texas, USA

2021

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