Video Article

Laboratory Simulation of an Iron(II)-rich Precambrian Marine Upwelling System to Explore the Growth of Photosynthetic Bacteria

Markus Maisch[1,2], Wenfang Wu[1], Andreas Kappler[1], Elizabeth D. Swanner[1,2]

[1]Department of Geosciences, University of Tuebingen

[2]Department of Geological and Atmospheric Sciences, Iowa State University

Correspondence to: Elizabeth D. Swanner at eswanner@iastate.edu

URL: http://www.jove.com/video/54251
DOI: doi:10.3791/54251

Keywords: Environmental Sciences, Issue 113, Geomicrobiology, Water column, Fe(II) oxidation, Photosynthesis, Archean ocean, Cyanobacteria, Great Oxidation Event, Banded Iron Formation

Date Published: 7/24/2016

Citation: Maisch, M., Wu, W., Kappler, A., Swanner, E.D. Laboratory Simulation of an Iron(II)-rich Precambrian Marine Upwelling System to Explore the Growth of Photosynthetic Bacteria. *J. Vis. Exp.* (113), e54251, doi:10.3791/54251 (2016).

Abstract

A conventional concept for the deposition of some Precambrian Banded Iron Formations (BIF) proceeds on the assumption that ferrous iron [Fe(II)] upwelling from hydrothermal sources in the Precambrian ocean was oxidized by molecular oxygen [O_2] produced by cyanobacteria. The oldest BIFs, deposited prior to the Great Oxidation Event (GOE) at about 2.4 billion years (Gy) ago, could have formed by direct oxidation of Fe(II) by anoxygenic photoferrotrophs under anoxic conditions. As a method for testing the geochemical and mineralogical patterns that develop under different biological scenarios, we designed a 40 cm long vertical flow-through column to simulate an anoxic Fe(II)-rich marine upwelling system representative of an ancient ocean on a lab scale. The cylinder was packed with a porous glass bead matrix to stabilize the geochemical gradients, and liquid samples for iron quantification could be taken throughout the water column. Dissolved oxygen was detected non-invasively via optodes from the outside. Results from biotic experiments that involved upwelling fluxes of Fe(II) from the bottom, a distinct light gradient from top, and cyanobacteria present in the water column, show clear evidence for the formation of Fe(III) mineral precipitates and development of a chemocline between Fe(II) and O_2. This column allows us to test hypotheses for the formation of the BIFs by culturing cyanobacteria (and in the future photoferrotrophs) under simulated marine Precambrian conditions. Furthermore we hypothesize that our column concept allows for the simulation of various chemical and physical environments — including shallow marine or lacustrine sediments.

Video Link

The video component of this article can be found at http://www.jove.com/video/54251/

Introduction

The Precambrian (4.6 to 0.541 Gy ago) atmosphere experienced a gradual build-up of photosynthetically produced oxygen (O_2), perhaps punctuated by step changes at the so-called "Great Oxidation Event" (GOE) at approximately 2.4 Gy ago, and again in the Neoproterozoic (1 to 0.541 Gy ago) as atmospheric O_2 approached modern levels[1]. Cyanobacteria are the evolutionary remnants of the first organisms capable of oxygenic photosynthesis[2]. Geochemical evidence and modeling studies support the role of shallow coastal environments in harboring active communities of cyanobacteria or organisms capable of oxygenic photosynthesis or oxygenic phototrophs, generating local oxygen oases in the surface ocean below a predominantly anoxic atmosphere[3-5].

The deposition of Banded Iron Formations (BIFs) from seawater throughout the Precambrian points to iron(II) (Fe(II)) as a major geochemical constituent of seawater, at least locally, during their deposition. Some of the largest BIFs are deep-water deposits, forming off the continental shelf and slope. The amount of Fe deposited is incompatible from a mass balance standpoint with predominantly continental (*i.e.*, weathering) source. Therefore, much of the Fe must have been supplied from hydrothermal alteration of mafic or ultramafic seafloor crust[6]. Estimates of the rate of Fe deposited outboard of coastal environments are consistent with Fe(II) supplied to the surface ocean via upwelling[7]. In order for Fe to be transported in upwelling currents, must have been present in the reduced, mobile form — as Fe(II). The average oxidation state of Fe preserved in BIF is 2.4 [8] and it is generally thought that BIF preserve Fe deposited as Fe(III), formed when upwelling Fe(II) was oxidized, possibly by oxygen. Therefore, exploring potential Fe(II) oxidation mechanisms along slope environments is important to understand how BIF formed. Moreover, refined geochemical characterization of marine sediments has identified that ferruginous conditions, where Fe(II) was present in an anoxic water column, were a persistent feature of the oceans throughout the Precambrian, and may not have been limited to just the time and place where BIF were deposited[9]. Therefore, for at least two billion years of Earth's history, redox interfaces between Fe(II) and O_2 in the shallow oceans were likely commonplace.

Numerous studies utilize modern sites that are chemical and/or biological analogs of different features of the Precambrian ocean. A good example are ferruginous lakes where Fe(II) is stable and present in sunlit surface waters while photosynthetic activity (including by cyanobacteria) was detected[10-13]. The results of these studies provide insight into the geochemical and microbial characteristics of an oxic

to anoxic/ferruginous chemocline. However these sites are generally physically stratified with little vertical mixing[14], rather than the chemical interfaces occurring in an upwelling system, and are thought to support the most oxygen production in Precambrian time[4].

A natural analogue to explore the development of a marine oxygen oasis beneath an anoxic atmosphere, and at an Fe(II)-rich upwelling system in sunlit surface water column is not available on the modern Earth. Therefore, a laboratory system that can simulate a ferruginous upwelling zone and also support the growth of cyanobacteria and photoferrotrophs is needed. The understanding and identification of microbial processes and their interaction with an upwelling aqueous medium that represents Precambrian seawater promotes the understanding and can complement the information gained from the rock record in order to fully understand the distinctive biogeochemical processes on ancient Earth.

Toward that end, a laboratory-scale column was designed in which Fe(II)-rich seawater medium (pH neutral) was pumped into the bottom of the column, and pumped out from the top. Illumination was provided at the top to create a 4 cm wide "photic zone" that supported the growth of cyanobacteria in the top 3 cm. Natural environments are generally stratified and stabilized by physicochemical gradients, like salinity or temperature. In order to stabilize the water column on a lab-scale, the column cylinder was packed with a porous glass bead matrix that helped to maintain the establishment of geochemical patterns that developed during the experiment. A continuous N_2/CO_2 gas flow was applied to flush the headspace of the column in order to maintain an anoxic atmosphere reflective of an ocean prior to the GOE[15]. After a constant flux of Fe(II) was established, cyanobacteria were inoculated throughout the column, and their growth was monitored by cell counts on samples removed through sampling ports. Oxygen was monitored *in situ* by placing oxygen-sensitive optode foils onto the inner wall of the column cylinder and measurements were made with an optical fiber from outside the column. Aqueous Fe speciation was quantified by removing samples from depth-resolved horizontal sampling ports and analyzed with the Ferrozine method. The abiotic control experiments and results demonstrate proof-of-concept — that a laboratory scale analog of the ancient water column, maintained in isolation from the atmosphere, is achievable. Cyanobacteria grew and produced oxygen, and the reactions between Fe(II) and oxygen were resolvable. Herein, the methodology for design, preparation, assembly, execution, and sampling of such a column are presented, along with results from an 84 hr run of the column while inoculated with the marine cyanobacterium *Synechococcus* sp. PCC 7002.

Protocol

1. Preparation of Culturing Medium

Note: Information on the required equipment, chemicals and supplies for the preparation of the culture medium is listed in **Table 1**. Italic alphanumerical codes in brackets refer to the equipment itemized in **Table 2** and shown in **Figure 1**.

1. Prepare 5 L of Marine Phototroph (MP) medium (referred to hereafter as "medium") following the protocol of Wu *et al.*[16]. Adjust the pH to 6.8 using anoxic and sterile 1 M HCl or 0.5 M $NaCO_3$. As a source for Fe(II), add 3.5 ml of a 1 M anoxic and sterile $FeCl_2$-solution to achieve a final Fe(II) concentration of 500 µM after filtering the medium solution in the next step.
2. Store the medium solution at 5 °C for 48 hr in order to precipitate Fe(II) carbonate and phosphate minerals. Fe(II) addition and Fe mineral precipitation results in a pH change, therefore readjust the pH back to 6.8. Filter the medium in an anoxic (100% N_2) glovebox through a 0.22 µm filter unit. Dispense the filtered medium into a sterile 5 L glass bottle (*E.'1*) inside a glovebox, and cap with a sterile butyl rubber stopper.
3. Flush the headspace of the medium bottle with N_2/CO_2(v/v, 90/10) by inserting one disposable needle connected to the gas line into the stopper, and a second needle that acts as a vent. Make sure to change the headspace volume 10 times. For example, with a constant gas flow of 10 ml/sec, flush the headspace volume of 50 ml for at least 50 sec (compare Hungate & Macy[17]).
4. Cover the medium bottle (*E*) that is now ready to use with aluminum foil and store at RT under dark conditions to prevent photooxidation of Fe(II). Allow 3 days for medium preparation.

2. Preparation of the Culture

Note: The culture of *Synechococcus* sp. PCC 7002 that is used in the column experiment is described as unicellular marine photoheterotrophic cyanobacteria genus[18]. It was provided by Dr. M. Eisenhut (Institute for Plant Biochemistry, University of Duesseldorf, Germany). For the current study the stock culture was grown on anoxic MP medium without additional Fe(II).

1. Prepare 100 ml MP medium following the protocol of Wu *et al.*[16] but substitute ferric chloride with 1 ml/L ferric ammonium citrate at 6 mg/ml.
2. Under anoxic conditions (glovebox, 100% N_2), dispense the medium into one 120 ml sterile serum bottle, cap with a sterile butyl rubber stopper and crimp with an aluminum cap. Change the headspace to N_2/CO_2(v/v, 90/10) (compare Hungate & Macy[17]) and inoculate with 5% of the stock culture. Subsequently store the culture in a light incubator at 25 °C and 600 Lux from a Tungsten light bulb.
3. Since *Synechococcus* sp. PCC 7002 is photosensitive, following transfer, cover the serum bottle with a thin paper towel for the first 24 hr within the light incubator. Allow the culture to grow for 6-8 days. Photosynthetic activity will result in the oxidation of Fe(II) and Fe(II) will not be static for the timescale of cellular growth, therefore transfer the culture after 7 days to maintain Fe(II) in the medium and the cells adapted to Fe(II).
4. Monitor the cell density by taking samples for optical density (OD) measurements: The cell density (cells/ml) of the culture can be determined via the absorbance of the cell suspension sample in a photo-spectrometer at a wavelength of 750 nm[19]. A linear relationship between OD_{750} and direct microscopic cell counts of a culture in log phase will determine the absolute cell density[20].
5. As soon as a cell density of ~10^8cells/ml is reached, wrap the serum bottle with aluminum foil in order to stop oxygen production by photosynthesis.
6. Remove the O_2 in the cell suspension by using a 0.22 µm sterile syringe filter attached to a long (100 mm) disposable needle inserted into the liquid medium. Flush the headspace and bubble the culture with N_2/CO_2 for 5 min (compare Hungate & Macy[17]). Keep the sample in dark until inoculation into the column.

3. Preparation of Items and Individual Parts for Experimental Set-up

Note: Information on the required equipment for the experimental set-up, quantities and specifications are listed in **Table 2**. Parts of the items that will be used for the experimental set-up are prepared in advance and are individually labeled with a single capital letter (A-G), listed in **Table 2** and shown as close-ups in **Figure 1** as well. Italic alphanumerical codes in brackets in the protocol refer to the equipment itemized in **Table 2** and are shown in **Figure 1**.

1. In order to prepare the sampling ports *(D)* for the column, close the ports with tight-fitting butyl rubber stoppers (*A.3*). Insert one stainless steel needle (*D.'*) into the butyl rubber stopper. Make sure that the tip of the needle is in the center of the column.
    1. Connect the needle to a rubber tube (*D.2*) and seal the connection with a heat-shrink tube (*D.3*). Attach the other end of the tube to a small Luer lock tube connector (*'D.5*), seal the connection with a heat-shrink tube (*D.3*) and cover the tube connector with an appropriate plastic cap (*D.6*).
    Note: Depending on the desired number of sampling ports it is necessary to provide one main port with various sampling ports — therefore insert the stainless steel needles (*D.1*) in an oblique angle into the butyl rubber stopper.

2. In order to connect the medium and discharge bottles to the column, modify butyl rubber stoppers following the next steps:
    1. Insert two stainless steel capillaries (*E.3; E.4*) into the butyl rubber stopper (*E.2*). Connect the appropriate rubber tubes (*E.6*) to these capillaries and seal the connection with heat-shrink tubes (*E.5*).
    2. Add a tube connector (*E.7*) to the other end of the tube of the longer capillary (*E.3*) and also fix this connector with a heat-shrink tube (*E.5*).
    3. Connect a stainless steel needle (*E.11*) to the free end of the other tube attached to the shorter capillary *(E.4)*, seal the connections with heat-shrink tubes (*E.5*), and insert the other end of the stainless steel needle into a smaller butyl rubber stopper *(E.10)*.
    4. Insert two stainless steel capillaries (*G.3*) into a large butyl rubber stopper *(G.2)* and attach the appropriate rubber tubes (*G.4; G.5*). Connect the free end of the shorter rubber tube (*G.4*) to a medium discharge bottle capillary (*w2*). Equip the free end of the longer tube (*G.5*) with a small tube connector (*G.6*). Repeat these steps in order to prepare another butyl rubber stopper for a second discharge bottle.

3. Prepare the stopper for the medium distribution panel (*F*) by inserting two stainless steel needles (*F.3*) into a butyl rubber stopper (*F.2*) in order to produce two media supply lines for the column. Attach a rubber tube *(F.4*) to each of the needles and connect one medium bottle capillary (*c1*) to one of the rubber tubes, respectively.
4. In order to prepare the glands for the medium supply line (*B)*, connect one medium supply capillary (*c2*) to a small tube connector (*B.3*) with a rubber tube (*B.4*). Repeat this step for another medium supply gland.
    1. Use the longer medium discharge capillary (*w1*) instead to prepare glands for the medium discharge line and follow the previous steps (compare (*B*) in **Figure 1**).
    Note: It is helpful to label inlet and outlet with different colors of tape to assist in proper assembly.

5. Assemble the parts for the headspace gas exchange panel (*C*) by inserting two long Luer lock stainless steel needles (*C.2*) into a rubber tube (*C.1*) and make sure that the tips of the needles reach 4 cm outside the other end of the tubing. Fill the tube with Polymers glue (*C.8*) and let the assembly dry for at least 6 hr.
    1. Loosely fill two Luer lock glass syringes (*C.3*) with cotton (*C.4*).
    2. Separately prepare two butyl rubber stoppers (*C.5*), one with a stainless steel needle inserted (*C.6*) and the other with a stainless steel needle (*C.7*). Do not connect to the glass syringes, yet.

6. Prepare a glass syringe (*E.8*) filled with cotton (*E.9*) for later use in line with the medium bottle and gas pack (*gp*).
7. Assemble the equipment for a 10 L gas pack (*gp*) by connecting a rubber tube (*gp.1*) onto the valve of the gas pack. Insert a tube connector (*gp.2*) into the free end of the rubber tube. Repeat this procedure for a second 10 L gas pack.

4. Sterilization of the Column and the Equipment

Note: Depending on the material properties, the equipment is sterilized by one of the following three methods:

1. Sterilize equipment made of glass by dry oven (180 °C for 4.25 hr):
    1. Sterilize the equipment for making medium (see **Table 1** and **section 1.2**) separately and in advance. Therefore, prepare 2 x 5 L glass bottles and cover the opening and the bottleneck with aluminum foil. Pack 4 x 5 ml and 5 x 2 ml glass pipettes into a heat resistant container and oven-sterilize all the equipment.
    2. Sterilize the equipment for the column set up in a second step. Wrap the glass syringes (*C.3; E.8; F.1* — prepared in **section 3**) with aluminum foil and make sure that the corresponding butyl rubber stoppers are removed.
    3. Place the glass beads (*A.2*) in a glass beaker and cover the top with aluminum foil. Also cover the transparent glass plate (*A.4*), 4 x large tube connectors (*B.2*) and the 3-way connector (*G.7*) with aluminum foil and oven-sterilize everything.

2. Sterilize autoclavable plastics and liquids by autoclave (120 °C, 10 bar, 20 min):
    1. In the first step, sterilize the Widdel flask with the medium, the $NaHCO_3$-buffer solution and 2 butyl rubber stoppers for the 5 L glass bottle.
    Note: Butyl rubber stoppers are first prepared by boiling in ultrapure water 3 times and then autoclaved in a glass beaker with a bit of water, covered with aluminum foil. The wet stoppers are easier to insert into glass bottles.
    2. In a second step sterilize the equipment for the experimental column set up. In order to prepare the column for sterilization in the autoclave, wrap the top opening, the media supply vents, the media discharge vents, and the headspace vent with aluminum foil before autoclaving.
    3. Cover the sampling ports (*D.'5*) with the appropriate plastic caps (*D.'6*) and make sure to remove the clamps (*D.4*) before autoclaving.

4. Wrap the butyl rubber stoppers and the corresponding capillaries for the medium and discharge bottles (*E.2; 2 x G.2* — prepared in **section 3**), the smaller stoppers for the glass syringes (2 x *C.5*; *E.'10'; F.'2* — prepared in **section 3**) and the capillaries connected to the medium supply and discharge glands (*s2; w1* — prepared in **section 3.4**) into aluminum foil and sterilize all the equipment in the autoclave.
5. After sterilization, dry the sterilized column and equipment in an oven at 60 °C for another 4 hr.

3. Since the pump tubing (*pt*) is not autoclavable, sterilize them in an Ethanol (EtOH) solution (80% EtOH, 20% water). Fill an appropriate beaker with EtOH-solution and place the pump tubing in it, ensuring that the tube is completely filled with EtOH-solution. Take out after 3 hr and wrap directly into pre-sterilized aluminum foil (oven-sterilized) and let dry at RT for 2 hr.

5. Assembly of the Column and the Equipment

1. Place the column (*A*) on a flat surface and stabilize with a laboratory stand and clamps. Make sure to work under sterile conditions (*e.g.*, within 40 cm of a Bunsen burner or under a laminar-flow hood). Carefully remove the aluminum foil from top opening and fill in the sterilized glass-beads (*A.2*).
    1. Prepare the watch glass (*A.4*) in order to tightly close the column by applying the Polymers glue (*A.5*) to the inner surface in the area where it will be in contact with the column. Lightly press the watch glass in place at the top of the column in order to glue both parts tightly together. Allow at least 6 hr for the installation to dry.
    Note: It is possible to place a sterile, fine pliable wire between the column edge and the watch glass, in order to easily remove the watch glass after the experiment.

2. While working under sterile conditions attach the following parts to the column:
    1. Connect the rubber tubes (*B.1*) and the corresponding tube connectors (*B.2*) to the medium supply and discharge vents (compare (*B*) in **Figure 1**).
    2. Connect the tube connectors of the glands (*B.3*) for media supply and discharge (prepared in **section 3.4**) to the corresponding connectors at the column (compare (*B*) in **Figure 1**).
    3. Attach the headspace gas exchange panel (compare (*C*) in **Figure 1** — prepared in **section 3.5**) to the headspace vent at the column and connect the glass syringes (*C.3*) to the corresponding stainless steel needles (*C.2*) and insert the appropriate butyl rubber stoppers (*C.6; C.7* — prepared in **section 3.5.2**) into the cotton-filled glass syringes (*C.3*).

3. Insert the butyl rubber stoppers for the discharge bottles (2 x *G.2* — prepared in **section 3.2**) into two sterile 3 L glass bottles (*G.1*), and connect the tube connectors of the bottles (*G.6*) to the 3-way connector (*G.7*).
4. Connect the free end of the medium discharge gland capillary (*w1*) to the free end of a discharge bottle capillary (*w2*) with pump tubing (*pt*). Repeat this procedure for the second medium discharge gland capillary and the second discharge bottle.
5. Connect the free end of one medium bottle capillary (*s1*) to the free end of the medium supply gland capillary (*s2*) with the pump tubing (*pt*). Repeat this procedure for the second medium bottle capillary and the second medium supply gland capillary.
6. Assemble the medium distribution panel by inserting the stopper with the corresponding capillaries (*F.2* — prepared in **section 3.3**) into the glass syringe of the medium distribution panel (*F.1*).
7. Connect the medium distribution panel to the connector of the butyl rubber stopper for the medium bottle (*E.7* — compare *F* in **Figure 1**).
8. Connect the N_2/CO_2 gas line to the headspace gas exchange panel to the Luer lock stainless steel needle (see position (*LLF*) in **Figure 1**), and flush the column installation and capillary system with N_2/CO_2 at low pressure (< 10 mbar). Maintain an outflow of gas at the open ends of the medium bottle capillary (*E.3*), the 3-way connector (*G.*7), and the sampling ports (*D.5*). Therefore, make sure to have the caps of the sampling ports (*D.6*) slightly open, to maintain sterile conditions through an overpressure of gas flowing out.
    1. Flush the complete installation for at least 20 min.
    Note: In addition, it is possible to close the outgassing needle (C.6) of the headspace gas exchange panel with an appropriate rubber tube and a clamp in order to increase the efficiency of flushing the complete installation.

9. Meanwhile, fill a 10 L gas bag (*gp*) with N_2/CO_2(v/v, 90/10), following 10 rounds of filling and deaerating (using a vacuum pump) the entire volume to ensure the bag is completely anoxic. Make sure to close the valve of the gas pack after the final fill. Repeat this procedure with a second gas pack but close the valve after deaeration (*i.e.*, leave it empty).
Note: The N_2/CO_2 filled gas pack will be connected to the medium bottle in order to compensate the increasing headspace volume due to medium loss by pumping. The N_2/CO_2 flushed, but empty gas pack, will later be connected to the discharge bottles in order to allow gas to escape the headspace due to increasing liquid discharge volume.
10. Insert the butyl rubber stopper (*E.10* — prepared in **section 3.2.3**) into the sterile glass syringe filled with cotton (*E.8* — prepared in **section 3.6**).
11. Place the filled and closed medium bottle (*E* — prepared in **section 1**) on the lab bench next to the stopper for the medium bottle (*E.2*), and prepare to connect the stopper to the medium bottle using the following procedure:
    1. Close the N_2/CO_2 gas flow into the capillary (*E.3*) by closing the corresponding rubber tube (*E.5*) with a hose clamp.
    2. Lightly lift the butyl rubber stopper off the medium bottle and flush the headspace with N_2/CO_2(50 mbar) by hanging a sterilized, cotton -filled syringe with a bent, long metal needle (1 mm x 140 mm) into the bottleneck of the medium bottle (compare Hungate & Macy[17]).
    3. Quickly remove the butyl rubber stopper from the medium bottle and insert the prepared butyl rubber stopper (*E.2*) into the medium bottle (*E*).
    4. Change the long metal needle of the syringe (previously used for flushing the headspace) to a disposable needle (0.9 mm x 45 mm; widely available) and inject into the stopper in order to flush the headspace of the medium bottle with N_2/CO_2.
    5. Make sure to have a slight outflow of gas at the open end of the gas syringe (*E.8* — assembled in **section 5.10**) connected to the stopper of the medium bottle. Flush the headspace of the medium bottle (*E*) and the glass syringe (*E.8*) for at least 4 min.

12. Meanwhile, tighten the plastic caps (*D.6*) of the sampling ports (*D.'5*) and close the corresponding rubber tubing (*D.2*) with a clamp (*D.4*).
Note: If necessary, open the outgassing needle (*C.6*) of the headspace gas exchange panel again in order to maintain an outflow of gas at the open end of the needle.

13. Place the 10 L gas pack, filled with N_2/CO_2(prepared in **section 5.9**), on the lab bench. Ensure that the tube connector is in a position next to the open end of the glass syringe (*E.8*) connected to the medium bottle.
14. After flushing the headspace of the medium bottle (*E*) for at least 4 min, close the N_2/CO_2 gas line of the flushing syringe and quickly pull the injection needle out of the stopper (*E.2*). The remaining overpressure of gas within the headspace of the medium bottle will be released through the glass syringe (*E.8*).
    1. Quickly open the valve of the gas pack and lightly press on the bag to maintain an outflow of N_2/CO_2 in order to flush the tube and the connector of the gas pack.
    2. As soon as the overpressure is released from the medium bottle, quickly connect the connector of the gas pack (*gp.3*) to the corresponding connector of the glass syringe (*E.8*).

15. Connect an empty 10 L gas pack (*gp*) that was previously flushed 10 times with N_2/CO_2 (prepared in section 5.9) to the free port of the 3-way connector (*G.7*) connected to the discharge bottles. Make sure to keep the valve of the gas pack closed.
16. Reduce the pressure of the N_2/CO_2 gas line (<0.1 mbar) in the headspace gas exchange panel (*C* in **Figure 1**; position LLF) to maintain an outflow of gas from the outgassing needle (*C.6*).
17. Insert the pump tubing (*pt*) into the pump (*P*) and remove the hose clamp from the corresponding rubber tube (*E.6*) of the medium bottle (*E*).
    1. Open the valve of the gas pack (*gp*) connected to the discharge bottles *(G*) to allow air to be released into the gas pack while the discharge bottles fill up with outflow medium.

18. Start the pump and monitor the medium distribution panel (see (*F*)) filling up with medium. Make sure to remove the remaining gas in the panel as it fills up by holding it in an inverted position. Gas is released through the capillaries that are connected to the pump.
19. Monitor the column as it fills with medium, and adjust the pump to a proper pumping rate of 0.45 L/day, which can be converted to a vertical flux of 10 mM Fe(II)/m^2/d, in order to simulate the chemical flux of interest.
20. Install the light source (*L*) 2 cm above the upper end of the column and cover the upper 10 cm around the column with a dark tape and/or aluminum foil in order to prevent the light from radiating out of the top of the column and illuminating the lower part of the column. Cover the whole installation with a dark textile cover in order to prevent illumination of the column from external light sources.

6. Inoculation of Bacteria into the Column

Note: For an abiotic control experiment this step is skipped.

1. Since the cell culture will be directly injected into the column through the butyl rubber stoppers of the main sampling ports along the side of the column, make sure to have prepared six syringes with needles that are long enough to reach the center of the column body.
2. Sterilize the outside of butyl rubber stoppers of the six main sampling ports at the column (*A.3*) with an EtOH solution (80%).
3. Have the serum bottle with the culture for inoculation ready (prepared in section 2) and sterilize the butyl rubber stopper by flaming several drops of EtOH solution. Flush the syringe with sterile N_2/CO_2 before taking an aliquot of the culture. Take 1 ml of the anoxic cell solution and inject it into the center of the column through the butyl rubber stoppers of the main sampling ports (*A.3*).
   Note: Depending on the bacterial growth, it may be necessary to adjust the pumping rate (or even stop pumping) during the first days of the lag-phase for cell growth and development to prevent the cultures from being washed out.

7 . Sampling

Note: In order to collect samples across the chemical gradients that develop inside the column, it is necessary to start sampling from the top sampling ports prior to the deeper ports, as volume loss occurs. Make sure to maintain sterile conditions (*e.g.,* by working within 40 cm of a Bunsen burner or under a laminar-flow hood).

1. Collect the first samples 24 hr after inoculation. Flush the syringe that will be used for sampling with sterile N_2/CO_2, in order to avoid oxygen injection into the sampling ports (*D*). Make sure to fill the syringe with N_2/CO_2 before sampling.
2. Quickly remove the plastic cap (*D.6*) and insert the sampling syringe into the tube connector (*D.5*). Maintain a small gap between syringe and tube connector. Release the gas from the syringe and flush the tube connector with N_2/CO_2.
    1. Firmly connect the syringe to the tube connector. Remove the clamp (*D.4*) and start taking a sample of about 1 ml.

3. After drawing the sample and before removing the syringe, attach the clamp (*D.4*). Then remove the sampling syringe and firmly close the tube connector (*D.5*) with the corresponding plastic cap (*D.6*).
4. Immediately repeat steps 7.1-7.3 for sampling from every port.
5. Collect the next set of samples every 24 hr.
   Note: For additional samples at different time steps, it is necessary to remove a small amount of sample (*e.g.*, 0.2-0.4 ml) initially before the sample can be taken, in order to remove residual media inside the sampling tube and to achieve representative samples from inside the column.

8. Methods of Analysis

1. **Oxygen quantification:**
   Note: The oxygen concentration within the flow-through medium and the headspace of the column is quantified non-invasively and non-destructively using the optical oxygen sensors and oxygen sensitive fluorescent foil patches of 0.5 x 0.5 cm (so called optodes), glued along the inner glass wall of the column using silicon glue (*A.6*). It was ensured that the oxygen sensitive foil patches are insensitive for Fe species in order to achieve reliable readings.

1. Make sure to calibrate the computer software with the appropriate calibration parameters for the optodes that are used in the column. The optical oxygen sensors come with a specific calibration for the measurement using a corresponding PC-controlled fiber optic oxygen meter.
2. Start the measurement and take care to hold the polymer optical fiber of the oxygen meter at a right angle to the oxygen sensitive foil that is glued inside the transparent glass wall of the column.
3. Repeat the measurement for every single O_2 measuring point throughout the column.

2. **Fe(II) and total Fe analysis of aqueous samples:**
   1. Since Fe(II) is rapidly oxidized by oxygen in air at neutral pH, stabilize the liquid samples for aqueous Fe(II) quantification immediately in 1 M HCl solution. For a final sample volume of 1 ml mix 0.5 ml liquid sample with 0.5 ml 2 M HCl.
   2. Quantify total Fe after incubating an aliquot of the 1 M HCl-stabilized sample with hydroxylamine hydrochloride (10% wt/v, in 1 M HCl) for 30 min. This reagent reduces all Fe(III) to Fe(II), which can then be quantified via the Ferrozine assay[21].
   3. Perform a Ferrozine Assay using a micro-titer plate reader. Measure the absorbance at a wavelength of 562 nm. Make sure to have standards within the range for detectable Fe(II) and total Fe concentrations.
      Note: If Fe concentrations in the liquid sample exceed standard calibrations, it is necessary to dilute the stabilized sample with 1 M HCl.
   4. Calculate the concentration of Fe(III) by the difference between total Fe and Fe(II).

Representative Results

Control experiment

Abiotic control experiments (10 days) demonstrated consistently low oxygen concentrations (O_2 <0.15 mg/L) with no significant fluctuations in the Fe(II)-profile throughout the upwelling water column. The formation of precipitates (presumably Fe(III)(oxyhydr-)oxides) in the medium reservoir and the slight decrease in the overall Fe(II) concentration from 500 µM to 440 µM over 10 days indicate some oxygen diffusion through connections made of rubber (*e.g.*, *E.6; gp.1* in **Figure 1**)[22]. For this experiment, the lowest oxygen concentrations that were reasonably achievable were ≤0.15 mg/L and is in the range for a sensitive oxygen quantification and above the detection limit of 0.03 mg/L. Oxygen values below 0.15 mg/L are for the remainder of this paper referred to as "anoxic".

Biotic experiment

Visible parameters, cell growth, and changes in the water column

Prior to inoculation on day 0 no precipitates were visible (**Figure 2A**). This indicated that the column was properly setup and that no oxygen was present (compare **Figure 3A**) that could lead to the oxidation of Fe(II) and the formation of Fe(III) precipitates. As a result, the Fe(II) concentration was constant throughout the upwelling water column as it is shown in the profile in **Figure 4A**. **Figure 2A** shows that the light gradient was narrowed to the upper 6 cm within the water column by using the glass beads matrix in the column cylinder.

The green color within the top 2.0 cm of the water column 84 hr after inoculation indicates the growth of the cyanobacteria (**Figure 2B**). The notable light orange band at a depth of -3 cm (highlighted by the arrow in **Figure 2B**) below the green band is due to the Fe-precipitates that formed during Fe(II) oxidation by molecular oxygen, produced by the cyanobacteria. Similar precipitates were also visible on surface of the water column. Light orange foam formed at the water column surface 84 hr after inoculation (**Figure 2B**) indicating the production of O_2 by cyanobacteria. The precipitates on the surface of the water column presumably formed due to the oxygen that is outgassing at the surface. Residual Fe(II) was eventually oxidized at the surface and formed precipitates on the glass bead matrix.

Oxygen gradient

Prior to inoculation on day 0, the initial O_2 concentration in the liquid medium was determined. **Figure 3A** clearly shows that the concentration for O_2 throughout the whole water column was consistently below the concentration present in the control experiment. The pre-inoculation O_2-concentration never exceeded values of 0.13 mg/L O_2($O_{2\,mean}$ = 0.099 ± 0.002 mg/L). This indicates that the column was anoxic prior to inoculation.

Figure 3B shows an increase of the O_2 concentration at 84 hr after inoculation with cyanobacteria. This, along with the visible green biomass (**Figure 2B**) are consistent with the photosynthetic production and the accumulation of O_2 in the column. The O_2 concentration after 84 hr achieved a maximum concentration for O_2 = 29.87 mg/L in a depth of -0.5 cm below the water column surface. The O_2 values in **Figure 3B** indicate that the O_2 levels were always above background concentration in the upper 8.5 cm within the water column (O_2 >0.15 mg/L). Noticeably high O_2 concentrations (>0.50 mg/L) were detected from -0.5 to -5.5 cm depth below the water column surface. Lower concentrations for O_2 ≤ 0.15 mg/L at depths below -10.5 cm, along with the lowest measured value for O_2 = 0.09 mg/L at a depth of -20.5 cm indicate that these areas were anoxic.

Fe(II) gradient

Figure 4A shows that the Fe(II) concentration on day 0, prior to inoculation with cyanobacteria, was constant throughout the water column with a mean concentration of $Fe(II)_{mean}$ = 282.6 ± 6.8 µM. The concentration in the medium reservoir on day 0 was $Fe(II)_{reservoir}$ = 320.4 ± 11.6 µM.

84 hr after inoculation with cyanobacteria the Fe(II) concentration decreased considerably in the upper 9 cm within the water column. **Figure 4B** shows a distinct Fe(II) gradient, where concentrations of Fe(II) decrease to the top surface of the water column. However, Fe(II) was still detectable at the surface of the water column. The lowest Fe(II) concentration detected was directly below the liquid medium surface at a depth of -0.9 cm. Fe(II) concentrations increased with depth from Fe(II) = 9.9 ± 2.8 µM at -0.9 cm to Fe(II) = 258.6 ± 3.1 µM at a depth of -8.9 cm, forming a steep positive linear Fe(II) gradient over depth ($[Fe(II)_d] = (d + 1.278) \cdot 0.031^{-1}$; d: depth (cm); $R^2 = 0.9694$) limited to the upper 6.8 cm. Areas in the liquid medium below -9 cm depth remain noticeably constant and show no significant decrease in their concentrations for Fe(II) compared to their initial values for Fe(II) on Day 0 (T-test; $p > 0.05$).

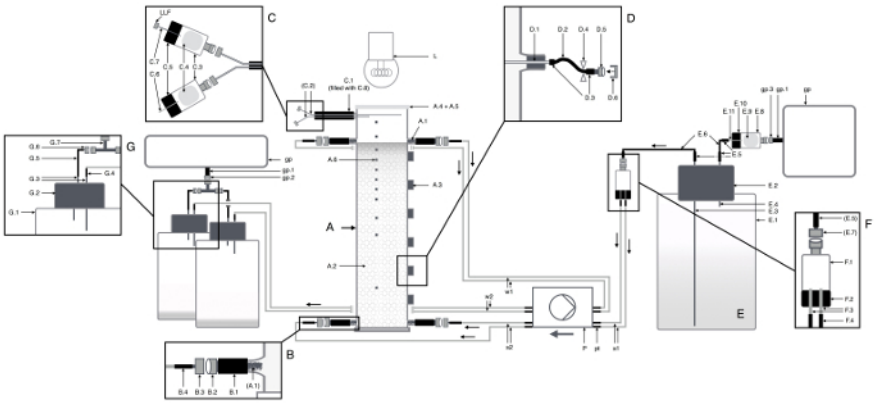

Figure 1. Schematic experiment set up. Alphanumeric codes for items refer to parts listed in **Table 2**. Please click here to view a larger version of this figure.

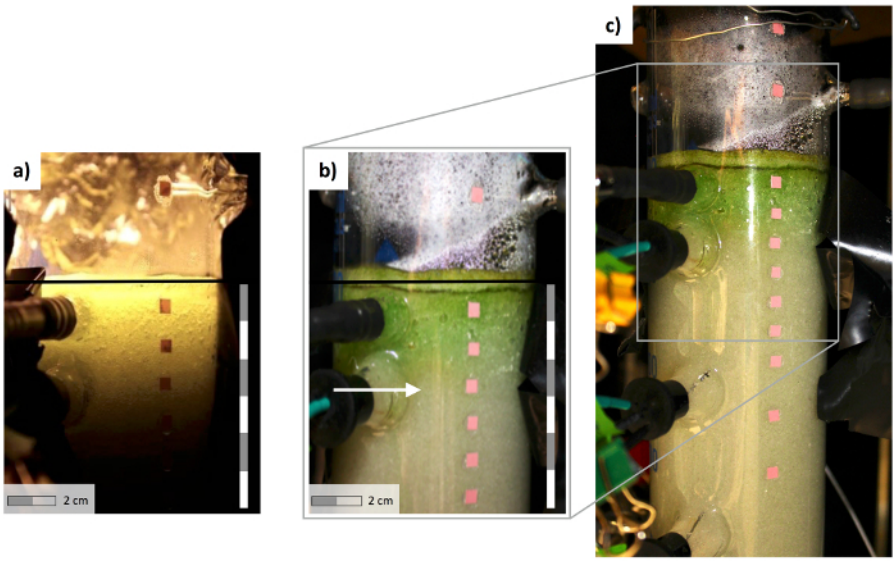

Figure 2. Visible changes in the glass bead matrix throughout the column cylinder before and 84 hr after inoculation with cyanobacteria. Pink squares are oxygen sensors. (**A**) Close up of the upper 6 cm in the column set up before inoculation. The liquid filled column showing a visible light gradient. The glass bead matrix narrows the visible light gradient to the upper 6 cm. (**B**) Close up of the upper 6 cm 84 hr after inoculation. Green indicates visible biomass, denser in the top of the column where light intensity is highest. The arrow points to a faintly visible orange band, which resulted from the formation of Fe(III) precipitates due to Fe(II) oxidation by molecular O_2 produced by the cyanobacteria. Faintly visible orange foam on top of the water column surface indicates Fe(III) is also precipitating there. O_2 outgassing through the surface causes foaming of the Fe(III) precipitates. (**C**) Overview of filled column cylinder. Visible growth of cyanobacteria is limited to the upper 4 cm due to limited light availability with depth. Please click here to view a larger version of this figure.

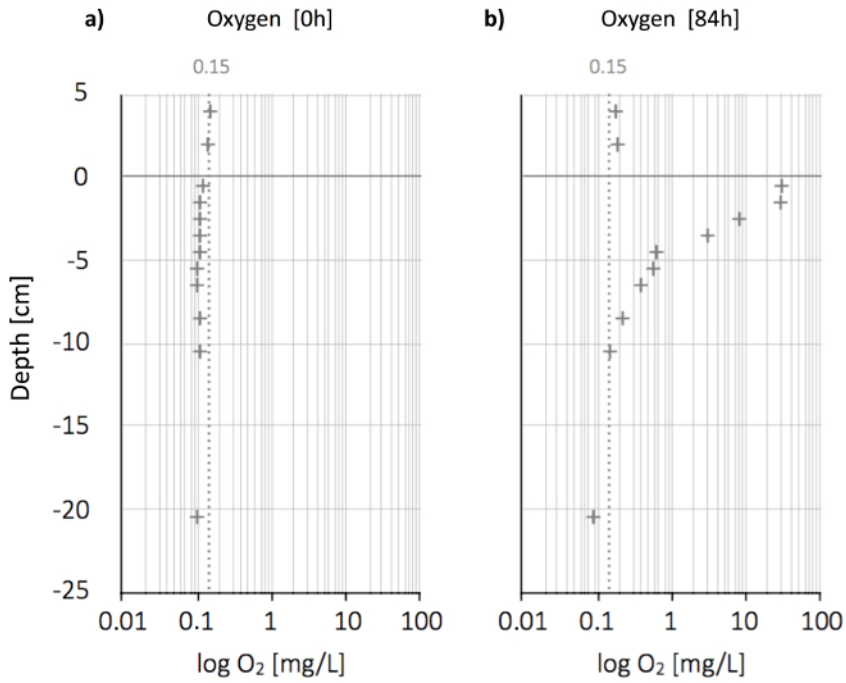

Figure 3. Oxygen profile within the water column before and 84 hr after inoculation with cyanobacteria. Zero cm for depth on the y-axis indicates the water column surface. Positive values for depths refer to the headspace above the liquid medium level, whereas negative values represent depths within the water column. Note the logarithmic scale for O_2 concentrations on the x-axis. The vertical dashed line indicates the threshold for anoxic conditions ($O_2 \leq 0.15$ mg/L). (**A**) Oxygen profile [0h] before inoculation. Values for O_2 were constantly below 0.13 mg/L throughout the water column. (**B**) Oxygen profile 84 hr after inoculation. O_2 was above 0.5 mg/L in the upper 5.5 cm of the water column. O_2 concentrations were higher than background concentrations (≥ 0.15 mg/L, dashed line) in areas above -8.5 cm depth. Deeper areas were anoxic with $O_2 \leq 0.15$ mg/L. Please click here to view a larger version of this figure.

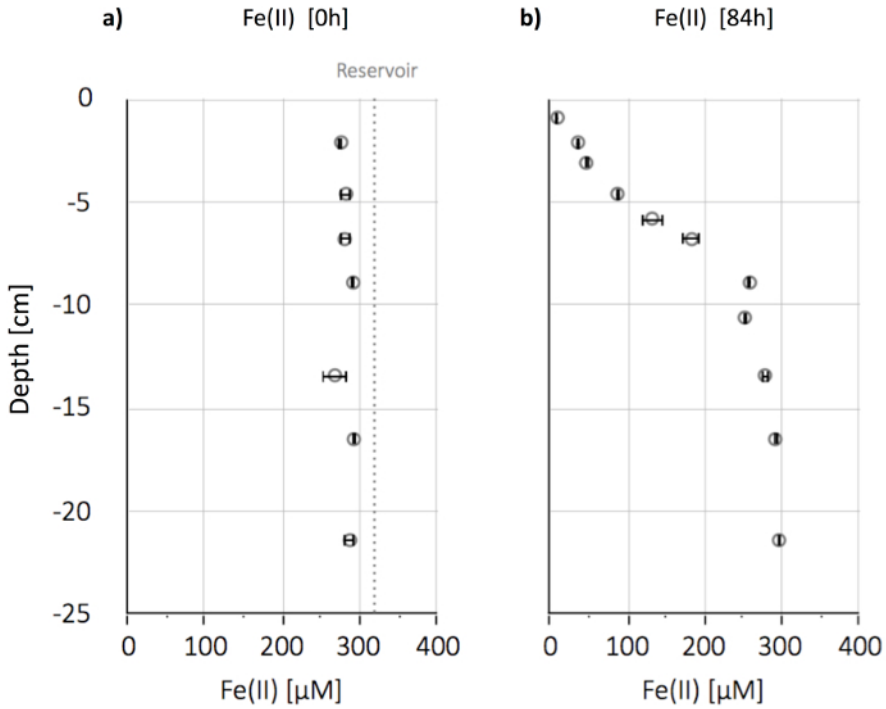

Figure 4. Fe(II) profile within the water column before and 84 hr after inoculation with cyanobacteria. Note: Error bars represent technical replicates deduced from triplicate measurements of one sample in the Ferrozine assay. (**A**) Fe(II) profile [0h] before inoculation. Values for Fe(II) were constant throughout the water column with a mean value for $Fe(II)_{mean}$ = 282.6 ± 6.8 µM. Variations in the Fe(II) profile result from single sample Fe(II) quantifications. Fe(II) quantification on sample triplicates would likely lead to less variation. (**B**) Fe(II) profile 84 hr after inoculation. Noticeably lower Fe(II) concentrations in the upper 6.8 cm within the water column. Fe(II) values below -8.9 cm depth show higher Fe(II) concentrations that do not significantly differ from initial Fe(II) values before inoculation with cyanobacteria (T-test; $p < 0.05$). Please click here to view a larger version of this figure.

Equipment	Quantity	Item description	Information details		
			Brand	**Order No.**	**Reference adress**
	1	Widdel flask (5 L)	Ochs	110015	labor-ochs.de
	2	Glass bottles (5 L)	Rotilabo	Y682.1	carlroth.com
	3	Glass pipettes (5 ml)		51714	labor-ochs.de
	1	0.22 µm Steritop filter unit (0.22 µm Polyethersulfone membrane)	Millipore	X337.1	carlroth.com
	0.5 m^2	Aluminum foil		–	
Supplies	-	N_2 - glovebox (100% N_2)		–	
	-	N_2/CO_2 - gas (90/10, v/v; 50 mbar)		–	
	1	Sterile Luer Lock glass syringe, filled with cotton		C681.1	carlroth.com
	1	Luer Lock stainless steel needles (150 mm, 1.0 mm ID)		201015	labor-ochs.de
Chemicals	4.8 L	MQ-water		–	
for 5 L medium solution	100 g	NaCl		433209	sigmaaldrich.com
	34 g	$MgSO_4$		208094	sigmaaldrich.com
	7.5 g	$CaCl_2$		C4901	sigmaaldrich.com
	1.25 g	NH_4Cl		A9434	sigmaaldrich.com
	0.34 g	KH_2PO_4		P5655	sigmaaldrich.com
	0.45 g	KBr		P3691	sigmaaldrich.com
	3.3 g	KCl		P9541	sigmaaldrich.com
	200 ml	Anoxic Na_2HCO_3-buffer solution (22 mM)		–	
	15 mg	Selenium and tungstate solution (comp. Wu *et al.*, 2014)		–	
	5 ml	$Na_2S_2O_3$ solution (1 M)		–	
	2.5 ml	Marine Phototroph (MP) vitamin solution (comp. Wu *et al.*, 2014)		–	
	5 ml	MP trace element solution (comp. Wu *et al.*, 2014)		–	
Reference					
Wu, W., Swanner, E. D., Hao, L. K., Zeitvogel, F., Obst, M., Pan, Y. X., & Kappler, A. (2014). Characterization of the physiology and cell-mineral interactions of the marine anoxygenic phototrophic Fe(II) oxidizer Rhodovulum iodosum - implications for Precambrian Fe(II) oxidation. Fems Microbiology Ecology, 88(3), 503-515.					

Table 1. Medium Preparation. Equipment list, supplies and chemicals for the preparation of culture medium.

	Qty.	Ref.	Item description	Information details			
for	**1**	**(A)**	**Glass cylinder**		Y310.1	carlroth.com	* custom modified by glass manufacturing facility
	2 g	(A.1)	Glass wool		7377.2	carlroth.com	
	1.03 L	(A.2)	Glass beads (ø 0.55 - 0.7 mm)		11079105	biospec.com	
	6	(A.3)	Butyl rubber stopper (ø 1.2 cm)		271024	labor-ochs.de	
	1	(A.4)	Petri Dish, glass (ø 8.0 cm)		T939.1	carlroth.com	
	40 ml	(A.5)	Polymers glue		OTTOSEAL S68	adchem.de	
	11	(A.6)	Optical oxygen sensor foil (for oxygen analysis, see below)		– on request –	presens.de	
for	**4**	**(B)**	**Medium Glands**				
	4	(B.1)	Rubber tubing (35 mm, 7 mm ID)		770350	labor-ochs.de	
	4	(B.2)	Luer Lock tube connector (3.0 mm, Luer lock male = LLM)		P343.1	carlroth.com	
	4	(B.3)	Luer Lock tube connector (3.0 mm, Luer lock female = LLF)		P335.1	carlroth.com	
	4	(B.4)	Rubber tubing (25 mm, 0.72 mm ID)		2600185	newageindustries.com	
for	**1**	**(C)**	**Headspace Gas Exchange Panel**				
	1	(C.1)	Rubber tubing (50 mm, 7 mm ID)		770350	labor-ochs.de	
	2	(C.2)	Luer Lock stainless steel needle (150 mm, 1.0 mm ID)		201015	labor-ochs.de	
	2	(C.3)	Luer Lock glass syringe (10 ml)		C680.1	carlroth.com	
	2 g	(C.4)	Loose cotton		–		
	2	(C.5)	Butyl rubber stopper (ø 1.75 cm)		271050	labor-ochs.de	
	1	(C.6)	Stainless steel needle (40 mm, 1.0 mm ID)	Sterican	4665120	bbraun.de	
	1	(C.7)	Luer Lock stainless steel needle (150 mm, 1.5 mm ID)		201520	labor-ochs.de	

		(LLF)	position: Luer Lock female connector part at C.7				
	10 ml	(C.8)	Polymers glue		OTTOSEAL S68	adchem.de	
for	**1**	**(D)**	**Sampling Port**				
	1	(D.1)	Stainless steel needle (120 mm, 0.7 mm ID)	Sterican	4665643	bbraun.de	
	1	(D.2)	Rubber tubing (40 mm, 0.74 mm ID)		2600185	newageindustries.com	
	2	(D.3)	Heat shrink tubing (35 mm, 3 mm ID shrunk)		541458 - 62	conrad.de	
	1	(D.4)	Tube clamp		STHC-C-500-4	tekproducts.com	
	1	(D.5)	Luer Lock tube connector (1.0 mm, LLF)		P334.1	carlroth.com	
	1	(D.6)	Luer Lock plastic cap (LLM)		CT69.1	carlroth.com	
for	**1**	**(E)**	**Medium bottle**				
	1	(E.1)	Glass bottle (5 L)	Rotilabo	Y682.1	carlroth.com	
	1	(E.2)	Butyl rubber stopper (for GL45)		444704	labor-ochs.de	
	1	(E.3)	Stainless steel capillary (300 mm, 0.74 mm ID)		56736	sigmaaldrich.com	
	1	(E.4)	Stainless steel capillary (50 mm, 0.74 mm ID)		56737	sigmaaldrich.com	
	4	(E.5)	Shrink tubing (35 mm, 3 mm ID shrunk)		541458 - 62	conrad.de	
	2	(E.6)	Rubber tubing (100 mm, 0.74 mm ID)		2600185	newageindustries.com	
	1	(E.7)	Luer Lock tube connector (1.0 mm, LLF)		P334.1	carlroth.com	
	1	(E.8)	Luer Lock glass syringe (10 ml)		C680.1	carlroth.com	
	1 g	(E.9)	Loose cotton		–		
	1	(E.10)	Butyl rubber stopper (ø 1.75 cm)		271050	labor-ochs.de	
	1	(E.11)	Stainless Steel needle (40 mm, 0.8 mm ID)	Sterican	4657519	bbraun.de	
for	**1**	**(F)**	**Medium distribution panel**				
	1	(F.1)	Luer Lock glass syringe (5 ml)		C679.1	carlroth.com	

	1	(F.2)	Butyl rubber stopper (ø 1.75 mm)		271050	labor-ochs.de	
	2	(F.3)	Stainless steel needle (40 mm, 0.8 mm ID)	Sterican	4657519	bbraun.de	
	2	(F.4)	Rubber tubing (40 mm, 0.74 mm ID)		2600185	newageindustries.com	
for	2	(G)	Discharge bottles				
	2	(G.1)	Glass bottle (2 L)	Rotilabo	X716.1	carlroth.com	
	2	(G.2)	Butyl rubber stopper (for GL45)		444704	labor-ochs.de	
	4	(G.3)	Stainless steel capillary (50 mm, 0.74 mm ID)		56736	sigmaaldrich.com	
	2	(G.4)	Rubber tubing (30 mm x 0.74 mm ID)		2600185	newageindustries.com	
	2	(G.5)	Rubber tubing (100 mm x 0.74 mm ID)		2600185	newageindustries.com	
	2	(G.6)	Luer Lock tube connector (1.0 mm, LLF)		P334.1	carlroth.com	
	1	(G.7)	Luer Lock 3-way connector (LLF, 2x LLM)		6134	cadenceinc.com	
additional equipment							
	1	(L)	Light source	Samsung	SI-P8V151DB1US	samsung.com	
	1	(P)	Peristaltic pump	Ismatec	EW-78017-35	coleparmer.com	
	4	(pt)	Pumping tubing (0.89 mm ID)		EW-97628-26	coleparmer.com	
	4	(s1/2)	Stainless steel capillary (200 mm, 0.74 mm ID)		56736	sigmaaldrich.com	
	4	(w3/4)	Stainless steel capillary (400 mm, 0.74 mm ID)		56737	sigmaaldrich.com	
	2	(gp)	Supel-Inert Foil (Tedlar - PFC) gas pack (10 L)		30240-U	sigmaaldrich.com	
with	2	(gp.1)	Rubber tube (30 mm, 6 mm ID)		770300	labor-ochs.de	
	1	(gp.2)	Luer Lock tube connector (3.0 mm, LLM)		P343.1	carlroth.com	
	1	(gp.3)	Luer Lock tube connector (3.0 mm, LLF)		P335.1	carlroth.com	

Supplies	2	-	N_2/CO_2 - gas line (90/10, v/v; 50 mbar)		–		
	2	-	Gas-tight syringe (20 ml)		C681.1	carlroth.com	
	1	-	Bunsen burner		–		
	1	-	Fiber optic oxygen meter for oxygen quantification	Presens	TR-FB-10-01	presens.de	
	1	-	Vacuum pump		–		
	1	-	Silicone glue for oxygen optodes	Presens	PS1	presens.de	
– : items marked with a dash (–) are generally available and not a specific item							

Table 2. Column set-up. Quantities, alphanumeric reference numbers and item descriptions of equipment for experimental set-up.

Discussion

Microbial communities in the Precambrian ocean were regulated by, or modified as a result of, their activity and the prevailing geochemical conditions. In interpreting the origins of BIF, researchers generally infer the presence or activity of microorganisms based on the sedimentology or geochemistry of BIF, *e.g.*, Smith *et al.*[23] and Johnson *et al.*[24]. The study of modern organisms in modern environments that have geochemical analogs to ancient environments is also a valuable approach, *e.g.,* Crowe *et al.*[11] and Koeksoy *et al.*[14]. A third approach is utilizing organisms in engineered laboratory systems that simulate processes taking place in the Precambrian ocean, *e.g.,* Krepski *et al.*[25]. This type of approach is useful to test specific hypotheses, and remove chemical or biological factors that might be present in modern systems, but were not part of the Precambrian ocean (*e.g.,* aquatic plants and animals). We therefore present a proof-of-concept method for a dynamic, laboratory upwelling system, in which the activity of (cyano-)bacteria and their influence on resulting geochemical profiles can be assessed under controlled laboratory conditions. Our column can be used to test hypotheses about the organisms and processes contributing to the deposition of BIF, and the biosignatures retained in BIF.

We optimized the protocol for the column setup so that assembly is comprehensible and easily conductible. However some steps in the protocol need to be addressed carefully and ideally conducted with the help of an assisting person. In particular, the connection of the medium bottles to the column cylinder needs to be performed quickly in order to avoid contamination of the medium solution with oxygen. The use of nonsterile equipment or working under nonsterile laboratory conditions will result in the contamination of the experiment and unreliable results. Therefore it is an absolute necessity to sterilize the equipment and maintain sterile conditions (working in a laminar flow hood or 40 cm next to a Bunsen burner) while setting up the experiment and collecting samples. In addition, some physical-chemical parameters of the column-material caused chemical changes over the long-time set-up in the column experiment. Parts that are made of rubber tubing seem to have a diffusion coefficient for oxygen that is high enough to significantly affect the medium reservoir bottle and lead to oxidation of Fe(II) and mineral precipitation in the medium solution. The abiotic consumption of >10% Fe(II) due to precipitation during the abiotic experiment over 10 days (compare: **REPRESENTATIVE RESULTS**) needs to be taken into account for future long-term experiments. The light source that was used in the current study created a downwelling light gradient within the upper 6 cm of the column. The light spectra covered the photosynthetic active wavelengths of chlorophyll a and b in *Synechococcus* and allowed growth and photosynthetic activity. In fact, the source of light is one of the most important parameters regarding phototrophic organisms since both, light quality and quantity can highly influence phototrophic bacteria[11,13]. Variations of wavelengths and spectral ranges, also considering the higher UV radiation during the Precambrian, may further allow insights into light dependent biogeochemical reactions. During light incubation experiments, we noticed that light was conducted through the glass wall of the column, emitting light through the sampling ports and at the bottom of the column. For future experiments, the glass at the top of the column should be replaced by non-light-conducting glassware. The light gradient must be measured in a mock set-up, as there was no easy and inexpensive way of measuring the light gradient within the closed column system available. We assume a significant change over time in the maximum penetration depth of light due to absorption of light by cells and minerals. Measuring the light gradient *in situ* during the experiment will be of interest for future experiments. The use of light-scattering beads in the glass beads matrix and the quantification of scattered light from the outside might be a possibility to quantify the relative light availability in certain depths over time. A further improvement would include a cover that does not need to be glued, but could be easily attached and removed with a flange and encircling clamp. A 4-point media supply and discharge port would result in a more homogenous flow field within the column. Narrower positioning of the main sampling ports for liquid samples would result in a higher resolution of sampling of the biological and geochemical gradients within the column.

Nevertheless, the first results demonstrated that the vertical flow-through column can be regarded as an appropriate experimental set-up to investigate microbial processes and geochemical changes in an upwelling system. We contend that this column serves as a prototype to prove the overall functionality of the system. Further, our results validate widely-held assumptions, modeling results, and inferences from sedimentary geochemistry that a chemocline between oxygen and Fe(II) results if cyanobacteria are present in an Fe(II)-rich upwelling system[20]. The anoxic conditions prior to inoculation reflect a Precambrian ocean before colonization by cyanobacteria or organisms capable of oxygenic photosynthesis. With the rise of the oxygen in surface waters, upwelling Fe(II) becomes oxidized and precipitates as Fe(III) minerals, such as occurred during deposition of BIF[26] .The establishment of a chemocline and the mineral formation can be evaluated to extrapolate geochemical processes into larger scale environments. However for upscaling the evaluated results to natural (ancient) environments, additional physical processes need to be considered. Advective lateral transport, for instance, might disturb the establishment of a chemocline, same as wind-induced turbulences in the surface waters.

The extraction of liquid samples from the water column for Fe(II), total Fe measurements, and the non-invasive O_2 quantification were able to track the evolution of a reaction front between these chemical species in a simple, fast, and reliable way. The low Fe(III) concentration in samples taken from the column set up in abiotic control experiments clearly indicate that although some oxidation occurred in the media bottle, the column itself was closed hermetically to external O_2 influx. Furthermore, these results indicate that our sampling protocol maintained anoxic samples for Fe(II) quantification. Changes in pH were not recorded during the column experiment and may have a dominant effect on Fe-speciation. However, the current flow-through system was buffered by 22 mM $NaHCO_3$ that is in equilibrium with the anoxic N_2/CO_2 atmosphere in the headspace and allows to maintain a circum-neutral pH for at least 84 hr. Nevertheless, the *in-situ* quantification of the pH may be an important parameter to fully understand geochemical processes in potential long-run experiments and the extrapolation to (ancient) open ocean systems. The glass bead matrix, used to stabilize the establishing geochemical gradients in the column cylinder, led to an accumulation of Fe-precipitates in the subsurface of the water column. We hypothesize that the accumulated precipitates do not have a dominant effect on our 84 hr experiment. However, degrading biomass might induce redox processes on Fe-precipitates that result in Fe cycling. This needs to be considered regarding potential long-run (<84 hr) experiments. In fact, light induced Fe-redox cycling and a release of Fe to the ferrous iron pool could be observed and quantified in replicate long-term (21 days) experiments (Wu, W., Maisch, M., Kappler, A., Pan, Y., & Swanner, E. D. Photochemical Fe(III) reduction stabilized Fe(II) in Archean oxygen oases. *Geology.* (in prep.)).

Future column experiments will incorporate both various microorganisms and variations in the culture medium composition. This allows simulation of diverse environmental conditions that are representative for different stages during the transformation of the Precambrian Ocean. For instance, silica could be added to the medium to simulate the concentrations of 0.67 to 2.2 mM that were present in Precambrian seawater[27]. Furthermore the concentration of sulphate in the medium solution could be changed to address variations in the composition of Precambrian seawater. Variations of the culturing medium will likely influence the physiology and effect of microorganisms on the geochemical patterns in the water column[19] that the current column setup allows us to investigate *in situ*. In addition to that, anticipated experiments will involve more complex microbial communities such as phototrophic Fe(II)-oxidizing bacteria (*e.g.,* Kappler *et al.*)[28], microaerophilic Fe(II)-oxidizing bacteria (*e.g.*, Krepski *et al.*)[29] and cyanobacteria. The column experiments will help to tease apart the individual contribution of these microbial processes to deposition of the Banded Iron Formations. However for the interpretation and extrapolation to ancient (and modern) environments needs to be derived very carefully. The microbial habitat that is simulated in the current study models only the basic features of a potential Precambrian upwelling ocean water column: vertical Fe(II) fluxes, a photic zone light gradient, anoxic atmosphere and cyanobacteria. In addition, the conditions in the artificial upwelling system potentially favor the growth of cyanobacteria, due to constant temperatures and 24 hr light conditions potentially leading to higher O_2 production rates, whereas the elevated O_2 concentrations subsequently leads to higher Fe(II) oxidation rates. Therefore the present study may not be interpreted as one experiment-fits-all hypotheses concerning BIF origin.

Nevertheless, the setup allows the *in-situ* investigation of various geochemical processes and the variation and simulation of certain boundary conditions (light availability, medium composition, fluxes). The quantification of single parameters and geochemical interactions under lab-controlled conditions may give insights into ancient and modern environments. Furthermore, the column system allows us to test hypotheses about how the geochemical conditions regulated microbial activity. For instance, it has been hypothesized that high Fe(II) concentrations in Precambrian upwelling systems may have limited photosynthetic oxygen production due to the toxicity of Fe(II) in sunlit, oxygenated environments[20]. Future investigations will additionally incorporate chemical fluxes and volumetric rates that allow qualitative and quantitative stoichiometric calculations of reaction kinetics in the artificial water column. Single observations will then be linked to evaluate a model for individual environmental simulations. With the column set up, we are now able to investigate the direct stress response of (cyano-)bacteria to fluxes of high Fe(II) and light in an *in-situ* upwelling system that represents marine Early Earth conditions[20]. The column can also be used to test hypotheses regarding the geochemical signatures produced by microbial activity, for instance the evolution of Fe isotope compositions along an upwelling system where Fe(II) is being oxidized (*e.g.,* Czaja *et al.*)[30]. In addition, the glass beads that stabilize the chemical gradients within the column could be replaced with sand or sediments. It is therefore also possible to apply this column for simulations of the geochemical gradients that might develop in marine or freshwater sediments inhabited by microorganisms (*e.g.,* Melton *et al.*)[31].

Disclosures

Authors have nothing to disclose.

Acknowledgements

Mark Nordhoff assisted in the design and implementation of tubing connections. Ellen Struve helped to select and acquire equipment used.

References

1. Lyons, T. W., Reinhard, C. T., & Planavsky, N. J. The rise of oxygen in Earth's early ocean and atmosphere. *Nature*. **506** (7488), 307-315 (2014).
2. Raymond, J., & Blankenship, R. E. The origin of the oxygen-evolving complex. *Coord. Chem. Rev*. **252** (3-4), 377-383 (2008).
3. Kendall, B., Reinhard, C. T., Lyons, T., Kaufman, A. J., Poulton, S. W., & Anbar, A. D. Pervasive oxygenation along late Archaean ocean margins. *Nature Geosci*. **3** (9), 647-652 (2010).
4. Olson, S. L., Kump, L. R., & Kasting, J. F. Quantifying the areal extent and dissolved oxygen concentrations of Archean oxygen oases. *Chem. Geol*. **362** (1), 35-43 (2013).
5. Satkoski, A. M., Beukes, N. J., Li, W., Beard, B. L., & Johnson, C. M. A redox-stratified ocean 3.2 billion years ago. *Earth Planet. Sci. Lett*. **430** (1), 43-53 (2015).
6. Holland, H. D. Oceans - Possible Source of Iron in Iron-Formations. *Econ. Geol*. **68** (7), 1169-1172 (1973).
7. Holland, H. D., Lazar, B., & Mccaffrey, M. Evolution of the Atmosphere and Oceans. *Nature*. **320** (6057), 27-33 (1986).
8. Klein, C., & Beukes, N. J. Time distribution, stratigraphy, and sedimentologic setting, and geochemistry of Precambrian iron-formations, In: Schopf, J.W., and Klein, C., eds., *The Proterozoic Biosphere.* Cambridge University Press, 139-146, (1992).

9. Poulton, S. W., & Canfield, D. E. Ferruginous Conditions: A Dominant Feature of the Ocean through Earth's History. *Elements*. **7** (2), 107-112 (2011).
10. Busigny, V. *et al.*, Iron isotopes in an Archean ocean analogue. *Geochim. Cosmochim. Acta*. **133**, 443-462 (2014).
11. Crowe, S. A. *et al.*, Photoferrotrophs thrive in an Archean Ocean analogue. *PNAS*. **105** (41), 15938-15943 (2008).
12. Jones, C. *et al.*, Biogeochemistry of manganese in ferruginous Lake Matano, Indonesia. *Biogeosciences*. **8** (10), 2977-2991 (2011).
13. Lliros, M. *et al.*, Pelagic photoferrotrophy and iron cycling in a modern ferruginous basin. *Sci. Rep.* **5** (13803), (2015).
14. Koeksoy, E., Halama, M., Konhauser, K. O., & Kappler, A. Using modern ferruginous habitats to interpret Precambrian banded iron formation deposition. *Int. J. Astrobiol.* **1-13**, (2015).
15. Canfield, D. E. A new model for Proterozoic ocean chemistry. *Nature*. **396** (6710), 450-453 (1998).
16. Wu, W. F. *et al.*, Characterization of the physiology and cell-mineral interactions of the marine anoxygenic phototrophic Fe(II) oxidizer Rhodovulum iodosum - implications for Precambrian Fe(II) oxidation. FEMS Microbiol. *Ecol*. **88** (3), 503-515 (2014).
17. Hungate, R. E., & Macy, J. The Roll-Tube Method for Cultivation of Strict Anaerobes. *Bull. Ecol. Res. Comm*. **17** (1), 123-126 (1973).
18. Van Baalen, C. Studies on marine blue-green algae. *Bot. mar*. **4** (1-2), 129-139 (1962).
19. Sakamoto, T., & Bryant, D. A. Growth at low temperature causes nitrogen limitation in the cyanobacterium Synechococcus sp. PCC 7002. *Arch. Microbiol*. **169** (1), 10-19 (1998).
20. Swanner, E. D., Mloszewska, A. M., Cirpka, O. A., Schoenberg, R., Konhauser, K. O., & Kappler, A. Modulation of oxygen production in Archaean oceans by episodes of Fe(II) toxicity. *Nature Geosci*. **8** (2), 126-130 (2015).
21. Stookey, L. L. Ferrozine - a New Spectrophotometric Reagent for Iron. *Anal. Chem*. **42** (7), 779-784 (1970).
22. Fitch, M. W., Koros, W. J., Nolen, R. L., & Carnes, J. R. Permeation of Several Gases through Elastomers, with Emphasis on the Deuterium Hydrogen Pair. *J. Appl. Polym. Sci*. **47** (6), 1033-1046 (1993).
23. Smith, A. J. B., Beukes, N. J., & Gutzmer, J. The Composition and Depositional Environments of Mesoarchean Iron Formations of the West Rand Group of the Witwatersrand Supergroup, South Africa. *Econ. Geol*. **108** (1), 111-134 (2013).
24. Johnson, C. M., Beard, B. L., Klein, C., Beukes, N. J., & Roden, E. E. Iron isotopes constrain biologic and abiologic processes in banded iron formation genesis. Geochim. *Cosmochim. Acta*. **72** (1), 151-169 (2008).
25. Krepski, S. T., Emerson, D., Hredzak-Showalter, P. L., Luther, G. W., & Chan, C. S. Morphology of biogenic iron oxides records microbial physiology and environmental conditions: toward interpreting iron microfossils. *Geobiology*. **11** (5), 457-471 (2013).
26. Posth, N. R., Konhauser, K. O., & Kappler, A. Microbiological processes in banded iron formation deposition. *Sedimentology*. **60** (7), 1733-1754 (2013).
27. Maliva, R. G., Knoll, A. H., & Simonson, B. M. Secular change in the Precambrian silica cycle: Insights from chert petrology. *Geol. Soc. Am. Bull*. **117** (7-8), 835-845 (2005).
28. Kappler, A., Pasquero, C., Konhauser, K. O., & Newman, D. K. Deposition of banded iron formations by anoxygenic phototrophic Fe(II)-oxidizing bacteria. *Geology*. **33** (11), 865-868 (2005).
29. Krepski, S. T., Hanson, T. E., & Chan, C. S. Isolation and characterization of a novel biomineral stalk-forming iron-oxidizing bacterium from a circumneutral groundwater seep. *Environ. Microbiol*. **14** (7), 1671-1680 (2012).
30. Czaja, A. D., Johnson, C. M., Beard, B. L., Roden, E. E., Li, W. Q., & Moorbath, S. Biological Fe oxidation controlled deposition of banded iron formation in the ca. 3770 Ma Isua Supracrustal Belt (West Greenland). *Earth. Planet. Sci. Lett*. **363** (1), 192-203 (2013).
31. Melton, E. D., Schmidt, C., & Kappler, A. Microbial iron(II) oxidation in littoral freshwater lake sediment: the potential for competition between phototrophic vs. nitrate-reducing iron(II)-oxidizers. *Front. Microbiol*. **3** (197), 1-12 (2012).

