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Resubmission of the manuscript "Inducing the Attachment of Cable Bacteria on Oxidizing Electrodes (bg-2019-334)"

Dear Editor,

Thank you for the opportunity to let us revise our manuscript: "**Inducing the Attachment of Cable Bacteria on Oxidizing Electrodes (bg-2019-334)**". We appreciate the reviewers' comments, encouragements, and suggestions to our manuscript. We believe that the revised manuscript is substantially improved after making edits according to reviewers' comments.

Following this letter are our point-to-point response to reviewers' comments including how and where our manuscript has been modified and a marked-up manuscript version showing the changes made in our manuscript.

Thank you for consideration of this manuscript for publication.

Sincerely,

Cheng Li, Ph.D.

Comments from reviewer #1	Authors response	Changes in manuscript
Can the cable bacteria exchange electrons with electrodes? The manuscript is well written and gives clear messages. The research is well done.	We thank the reviewer for such positive comments. Our experimental results showed that cable bacteria migrated out of sediments and attached to poised electrodes where they most likely contributed to measured currents, implying they can perform electron transfer to an electrode. However, since they were only part of a mixed-species biofilm we cannot say definitively that this experiment shows they can exchange electrons with electrodes. The complicated environment on the electrode surface (e.g. the deposited minerals and other types of electrode-associated bacteria) hindered such a certain conclusion.	This comment has given us a clear direction for the revision of our manuscript. We will revise the implication and conclusion sections to clearly state how the results from this study and other published research only indicate cable bacteria may exchange electrons with electrodes, and we will point out potential directions for upcoming experiments to observe definitively cable bacteria's electron transfer to electrodes. Line 359 to 390
However, I am wondering what controls are used? Such as is there any electrode without polarization to see if the cable bacteria still grow. I believe discussion of control would critically improve the manuscript. I am wondering, what would happen the shape of the profiles (DO, S2-, and pH) if the polarization was stopped. Even without these controls, the manuscript is critically important to advance our knowledge on cable bacteria. I believe, this manuscript will generate many new research questions.	In our experimental setup, one of the 3 electrodes inside of the anodic chamber was maintained at open circuit as a control electrode (as is stated in the section on Reactor Configuration and Operation). Scanning electron microscopy showed that the surface of this control electrode surface stayed relatively clean without any filamentous bacteria biomass or mineral deposition (Fig. 5i). We did conduct profiling with microelectrodes in the reactor after the anodes were poised but, in the process, broke our pH microelectrode. The profiles of O_2 and H_2S were predictable due to the imposed anoxia and the presence of the anodes as a high-area oxidizing surface: dissolved oxygen was below detection in the overlying seawater and sulfide concentrations were also below detection limit in overlying seawater but could be detected when the microelectrode entered sediment surface. We decided not to include this profile data because it was not very visual (zeros) and does not reveal what was happening on the electrode surface. When we dissembled the	We will also revise the Results and Discussion to give more information about microprofiling results in the closed reactors. Line 139 to 144 Line 272 to 276 Also see Fig. 2d

	reactor, seawater inside of the reactor had a pH near 6.2.	
Comments from reviewer #2	**Authors response**	**Changes in manuscript**
The study of Li et al. is a follow up study on the Reimers et al. (20xx) where cable bacteria attachments to the anodes in microbial fuel cells were reported. Li and coworkers aim to reproduce the observations by establishing a microbial fuel cell in the lab and then investigate if cable bacteria attach to the anode. After 135 days of incubation cable bacteria attachments were observed through SEM imaging and CARD FISH. In addition to the primary work Li and Co-workers report the presence of cable bacteria at their study site Yaquina Bay by means of pH, O2 and H2S profiling, SEM, FISH and 16sRNA analysis. In general, I think that the overall aim of this study is only loosely approached. The story goes in many directions and is not well focused: There are two lines one is the MCF line another is the report on cable bacteria in Yaquina Bay. If the primary aim was to investigate if cable bacteria can grow on anodes, why not tone the latter story line a bit more down to avoid confusions about the experimental goals?	We appreciate the reviewer's critical and insightful comments. Firstly, we agree with the reviewer that the aim of study needs to be made clearer by focusing on the reactor experiment rather that the broader characterizations of incubations from Yaquina Bay, Oregon. This part of the study was conducted to confirm that the sediment within Yaquina Bay can harbor a population of cable bacteria, and it will be toned down in revision.	Firstly, we will tone down presentation of the sediment core incubations from Yaquina Bay as suggested by the reviewer. In the revised manuscript, we will only present incubation and phylogenetic results from samples from the intertidal mudflat sediment which was used in the reactor incubation (eliminating the OFS site from figures and discussion). We will maintain the focus on determining if cable bacteria can grow on anodes and conditions in the anodic chamber that may trigger electrode attachment. Line 6 to 23 Line 72 to 77 Line 91 to 93 Line 204 to 260 (section 3.1) Line 359 to 390 Also see Fig. 2 and Fig. 3
It is drawback that the authors do not present quantitative estimates of cable bacteria density on the electrodes and that they only present SEM images. I think that it would be better and more convincing with FISH or molecular tools (qpcr) that allows for both identification and quantification of cable bacteria on the anodes and on the control electrodes. This could allow for a more robust comparison of the two types of systems and thus stronger	Secondly, we also agree with the reviewer that a quantitative estimation of cable bacteria density coupled with SEM images would ideally be useful for a convincing argument that cable bacteria can be attracted to an oxidizing electrode. However, in the present study the density was quite low and thus CARD-FISH along with morphological observation by SEM were necessary to confirm the presence of cable bacteria on the electrodes. The SEM method was considered	Secondly, additional quantitative analyses will not be performed as an addition to the present manuscript. Such analyses would not be fruitful since the cable bacteria density was low and affected by the mineral precipitation observed on the poised electrodes. No change was proposed

conclusions. The techniques were used in the sediment studies, why where they not applied on in the experiment?	key, since no other member in the family of *Desulfobulbaceae* forms filaments with ridges along their longitudinal axis. More important than showing a high density, at present stage of the study, we were trying to reconfirm that the cable bacteria can be drawn from sediments to grow on an oxidizing electrode and to deliver critical information about the conditions that trigger the attachment. Quantitative analyses such as qPCR will be employed in our next stage of experiment that utilizes electrochemical reactor without sediment to quantify the cable bacteria abundance on electrode.	
Some of the citations are incorrect e.g. Risgaard-Petersen et al. 2015 is cited for observations that cable bacteria can deplete iron sulfide, but this paper report the discovery of cable bacteria in freshwater sediment. Should be Risgaard-Petersen 2012. Bjerg et al. 2016 and Pfeffer et al. 2012 are cited to document that D. propionicus can transfer electrons to electrode and/or to insoluble Fe (III)-oxides. This was not shown in these papers, which are on cable bacteria motility and on the discovery of the cable bacteria.	Thirdly, we thank the reviewer for pointing out our mistakes when sorting references. We did mean to cite Bjerg et al. 2016 and Pfeffer et al. 2012 in our manuscript. We cited Holmes et al. 2004 to indicate that *D. propionicus* can utilize an electrode as an electron acceptor to oxidize S^0, H_2, and organic acids like pyruvate, lactate, and propionate.	Thirdly, we will carefully reexamine our referencing to make sure that each citation accurately represents preceding statements. We will also add in new references and revise the introduction to keep our information about the current cable bacteria research up-to-date. Suggested references have been revised and added. Line 62 to 63 Line 341 Line 406 to 408 Line 420 to 424 Line 478 to 479 We also added Meysman et al., 2019 and Kjeldsen et al., 2019. Line 62 to 63 Line 431 to 434 Line 464 to 468
Some statements are highly speculative and not supported by the presented data (l 293) “In summary, when growing on an electrode poised at an oxidative potential, cable bacteria may no longer require long filaments or be able to maintain them due to the nature of the potential gradient” There are no data in the study that can document such statement.	Lastly, the statement pointed out by the reviewer is speculative, though this statement was deduced from observations from our study and another study by Aller et al. 2019. We will make this statement less speculative in revision by only commenting on the observations of Aller et al.	Lastly, we will revise the speculative statement pointed out by the reviewer. We will integrate observations from our study with those presented by Aller et al. 2019 without further speculation. Line 329 to 335

Inducing the Attachment of Cable Bacteria on Oxidizing Electrodes

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Abstract. ~~The scope of~~Cable bacteria (CB) are multicellular, filamentous bacteria within the family of *Desulfobulbaceae* that transfer electrons longitudinally from cell to cell to couple sulfide oxidation and oxygen reduction in surficial aquatic sediments. In the present study~~ is to introduce,~~ electrochemical reactors that contain natural sediments are introduced as a tool for investigating the growth of ~~novel filamentous cable bacteria and their unique extracellular electron transfer ability. New evidence that cable bacteria are widely distributed in sediments throughout~~CB on electrodes poised at an ~~estuarine system connected to the NE Pacific Ocean is also presented. Cable bacteria found within~~ oxidizing potential. Our experiments utilized sediments from Yaquina Bay, Oregon, USA, ~~appear to~~and we include new phylogenetic analyses of separated filaments to confirm that CB from this marine location cluster with the genus, *Candidatus Electrothrix*. These CB may belong to a distinctive lineage, however, because their filaments contain smaller cells and a lower number of longitudinal ridges compared to cables described from other locales. Results of a 135-day bioelectrochemical reactor experiment ~~confirm a previous observation~~confirmed that ~~cable bacteria~~these CB can migrate out of reducing sediments and grow on oxidatively poised electrodes suspended in anaerobic seawater~~ above reducing sediments. However,~~. CB filaments and several ~~diverse~~other morphologies of *Desulfobulbaceae* ~~filaments,~~ cells~~, and colonies~~ were observed ~~on the carbon fibers of the suspended electrodes including encrusted chains of cells. These observations~~by scanning electron microscopy and fluorescence in situ hybridization on electrode surfaces, although in low densities and often obscured by mineral precipitation. These findings provide new information to suggest what conditions will induce ~~cable bacteria~~CB to perform electron donation to an electrode surface, further informing future experiments to culture ~~cable bacteria~~CB apart from a sediment matrix.

1 Introduction

Long distance electron transfer (LDET) is a mechanism used by certain microorganisms to generate energy through the transfer of electrons over distances greater than a cell-length. These microorganisms may pass electrons across dissolved redox shuttles, nanofiber-like cell appendages, outer-membrane cytochromes, and/or mineral nanoparticles to connect extracellular electron donors and acceptors (Li et al., 2017; Lovley, 2016). Recently, a novel type of LDET exhibited by filamentous bacteria in the family of *Desulfobulbaceae* was discovered in the uppermost centimeters of various aquatic, but mainly marine, sediments (Malkin et al., 2014; Pfeffer et al., 2012). These filamentous bacteria, also known as "cable bacteria (CB)", electrically connect two spatially separated redox half-reactions and generate

electrical current over distances that can extend to centimeters, which is an order of magnitude longer than previously recognized LDET distances (Meysman, 2017).

The unique ability of ~~cable bacteria~~CB to perform LDET creates a spatial separation of oxygen reduction in oxic surface layers of organic-rich sediment from sulfide oxidation in subsurface layers (Meysman, 2017)~~. The spatial separation of these two half-reactions also creates localized porewater pH extremes in oxic and sulfidic layers, which induces a series of secondary reactions that stimulate the geochemical cycling of elements such as iron, manganese, calcium, phosphorus, and nitrogen (Kessler et al., 2018; Rao et al., 2016; Seitaj et al., 2015; Sulu-Gambari et al., 2016b, 2016a). In addition to altering established perceptions of sedimentary biogeochemical cycling and microbial ecology~~. The spatial separation of these two half-reactions also creates localized porewater pH extremes in oxic and sulfidic layers, which induces a series of secondary reactions that stimulate the geochemical cycling of elements such as iron, manganese, calcium, phosphorus, and nitrogen (Kessler et al., 2018; Rao et al., 2016; Seitaj et al., 2015; Sulu-Gambari et al., 2016a, 2016b). In addition to altering established perceptions of sedimentary biogeochemical cycling and microbial ecology (Meysman, 2017; Nielsen and Risgaard-Petersen, 2015)~~, cable bacteria also possess intriguing structural features that may inspire new engineering applications in areas of bioenergy harvesting and biomaterial design (Lovley, 2016). Much is still unknown about the basic ability of cable bacteria to perform LDET. It has been suggested that when long filaments form, a chain of cells at the sulfidic terminal catalyzes anodic half reactions (½ $H_2S + 2H_2O \rightarrow ½\ SO_4^{2-} + 4e^- + 5H^+$) while a cathodic half reaction ($O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$) is catalyzed by cells at the oxic terminal. Electron transfer then occurs along the longitudinal ridges of cable bacteria filaments via electron hopping promoted by extracellular cytochromes positioned within a redox gradient (Bjerg et al., 2018; Meysman et al., 2015; Pfeffer et al., 2012). However, this hypothesis has not been directly verified and the electrical conductivity of cable bacteria filaments or their longitudinal ridges has not been measured. Furthermore, cable bacteria remain uncultured and difficult to grow outside of sediment, complicating efforts to study them using different techniques, such as electrochemical assays and metatranscriptomics.~~, CB also possess intriguing structural features that may inspire new engineering applications in areas of bioenergy harvesting and biomaterial design (Lovley, 2016; Meysman et al., 2019).

Much is still unknown about the basic mechanism(s) that CB use to perform LDET. It has been suggested that when long filaments form, a chain of cells at the sulfidic terminal catalyzes anodic half reactions (e.g., ½ $H_2S + 2H_2O \rightarrow ½\ SO_4^{2-} + 4e^- + 5H^+$) while a cathodic half reaction ($O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$) is completed by cells at the oxic terminal. Electron transfer then occurs along the longitudinal ridges of CB filaments via electron hopping promoted by extracellular cytochromes positioned within a redox gradient or via conductive electronic structures such as pili (Bjerg et al., 2018; Kjeldsen et al., 2019; Meysman et al., 2015, 2019; Pfeffer et al., 2012). These hypotheses await further verification, and CB remain uncultured and difficult to grow outside of sediment. This difficulty complicates efforts to study them using different techniques, such as electrochemical assays and metatranscriptomics.

In a previous benthic microbial fuel cell (BMFC) experiment in a marine estuary (Reimers et al., 2017), we serendipitously observed the attachment of ~~cable bacteria to carbon fibers serving as an anode in an anaerobic environment. This finding suggested that cable bacteria possess the ability to donate electrons to solid electron acceptors. While the understanding of cathodic potentials utilized by cable bacteria has been extended, further~~

~~investigation is still needed to study the conditions that trigger the attachment of cable bacteria to a poised electrode in the laboratory and to provide information about the ability of cable bacteria to use an electrode as an electron acceptor. In the present study~~CB to carbon fibers serving as an anode in an anaerobic environment above sediments. This finding suggested that CB possess the ability to donate electrons to solid electron acceptors, and it indicated a range of cathodic potentials favourable for electron transfer (Reimers et al., 2017). However further investigations are still needed to study the conditions that allow the attachment of CB to a poised electrode and to document electron transfer mechanisms at their cathodic terminus. In the present study, we first clarify the phylogenetic placement of CB found in sediments from Yaquina Bay, Oregon, where the BMFC was previously deployed. Then, we describe the design of a bioelectrochemical reactor configured to mimic the environment in the anodic chamber of a BMFC and verify conditions that can induce ~~cable bacteria~~CB attachment on electrodes. ~~As part of this research, we also clarify the phylogenetic placement of cable bacteria found in sediments from Yaquina Bay, Oregon.~~ Results assert that when oxygen is not available ~~these cable bacteria~~, CB can glide through ~~different redox layers~~sediments and seawater to an electrode poised at oxidative potentials. Thus, the present study provides new information about the chemotaxis of ~~cable bacteria~~CB in environments other than sediments, revealing key conditions for their attachment to surfaces and growth ~~of cable bacteria~~ in both natural and engineered environments.

2 Materials and Methods

2.1 Study Site and Sediment Collection

Several studies suggest that ~~cable bacteria~~CB may be found widely in coastal sediments possessing high rates of sulfide generation coupled with ~~high rates of~~ organic matter mineralization (Larsen and Nielsen, 2015; Malkin et al., 2014; Pfeffer et al., 2012). Therefore, to initiate this enquiry, sediment with these two characteristics was collected from Yaquina Bay, Oregon, USA using a hand shovel at a site on an intertidal mud flat (IMF, Latitude 44˚37’30 N, Longitude 124˚00’26 W~~), and secondly using a sediment grab to recover subtidal deposits from downstream of a commercial oyster farm (OFS, Latitude 44°34’37.0 N, Longitude 123°59’21.4 W) (Fig. 1). The IMF and OFS sites are located about 3 km and 7 km, respectively,~~). The IMF site is located about 3 km upstream from the site where the BMFC was deployed in the abovementioned study (Reimers et al., 2017). The top 20 cm of these sediments were sieved through a 0.5 mm mesh size metal screen to remove macrofauna and shell debris. Then the sieved sediments were allowed to settle and stored in sealed buckets in a cold room at 5˚C.

2.2 Sediment Incubation

To cultivate ~~cable bacteria~~CB of Yaquina Bay, IMF ~~and OFS sediments were each~~sediment was initially incubated for 60 days. These first incubations were started 2-5 days after collection and performed after homogenizing the sieved sediments under a flow of N_2 and then packing the sediment into triplicate polycarbonate tubes (15 cm height and 9.5 cm inner diameter). These cores were submerged in an aquarium containing aerated seawater collected from Yaquina Bay and held at 15˚C, a temperature that is about average on the mudflats of Yaquina Bay (Johnson, 1980). Once a

distinctive suboxic layer was evident from color changes in the top centimeters of the cores, profiles of porewater pH, O_2, and H_2S were measured to 2-3 cm depth with commercial microelectrodes (Unisense A.S., Aarhus, Denmark) to confirm geochemical evidence of ~~cable bacteria~~CB activity (see below). ~~Small~~Multiple small subcores (0.5 cm diameter, 3 cm in length) were then taken out from each incubated core using ~~cutoff~~cut-off syringes. ~~The~~Some of these sediment plugs were washed gently to reduce the volume of fine particles, and ~~cable bacteria~~CB biomass was further separated out from the sediment matrix by using custom-made tiny glass hooks after Malkin et al. (2014). ~~Washed sediment~~Sediment plugs and separated filamentous biomass were frozen or fixed for subsequent phylogenetic and microscopic characterizations.

2.3 Reactor Configuration and Operation

To mimic the conditions where ~~cable bacteria~~CB were found attached to electrode fibers in a BMFC (Reimers et al., 2017), a bioelectrochemical reactor was assembled from a polycarbonate core tube (15 cm height and 11.5 cm inner diameter, Fig. ~~2~~1) as a second phase of this research. A lid, a center rod to locate and support the electrodes, and a perforated bottom partition were made from polyvinyl chloride (PVC, McMaster-Carr, Elmhurst, IL). Three carbon brush electrodes, that would serve as two anodes and a control electrode (Mill-Rose, Mentor, OH, 2 cm in diameter and 8.9 cm of total length~~))~~), were inserted through septa within holes in the core lining to meet the center rod and were spaced radially at 120˚ angles from each other.

To initiate the experiment, the reactor was placed inside an 8L plastic beaker (with perforated walls) containing 3 cm of IMF sediments at the bottom. Enough additional IMF sediment was then placed inside the reactor to form an 8 cm thick layer after settling/compacting. In this configuration, the sediment-water interface was approximately 1 cm away from the lower extent of the carbon brush electrodes. The beaker was then gently lowered inside an aquarium filled with Yaquina Bay seawater until fully submerged, and the reactor was left uncapped. Seawater in the aquarium was maintained at 15˚C and bubbled to maintain air-saturation. A fuel cell circuit was completed by placing a 10 cm-long carbon-fiber brush cathode (Hasvold et al., 1997) and a reference electrode (Ag/AgCl [3 M KCl], MI-401F, Microelectrodes, Inc., Bedford, NH) into the seawater outside the reactor tube (Fig. ~~2~~1a).

The reactor was monitored in an open circuit state for 31 days to allow the development of a ~~cable bacteria~~CB population within the top centimeters of sediment as had been observed in the previous incubations. Microelectrode profiling was used to characterize the vertical distribution of porewater pH and concentrations of O_2 and H_2S on days 13 and 24 of reactor incubation. On day 31, carbon fiber samples were trimmed off the unpoised anode brushes as ~~control~~initial reference samples, and the reactor was sealed to create fully anoxic conditions. Beginning on day 44, under seal, cathode versus anode potentials were poised at 300 mV by regulating two of the three anode carbon brushes with an individual custom-designed potentiostat circuit board (NW Metasystems, Bainbridge Island, WA) (Fig. ~~2~~1b). The third brush was kept at open circuit as a continuing control. Electrode potentials of the anode (versus reference) and whole cell, and the current flow between anodes and cathode were monitored and recorded every 7 min with a multichannel datalogger (Agilent Technologies, Santa Clara, CA, model 34970A fitted with two 34901A multiplexer modules) wired to the potentiostat outputs. The electrodes were poised for more than 3 months. On day 48 microelectrode profiling was repeated by lowering the sensors through the ports in the reactor lid (Fig. 1c). ~~On day~~

~~135, they~~The pH microelectrode was broken at the start of this profile and therefore no pH or calculated total sulfide are reported (only H_2S). On day 135, the anodes and control electrode were extracted through the side openings in the bioreactor tube for SEM and CARD-FISH analyses (described below). At the experiment's end, the final pH of the seawater inside of the anodic chamber was measured by microelectrode (see below).

2.4 Microelectrode ~~Profiling~~Measurements

The sediments incubated in open cores and in the bioelectrochemical reactor were each profiled with ~~pH,~~ O_2, pH and H_2S microelectrodes to ~~provide signatures~~show through geochemical signatures evidence of ~~cable bacteria~~CB activity (Malkin et al., 2014). Microelectrodes had tip diameters of 100 µm. The O_2 microelectrodes were calibrated in air-purged seawater (as 100% air saturation) and in a solution of sodium ascorbate and NaOH (both to a final concentration of 0.1 M, as 0% O_2 saturation). Vertical oxygen microprofiles were recorded starting from 2 mm above either the sediment-water interface, or in the reactor above the carbon brush, at a step size of 400 µm. Vertical pH and H_2S microprofiles were measured concurrently at the same spatial interval. The pH microelectrode was calibrated by using standard pH 4, 7, and 10 buffer solutions (Ricca Chemical, Arlington, TX, United States). H_2S microelectrodes were calibrated by generating an 11-point calibration relationship by standard addition, from 0 to 7.48 µM H_2S at pH = 1.6. Total sulfide concentration at each profile depth was derived from pH and H_2S according to equilibrium relationships given in Millero et al. ~~(1988) (Millero et al., 2018~~1988).

2.5 SEM

To confirm the presence of CB and to examine the characteristic longitudinal ridges and cell-cell junctions of ~~cable bacteria~~CB, filaments extracted from the sediments and carbon fibers from the reactor electrodes were visualized by scanning electron microscopy (SEM). Samples were dehydrated in a graded series of ethanol solution from 10 to 100%. Specimens were then mounted on aluminum SEM stubs with double-sided carbon tape, critical-point dried using an EMS 850 Critical Point Dryer, and sputter-coated with gold and palladium using a Cressington 108 sputter coater. The resultant specimens were observed under a FEI Quanta 600FEG ESEM at 5–15 kV. This instrument also provided elemental spectra by X-Ray Energy Dispersive Spectrometry (EDS).

2.6 CARD-FISH

Catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH) was used to microscopically identify *Desulfobulbaceae* filaments using a *Desulfobulbaceae*-specific oligonucleotide probe (DSB706; 5′-ACC CGT ATT CCT CCC GAT-3′) labelled with horseradish peroxidase (Lücker et al., 2007). ~~-~~In preparation for CARD-FISH, sediment samples were fixed with a 1:1 (vol: vol) ethanol and phosphate-buffered saline solution and stored at -20 ˚C until analysis. Extracted bacterial filaments and carbon fibers cut from the carbon brush electrodes were treated with a fixative solution containing 1.25% glutaraldehyde and 1.3% osmium tetroxide. Fixed samples were stored at -20 ˚C until analysis. Sediment and bacterial filament samples were first retained on polycarbonate membrane filters and then mounted onto a glass slide by using 0.2 % agarose (Malkin et al., 2014). Carbon fiber samples were mounted directly onto a glass slide without first retaining on a filter. Mounted samples were sequentially permeabilized by 10 mg/mL

of lysosome (2 hrs at 37°C) and achromopeptidase (1 hr at 37°C). After ~~permeablization~~permeabilization, glass slides were incubated in H_2O_2 (0.15% in methanol) for 30 mins at room temperature (~25°C) to inactivate the endogenous peroxidases. The hybridization process was performed in a standard hybridization buffer at 46°C with 45% formamide for 7 ~~hrs(Wendeberg, 2010)~~hours (Wendeberg, 2010). Alexa Fluro 488 (ThermoFisher, Waltham, MA, United States) was deposited on samples in the presence of 0.15% H_2O_2.

Two-color CARD-FISH was performed on some carbon fiber samples to look for a previously observed co-occurrence of ~~cable bacteria~~CB and other electroactive bacteria on electrode surfaces (Reimers et al., 2017). To perform the ~~two-color~~ CARD-FISH, horseradish ~~peroxidase~~peroxidases on the hybridized DSB706 probes were inactivated by 0.15% H_2O_2. The inactivated samples were then hybridized with a *Desulfuromonadales*-specific oligonucleotide probe (DRM432; 5′- CTT CCC CTC TGA CAG AGC−3′) modified with horseradish peroxidase in standard hybridization buffer at 46°C with 40% formamide for 5 hrs and sequentially stained with Alexa Fluro 555 (ThermoFisher, Waltham, MA, United States). A counter stain, 4′,6-diamidino-2-phenylindole (DAPI), was applied to all samples after the deposition of fluorescent probe(s). Hybridization samples were visualized using confocal laser scanning microscopy (CLSM) (LSM 780, Zeiss, Jena, Germany).

2.7 Microbial Community Characterizations

To investigate the phylogeny of the ~~cable bacteria~~CB discovered in Yaquina Bay, genomic DNA was extracted from ~~washed~~3 sediment plug samples and from ~~tangles of bacterial filaments~~2 separated ~~from sediments~~filamentous biomass samples using a MoBio PowerSoil DNA Extraction Kit. To avoid insufficient cell lysis, all samples went through 5-7 freeze-thaw cycles before the use of the extraction kit (Roose-Amsaleg et al., 2001). Bacterial 16S rRNA genes were amplified by PCR with random primers 357wF (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′). Amplification and sequencing of DNA (Illumina MiSeq Reagent Kit v3, 2 × 300 bp) was performed by the Center of Genome Research and Biocomputing at Oregon State University. Sequences were processed using DADA2 (v.1.10) in R (3.5.0) as described in a previous study (Callahan et al., 2016). Sequences were aligned to the Silva SSU Ref NR database (v.132) and clustered into operational taxonomic units (OTUs) at 97% similarity. Representative sequences classified to the family of *Desulfobulbaceae* were tagged and aligned to 16S rRNA gene sequences from previously identified ~~cable bacteria~~CB (Trojan et al., 2016). A phylogenetic tree was constructed using RaxML with 1,000 bootstraps (Stamatakis, 2014). Sequences from this study were deposited to the Genbank's Sequence Read Archive (MK388690-MK388723, PRJNA587126).

3 Results and Discussion

3.1 Cable Bacteria Activity in the Sediments of Yaquina Bay

During the initial open incubations of IMF ~~and OFS sediments, the top centimeter of each core changed from dark to light gray, and a layer of brownish mineral formed 2 mm the sediment-water interface to ~0.2 cm depth. These changes indicate the depletion of iron sulfides and the formation of iron oxy(hydroxide)s, respectively (Risgaard-Petersen et al., 2015). Hallmarks of the activity of cable bacteria were documented by microelectrode profiling after~~

~~53 and 34 days of culture in IMF and OFS sediments, respectively.~~sediment, the top centimeter of each core changed from dark to light gray, and a brownish layer formed from the sediment-water interface to ~0.2 cm depth. Hallmarks of the activity of CB were documented by microelectrode profiling after 53 days of culture. These hallmarks were a sulfide-free suboxic zone and opposing pH extremes at approximately 0.2 cm and 1-1.5 cm deep (Fig. ~~3a & 3b~~2a). Although a faint smell of sulfide was detected during collection of the sediment, total sulfide concentrations detected by microelectrode profiling were low compared to previous studies of marine sediments hosting ~~cable bacteria (roughly above 200 µmol/L)~~CB (Malkin et al., 2014). The pH ~~minima~~minimum within the ~~sulfidic~~anoxic layers of cultured sediment ~~were also lower than observed previously (roughly around~~was 6.~~5). It is likely that the relatively long times of our incubations contributed~~0 indicating acidification coupled to ~~these low values~~sulfide or iron-sulfide oxidation.

~~After the geochemical hallmarks of cable bacteria were observed, SEM performed on extracted sediment and bacterial filament samples revealed that cells within extracted filaments were 0.5 to 1.2 µm wide and 2 to 3 µm long (Fig. 4a, b, & c). Typical morphological features of cable bacteria including the longitudinal ridges and the cell-cell junctions were observed (Pfeffer et al., 2012).~~SEM revealed that cells within extracted filaments were 0.5 to 1.2 µm wide and 2 to 3 µm long (Fig. 3a, b, & c). Typical morphological features of CB including longitudinal ridges and cell-cell junctions were observed, though a smaller number of ridges (8-10) were spotted compared to 16-58 in other characterizations (Malkin et al., 2014). Certain filaments extracted from sediments were covered by heterogeneous coatings of mineral particles as was observed recently by Geerlings et al. ~~(Fig 4c) (Geerlings et al., 2018).~~(2018) (Fig. 3c). These particles have similar elemental compositions to some authigenic clays (Sturz et al., 1998), showing enrichments of silicon, aluminum, magnesium, and iron. In our open incubation samples, some thinner filaments were also seen that displayed no obvious longitudinal ridges, although cell-cell junctions were still visible (Fig. ~~4b). Extracted filaments reacted positively to the DSB 706 probe and DAPI (Fig. 4d). Certain filaments seemed to be thicker (3-3.5 µm diameter) when visualized using FISH (SI Fig. 1a). Granules that appeared to be impenetrable to light were also observed along filaments when viewed with a transmitted light microscope. Most of these granules had high affinity to DAPI stain (SI Fig.~~ 3b). Extracted filaments reacted positively to the DSB 706 probe and DAPI (Fig. 3d and SI Fig. 1).~~1c). Similar granules reported in a recent study, were suggested to be intracellular polyphosphate granules (Geerlings et al., 2018). Together, these observations indicate that the population of the cable bacteria, widely distributed along the NE Pacific estuarine system of Yaquina Bay, Oregon, has characteristics very similar to those observed on other coasts.~~

~~3.2 Phylogenetic Analysis of the Cable Bacteria in Yaquina Bay~~

~~After identifying cable bacteria filaments through SEM and FISH, we also sequenced the 16S rRNA gene in genomic DNA extracted from separated aggregations of cable bacteria biomass from the IMF sediment, and from washed sediments from the top 3 cm of both IMF and OFS cores that were incubated (Fig. 4e). Sequencing of the 16s rRNA gene yielded 725 OTUs in DNA extracted from the aggregations of cable bacteria biomass, in which the family of *Desulfobulbaceae* was predominately abundant (> 80%). Identified OTUs from the extracted biomass of cable bacteria from IMF sediments were predominately *Candidatus* Electrothrix. Sequencing of the 16s rRNA gene yielded 1520~~

~~and 1524 OTUs respectively in DNA extracted from the top 3 cm of the IMF and OFS sediments. Among these OTUs, 96 and 69 of them respectively from IMF and OFS can be assigned to the family of *Desulfobulbaceae*. When the most abundant OTU in the family of *Desulfobulbaceae* in all extracted DNA were aligned with a previously established taxonomy framework of cable bacteria, they clustered with *Candidatus* Electrothrix, a candidate genus that has been recently proposed for the cable bacteria.~~ When analyzing the 16S rRNA gene sequence data, we found that one of the candidate CB genera, *Candidatus* Electrothrix, was relatively abundant in sediment plug samples (2.9%) and predominate (83.5%) in separated filamentous biomass samples. The most abundant *Desulfobulbaceae* OTUs within these samples were aligned with a previously established taxonomy framework of CB (Trojan et al., 2016)~~. An OTU in OFS sediment clustered with the genus of *Candidatus* Electronema, which is associated with freshwater cable bacteria. As the OFS site is in the mid estuary zone of the Yaquina River, it is under the influence of low salinity seawater in winter, and therefore may be expected to have a community composition distinct from the IMF site.~~ (Fig. 3e). Partial 16s rRNA sequences of ~~cable bacteria~~CB have been discovered in sediment samples from the US east coast, Gulf of Mexico, and certain sites on the US west coast from SILVA or GenBank databases (Trojan et al., 2016). Our studies have provided the first combined microscopic and genetic observations of ~~cable bacteria~~CB in sediments from the NE Pacific coast of the United States, reinforcing the suggestion that ~~these filamentous~~CB bacteria are distributed globally. This result also indicates that Yaquina Bay, OR, where we deployed the ~~previous~~ BMFC, indeed harbors a rich population of ~~cable bacteria~~CB.

3.~~3~~2 Encouraging the Growth of Cable Bacteria on Poised Electrodes

~~We chose the IMF sediment to seed our bioelectrochemical reactor because these sediments developed a higher relative abundance of cable bacteria compared to the OFS sediment (data not shown) and their location was closer to the deployment site of the previous BMFC.~~ Geochemical hallmarks of ~~cable bacteria~~CB developed within two weeks of culture within the bioelectrochemical reactor~~.~~ (Fig. 2b). The pH minimum ~~at~~within the sulfidic layer and the pH maximum ~~at~~in the ~~subsurface~~oxic layer of sediment became more extreme ~~at~~by day 24~~,~~ (Fig. 2c), indicating that a ~~cable bacteria~~CB population was actively mediating electrogenic sulfide oxidation and transporting electrons to reduce oxygen. After sealing the reactor ~~was closed~~, oxygen concentration in the overlaying seawater dropped below detection limits and the open circuit anode potential fell to ~~more negative than -100~~-104 mV (vs Ag/AgCl). Once poised with the potentiostat, the cathode and anode potentials became stable at ~330 mV and ~30 mV versus Ag/AgCl, respectively. When microelectrode profiling was performed on day 48, the measurements indicated that the overlying seawater was anoxic (except right below the sample port) and that free H_2S was detectable right below the sediment-water interface but not in the water-column (Fig. 2d). The pH of the seawater measured inside of the reactor chamber at the experiment's end was 6.2, consistent with sulfide oxidation under anaerobic conditions within the anolyte seawater. Current collection started to increase once the anodic potential became stable, indicating that the anode brushes were being used as an electron acceptor. Current records collected from duplicate electrodes ~~was~~were similar, and the current in each steadily increased to ~ 30.5 ± 2.5 µA by day 86, stabilized, then rose again to a peak of ~ 75 ± 8 µA on day 101. After this maximum, current decreased and restabilized at ~ 30 ± 5 µA. These electrochemical results are portrayed in Fig. ~~5~~4. The cause of the current rise and subsequent fall (Fig. ~~5~~4) is unknown but is a common

occurrence in marine BMFC experiments (Nielsen et al., 2009; Ryckelynck et al., 2005). It is likely that such behavior is a result of a varying supply of reductants from the underlying sediment, changes in the anodic biofilm, and finally loss of anode surface area ~~loss caused by~~due to mineral deposition induced by microbial activity and/or the applied electrical potential. Coatings containing iron, phosphorus, sulfur, silicon, and aluminum are often found on anode surfaces of ~~bioelectrochemical reactors in anoxic marine environments (Nielsen et al., 2008) and were seen by SEM (see below).~~BMFCs in marine environments and were seen by SEM in the present study (see below). Cyclic voltammetry (CV, SI Fig. 2a) performed on the anode brushes at day 52 and 100 yielded broad and poorly defined electrochemical signals. The interpretation of such voltammograms may be complicated by a high uncompensated resistance between working electrode and reference electrode (Babauta and Beyenal, 2015a). While an oxidation peak can be clearly identified at potentials near where the anode was held, the peak current did not increase with an increase of scan rate (SI Fig. 2b). The peak oxidation current also did not change much between day 52 and day 100. This CV behavior suggests that any current generated by the biomass of electroactive bacteria, including ~~cable bacteria~~CB, was obscured during scans by current arising from irreversible redox reactions, such as oxidation of dissolved iron. ~~The~~A reduction peak was unidentifiable throughout the ~~scan~~scans, a common phenomenon in benthic and sediment MFCs (Babauta and Beyenal, 2015b). Taken together, these results ~~suggest~~demonstrate that the electrode surface was altered during the course of the bioreactor experiment by mineral/chemical precipitate deposition (Imran et al., 2019).

3.~~4~~3 Examining the Attachment of Cable Bacteria on the Anode

The hypothesis that led to the bioreactor experiments in this study was that an electrode poised at an oxidative potential can produce redox conditions and geochemical gradients that attract CB and ~~signal the~~that will lead to their electron donation ~~of cable bacteria.~~to an electrode. Several observations that were made on harvested electrodes affirm this hypothesis. ~~First~~Firstly, under SEM, bacteria filaments with visible longitudinal ridges and cell-cell junctions were found integrated into biofilms on the surfaces of poised electrodes (Fig. ~~6a~~5a & b). ~~Some~~As observed in the initial IMF sediment examinations, filaments appeared to contain a smaller number of ridges (8 to 10) compared to previously reported ~~cable bacteria~~CB filaments and others were without pronounced ridges along their longitudinal axes. The latter examples did show cell-cell junctions and appeared to have wrinkled surfaces ~~that were similar to~~ (Fig. 5c, d, e, &f). Secondly, many of the ~~thin cable~~ bacteria filaments ~~extracted from our cultured sediment cores (Fig. 6c & 6d). Secondly, many bacteria filaments~~ observed on the electrode surfaces were encrusted, suggesting mineral deposition similar to that observed at the oxic terminal of ~~cable bacteria~~ CB filaments in sediments (Fig. ~~6c & 6f~~5g & h). EDS indicated that these deposits contained iron, phosphorus, oxygen, and silicon (SI Fig. 3b). The control electrode that was not positively poised displayed no mineral deposition and nearly no cell growth (Fig. ~~6). Third, many~~5i). Thirdly, most of the bacterial filaments on the poised electrode surfaces reacted positively with the *Desulfobulbaceae*-specific probe. CARD-FISH performed in the present study ~~suggested~~revealed that the anodic carbon fibers harbored many short bacterial filaments ~~and~~as well as colonies belonging to the family of *Desulfobulbaceae* (Fig. ~~7a~~6a, b, c, d, e, &f). Clear cell-cell junctions were observed along many of the fluorescent filaments. However, the complexity of the carbon fiber samples often hampered clear microscopic visualization~~.~~ of fluorescent cells. Application of an additional *Desulfuromonadales*-specific oligonucleotide probe (DRM432)

confirmed the presence of other known electrogenic bacteria on the carbon fibers near *Desulfobulbaceae* cells as well (Fig. 7 b, c & d).

Though a global occurrence has recently been indicated (Malkin et al., 2014), ~~cable bacteria~~CB successfully evaded microbiological survey for quite a long time. One of the reasons is likely that the phylogeny of ~~cable bacteria~~CB is shadowed under the broad family of *Desulfobulbaceae*, which are often highly abundant in marine sediments (Kuever, 2014). Another reason may be a resistance of the cells of ~~cable bacteria~~CB to routine cell lysing techniques that have been used with many DNA extraction kits (Trojan et al., 2016). Therefore, ~~the~~their identification in various studies has relied on microscopic observations of ~~the~~their unique filamentous form and morphological features (ridges and cell-cell junctions~~) of cable bacteria,~~), combined with fluorescence *in situ* hybridization ~~labeling have been employed to identify their presence in various studies~~labelling (Malkin et al., 2014, 2017; Malkin and Meysman, 2015). The electrochemical reactor in this study was anoxic for more than 100 days. The observation of ~~cable bacteria~~CB on the anodic carbon fibers ~~confirmed~~at the end of this experiment confirms that, although they may not have been abundant, they can survive under such conditions and were likely using the anode as electron acceptor (as suggested previously ~~(~~by Reimers et al~~,~~., 2017). Besides the recognized forms of CB, short filaments within the family of *Desulfobulbaceae* that possessed different morphologies were also observed on the anode surface. Aller et al. (2019) suggest that redox environment may play an important role in controlling the length of CB filaments. For example, in the bioturbated zone associated with the tube worm *Chaetopterus variopedatus*, in which redox conditions often oscillated between oxic and hypoxic, CB were present predominately in short filaments. Assuming the CB can use an electrode as an electron acceptor, the distance between the electron donor and acceptor utilized by CB may be short, reducing the advantage of forming long filaments.

The closest culturable relative to CB, *Desulfobulbus propionicus*, can utilize an electrode as an electron acceptor to oxidize S^0, H_2, and organic acids like pyruvate, lactate, and propionate (Holmes et al., 2004). While ~~cable bacteria~~CB appear to possess features like motility and an ability to form loops and bundles that are similar to large sulfur bacteria (but distinct from the *D. propionicus*), our SEM and CARD-FISH examinations suggest that ~~cable bacteria~~CB on oxidative electrode surfaces may produce extracellular structures to transfer electrons to an electrode and/or to insoluble Fe(III)-oxides similar to *D. propionicus* (Bjerg et al., 2016; Holmes et al., 2004; Jørgensen, 2010; Pfeffer et al., 2012). Admittedly, indisputable proof of electron transfer from ~~cable bacteria~~CB to electrodes still awaits growth in purer biofilms and cultures.

~~The present study demonstrated the possibility of drawing cable bacteria out of sediments for further culture on an electrode. Bjerg et al. (2016) have suggested cable bacteria form twisted loops to move out of sediment when oxygen becomes unavailable. How the cable bacteria adjust themselves when the loop lands on a new location where electron acceptors and donors are available remains unknown. In the microenvironment induced by the poised anode, assuming the cable bacteria can use electrode as an electron acceptor, the distance between the electron donor and acceptor utilized by cable bacteria may be short, reducing the advantage of long filaments. Recently, Aller et al. (2019) discovered the presence of short cable bacteria filaments in a bioturbated zone associated with the tube worm *Chaetopterus variopedatus* likely as a response to the redox microenvironment, prompting a similar suggestion about what may control filament length and morphologies. Additionally, a change of electron transfer mechanism could~~

~~alter the gene expression, as have been suggested in *Geobacter sulfurreducens* growing at different electrical potentials (Malvankar et al., 2011; Yi et al., 2009). In summary, when growing on an electrode poised at an oxidative potential, cable bacteria may no longer require long filaments or be able to maintain them due to the nature of the potential gradient.~~

4 Summary and Implications for the Electrode Associated Growth of Cable Bacteria

~~The present study introduces electrochemical reactors as a unique approach for investigation of filamentous cable bacteria and their unique ability to transfer electrons. In addition, we confirm that an active population of the filamentous cable bacteria are widely distributed along the estuary of Yaquina Bay, Oregon. Cable bacteria OTUs found in Yaquina Bay clustered closely with the genus of *Candidatus* Electrothrix, contributing a new location to the global distribution of cable bacteria. Moreover, by incubating intertidal sediment collected from Yaquina Bay in a bioreactor mimicking the anodic chamber of a BMFC, we observed that this group of bacteria can be drawn to electrodes at oxidative electrical potentials and that they likely will use an electrode as an electron acceptor in the absence of dissolved oxygen (Nielsen et al., 2008; Reimers et al., 2017). Beside identified cable bacteria, filaments and cells within the family of *Desulfobulbaceae* that possessed different morphologies were also observed on the anode surface, suggesting that cable bacteria may be able to alter their morphologies depending on the redox microenvironment. Heavy mineral encrustation observed on filaments attached to electrode was similar to that reported as occurring at the oxic terminal of cable bacteria in other marine sediments, suggesting that an electrode poised at an oxidative potential creates a similar redox environment to that created by oxygen diffusion and reaction at the interface of sulfidic sediments. However, more work is needed to determine potentials and conditions that will not also lead to mineral precipitation on electrode surfaces. Cable bacteria greatly influence sediment habitats by performing electrogenic sulfur oxidation. Developing *ex situ* culture techniques and gaining insight into their electron transfer will contribute to the overall understanding of this group of bacteria and their survival in both natural and engineered environments.~~

The present study introduces bioelectrochemical reactors as an approach to investigate filamentous cable bacteria and their unique ability to transfer electrons. Furthermore, we confirm that an active population of filamentous CB are present in Yaquina Bay, Oregon USA, where CB were previously found attached to carbon-fiber electrode surfaces within a BMFC (Reimers et al., 2017). Moreover, by incubating intertidal sediment collected from Yaquina Bay in a reactor mimicking the anodic chamber of a BMFC, we observed that CB can be drawn to electrodes at oxidative electrical potentials. Thus, we have further evidence that CB can survive under anoxic conditions in the presence of an oxidative electrode serving as an electron acceptor. The bioelectrochemical reactor study also showed attachment of CB to an oxidative electrode when the surrounding seawater was stripped of hydrogen sulfide and at a pH ~6.2. However, the observed CB density and the overall density of recognizable cells were relatively low on electrode surfaces, as the respirable surface area appeared to become limited by the deposition of mineral coatings. More work is needed to determine conditions or experimental designs that may attract CB to an electrode while not also leading to excessive mineral precipitation on electrode surfaces. Developing *ex situ* culture techniques of CB and using these

approaches to gain insight into their electron transfer will contribute to the overall understanding of this group of bacteria, their genomic makeup, and their survival in both natural and engineered environments.

Author contribution

CL and CR conceived the ~~presented idea.~~study. YA designed and assembled the bioelectrochemical reactor. CL performed the microscopic ~~examination, analysed~~examinations and microprofiling, and he analyzed the microbial community and phylogenies~~, and~~. CL also wrote the manuscript~~.~~ with major rewrites and editing contributed by CR.

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~~**Figure 1. Detailed satellite image of Yaquina Bay estuary with study locations superimposed. Insert: North America topographic map indicating the location of Newport, Oregon. IMF: intertidal mud flat sediment, OFS: oyster farm sediment, and BMFC: previous benthic microbial fuel cell deployment site.**~~

~~Figure 2.~~

Figure 1. Schematic of the bioelectrochemical reactor design used in this study: (a) lateral view of the reactor; (b) electrical circuit of the reactor and (c) birds eye view of the reactor cap and electrode arrangement~~; and (c) electrical circuit of the reactor~~. Dimensions are in cm. A_1 and A_2 represent the current monitored in duplicate anodes; V_1 represents the potential monitored between the duplicate anodes and cathode; and V_2 and V_3 represent potentials monitored between the duplicate anodes and the reference electrode.

Figure ~~3~~2. Representative microelectrode depth profiles of oxygen (blue), pH (red), and ΣH_2S or H_2S(yellow) in (a) IMF ~~and (b) OFS sediments~~sediment after 53 ~~and 34~~ days of incubation~~, respectively;~~ and in the bioelectrochemical reactor at (b) day 13, (c) day ~~13~~24, and (d) day ~~24.~~ 48.

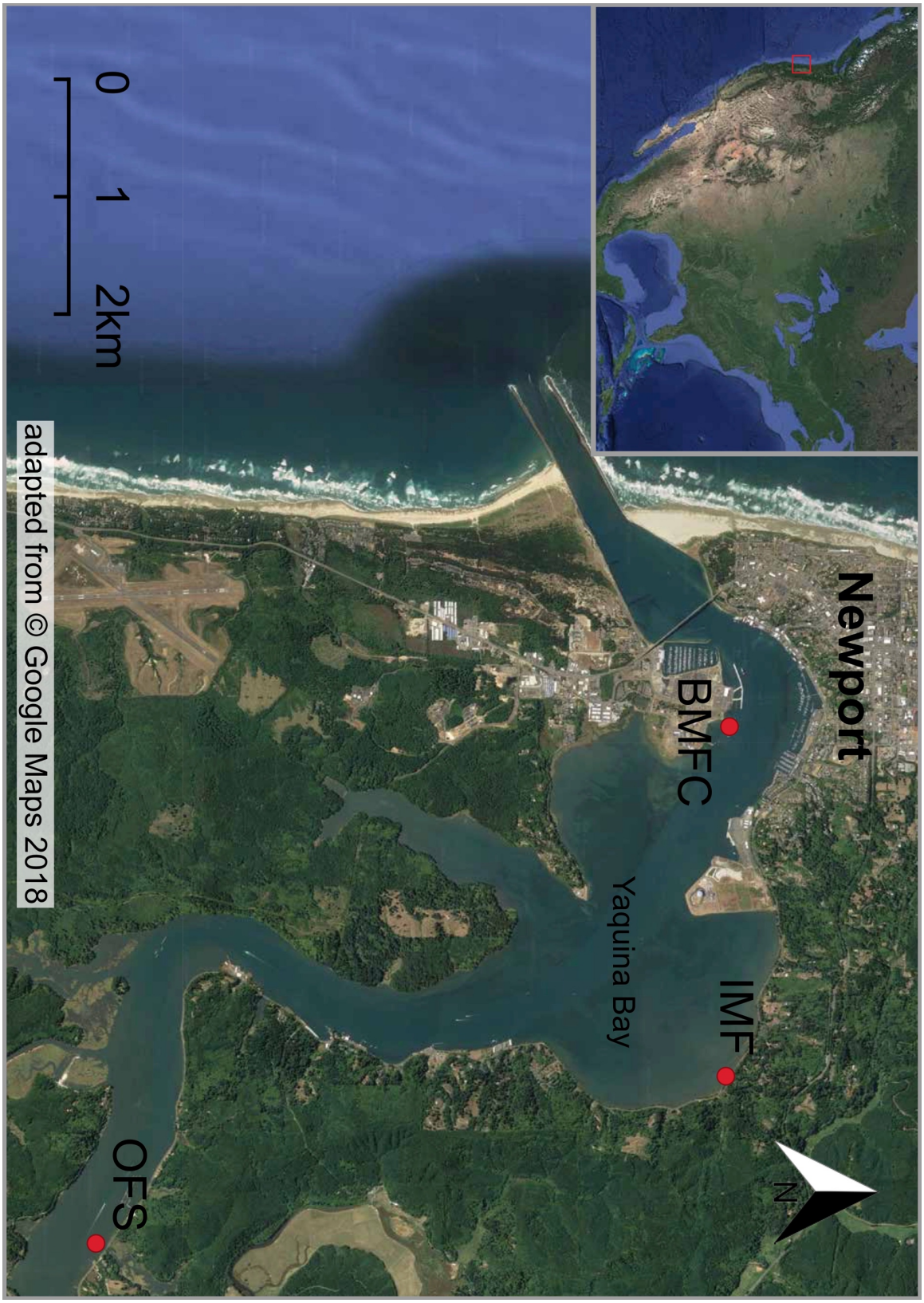
Figure ~~4~~3. Cable bacteria filaments ~~in~~recovered from Yaquina Bay sediments. (a) A cable bacteria filament under SEM. (b) A thin type of cable bacteria filament under SEM. (c) Multiple filaments of cable bacteria clumped together under SEM. Blue arrow indicates a ~~thick type~~section of cable ~~bacteria~~, and red arrow indicates a cable bacteria filament ~~incorporated in the observed~~covered with a mineral coating. (d) Identification of the filaments belonging to *Desulfobulbaceae* using Catalyzed reporter deposition-fluorescence *in situ* hybridization (DSB 706 probe + Alexa Fluor 488, green and DSB DAPI, blue). (e) Phylogenetic tree of *Desulfobulbaceae* 16s rRNA gene sequences recovered from IMF ~~and OFS~~ sediment ~~samples~~ and extracted biomass ~~from IMF~~samples. Color boxes indicate previously recognized species of cable bacteria. Scale bar shows 5% sequence divergence.

Figure ~~5~~4. The current production (blue), the anodic potential (black), and cathodic potential (orange) over time during the reactor experiment. The reference electrode was an Ag/AgCl electrode with saturated KCl filling solution. Figure only shows measurements associated with one of the duplicate electrodes.

Figure ~~6~~5. SEM images illustrating (a) & (b) cable bacteria filaments with visible ridges and cell-cell junctions incorporated into the biofilms on carbon fiber electrode surfaces. Red ~~triangles~~pointers indicate ~~the~~ cell-cell junctions. (c~~) &~~), (d~~) Long~~), (e) & (f) Short bacterial filaments without typical morphological features of cable bacteria. ~~(e) & (f)~~ Yellow arrows indicate the locations of elongated cells. (g) & (h) Mineral encrusted bacterial filaments. ~~(g) & (h) Colonies of long cells.~~ (i) Image of control electrode surface after culture~~. Yellow arrows indicate the locations of long cells~~.

Figure ~~7~~6. (a), (b), and (c) Confocal microscope images illustrating cable bacteria filaments on the carbon fibers that served as an anode. (d), (e), & (f) Colonies of cells belonging to *Desulfobulbaceae*. Red circles indicate possible doublet of the long cells. Cells were visualized using Catalyzed reporter deposition-fluorescence *in situ* hybridization (DSB 706 probe + Alexa Fluor 488, green; DRM 432 + Alexa Fluor 555, red; and DAPI, blue).

Fig. 1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IMF
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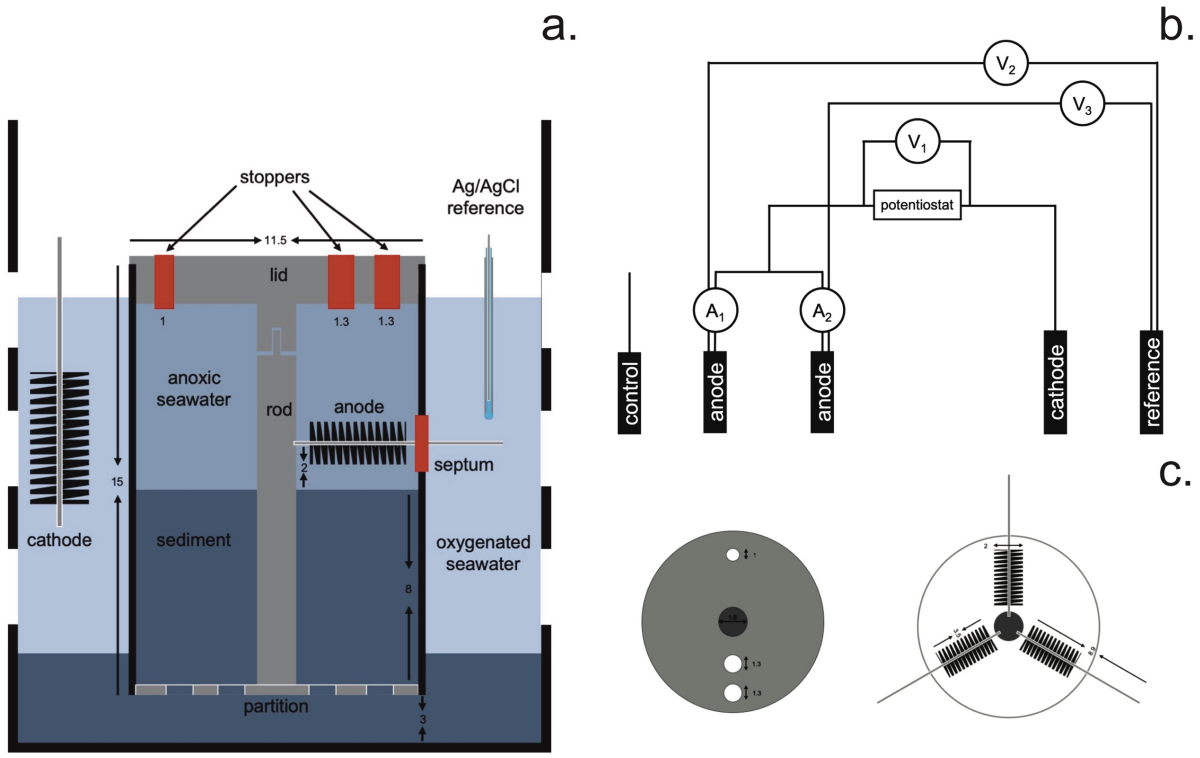
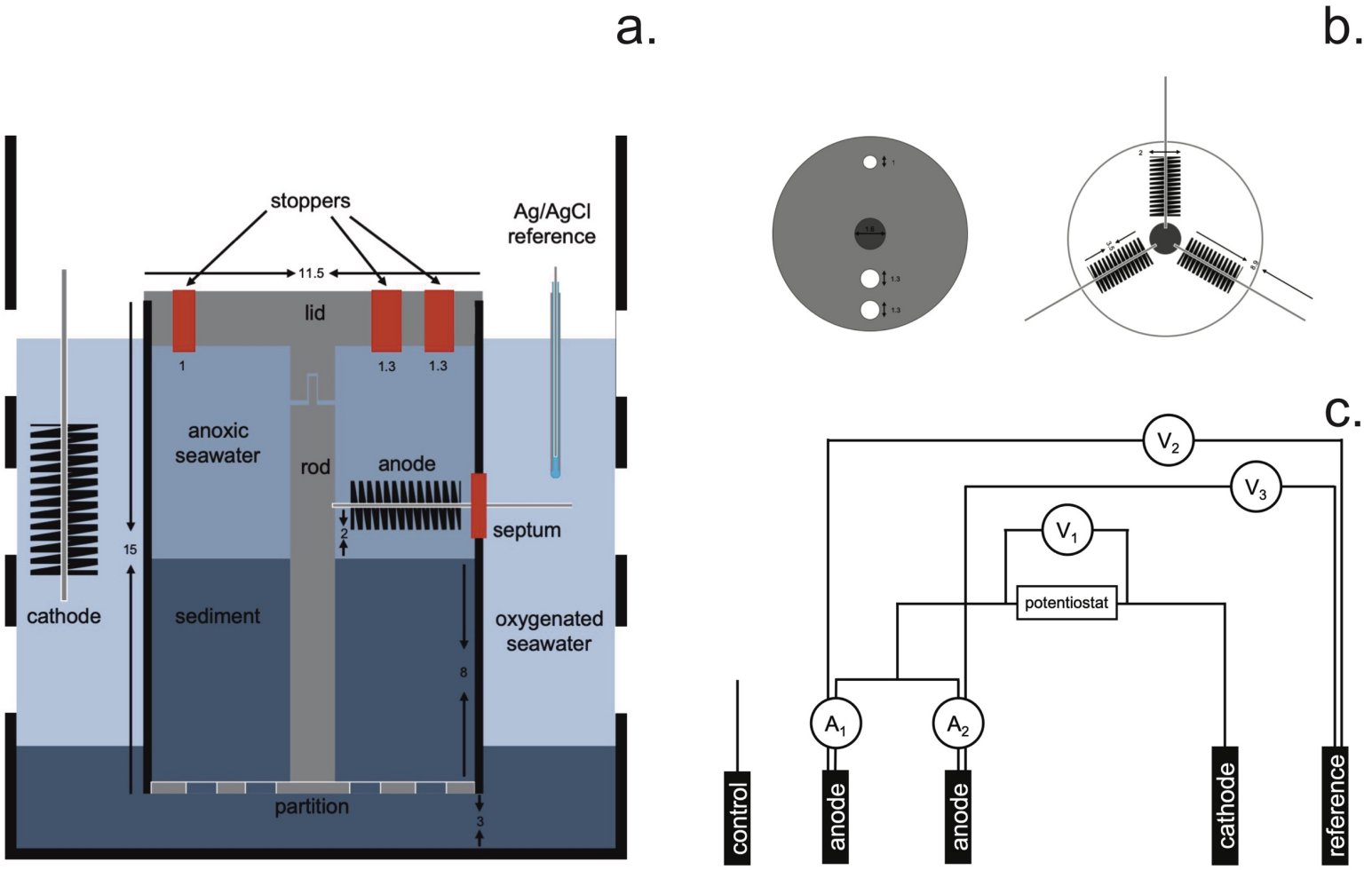

a.
b.
c.
stoppers
Ag/AgCl
reference
11.5
lid
1
1.3
1.3
anoxic
seawater
rod
anode
septum
15
cathode
sediment
oxygenated
seawater
8
partition
3
2
V2
V3
V1
potentiostat
A1
A2
control
anode
anode
cathode
reference

Fig. 2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septum
2
15
cathode
sediment
oxygenated
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V3
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A1
A2
control
anode
anode
cathode
reference

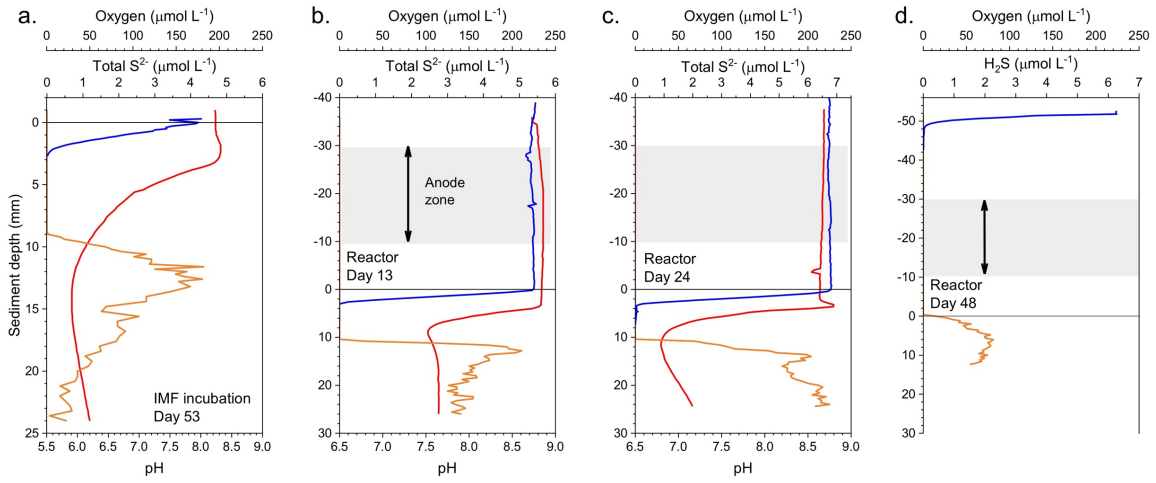
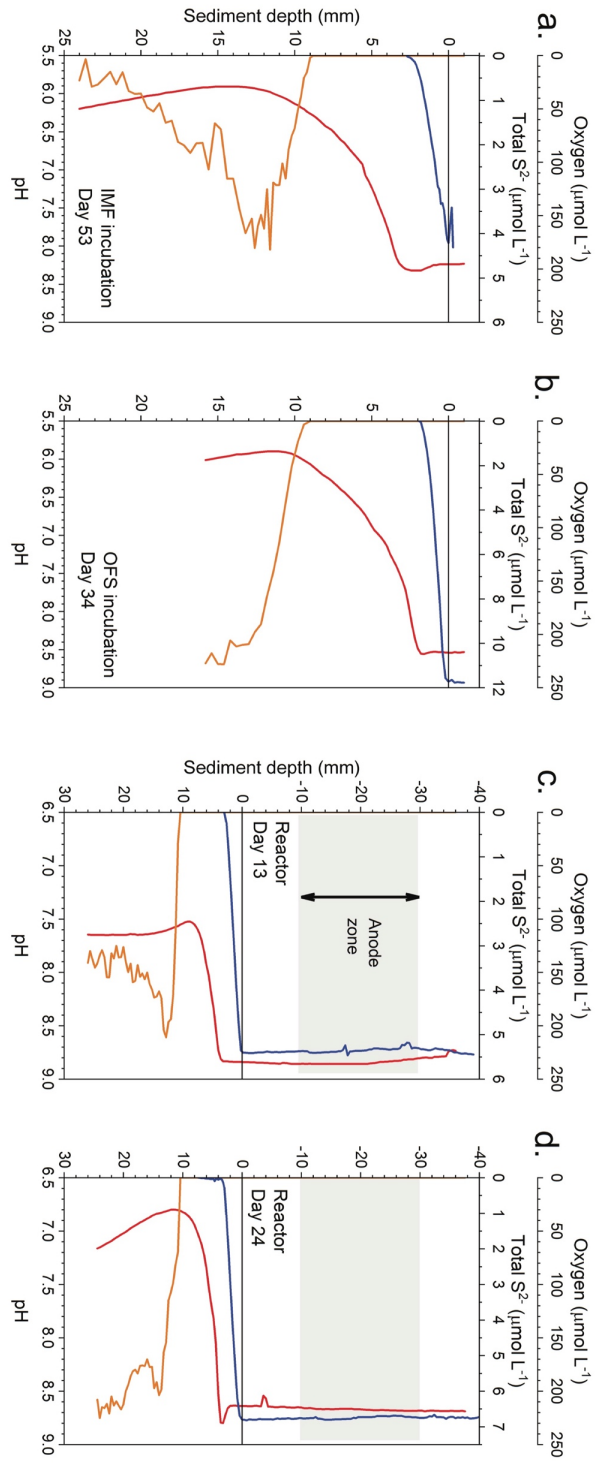
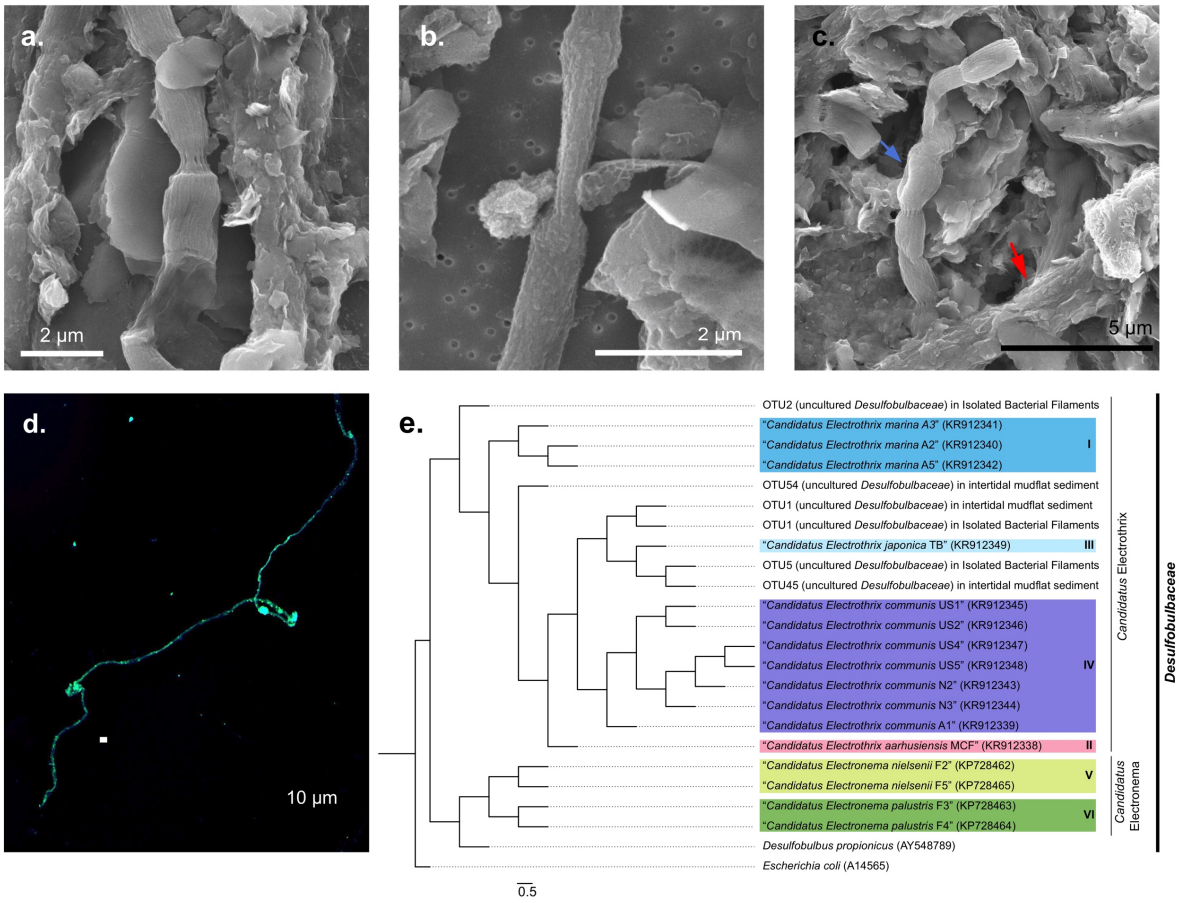

a.
Oxygen (μmol L-1)
Total S2- (μmol L-1)
Sediment depth (mm)
IMF incubation
Day 53
pH
b.
Oxygen (μmol L-1)
Total S2- (μmol L-1)
Anode
zone
Reactor
Day 13
pH
c.
Oxygen (μmol L-1)
Total S2- (μmol L-1)
Reactor
Day 24
pH
d.
Oxygen (μmol L-1)
H2S (μmol L-1)
Reactor
Day 48

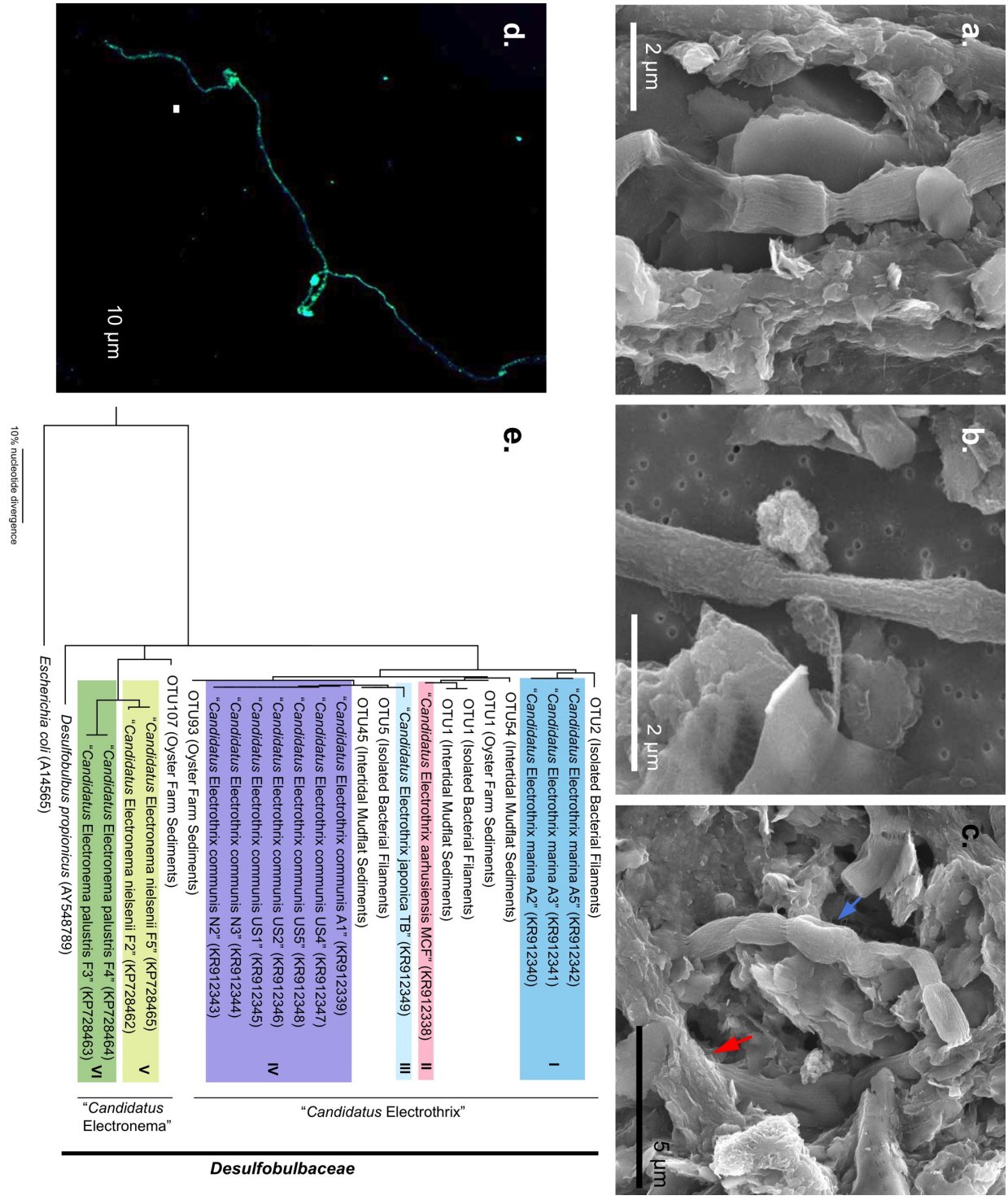
Fig. 3

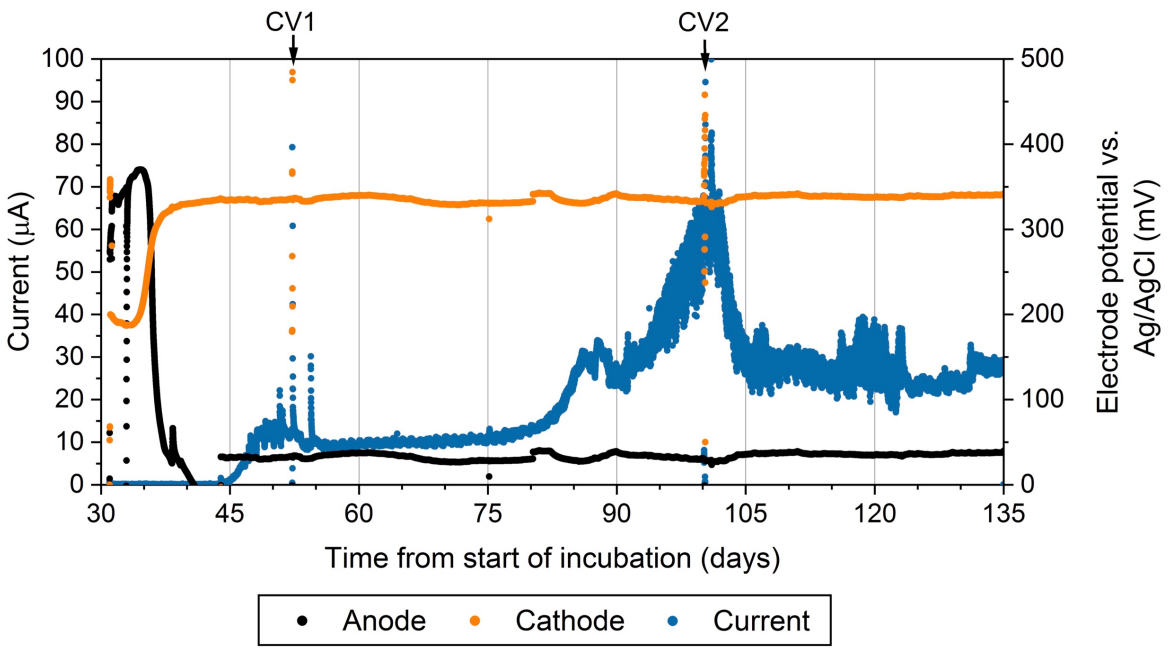

a.
b.
c.
d.
Sediment depth (mm)
Oxygen (µmol L-1)
Total S2- (µmol L-1)
pH
IMF incubation
Day 53
OFS incubation
Day 34
Reactor
Day 13
Reactor
Day 24
Anode
zone


a.
2 µm
b.
2 µm
c.
5 µm
d.
10 µm
e.
OTU2 (uncultured Desulfobulbaceae) in Isolated Bacterial Filaments
"Candidatus Electrothrix marina A3" (KR912341)
"Candidatus Electrothrix marina A2" (KR912340)
"Candidatus Electrothrix marina A5" (KR912342)
I
OTU54 (uncultured Desulfobulbaceae) in intertidal mudflat sediment
OTU1 (uncultured Desulfobulbaceae) in intertidal mudflat sediment
OTU1 (uncultured Desulfobulbaceae) in Isolated Bacterial Filaments
"Candidatus Electrothrix japonica TB" (KR912349)
III
OTU5 (uncultured Desulfobulbaceae) in Isolated Bacterial Filaments
OTU45 (uncultured Desulfobulbaceae) in intertidal mudflat sediment
"Candidatus Electrothrix communis US1" (KR912345)
"Candidatus Electrothrix communis US2" (KR912346)
"Candidatus Electrothrix communis US4" (KR912347)
"Candidatus Electrothrix communis US5" (KR912348)
"Candidatus Electrothrix communis N2" (KR912343)
"Candidatus Electrothrix communis N3" (KR912344)
"Candidatus Electrothrix communis A1" (KR912339)
IV
"Candidatus Electrothrix aarhusiensis MCF" (KR912338)
II
"Candidatus Electronema nielsenii F2" (KP728462)
"Candidatus Electronema nielsenii F5" (KP728465)
V
"Candidatus Electronema palustris F3" (KP728463)
"Candidatus Electronema palustris F4" (KP728464)
VI
Desulfobulbus propionicus (AY548789)
Escherichia coli (A14565)
0.5
Candidatus Electrothrix
Candidatus Electronema
Desulfobulbaceae

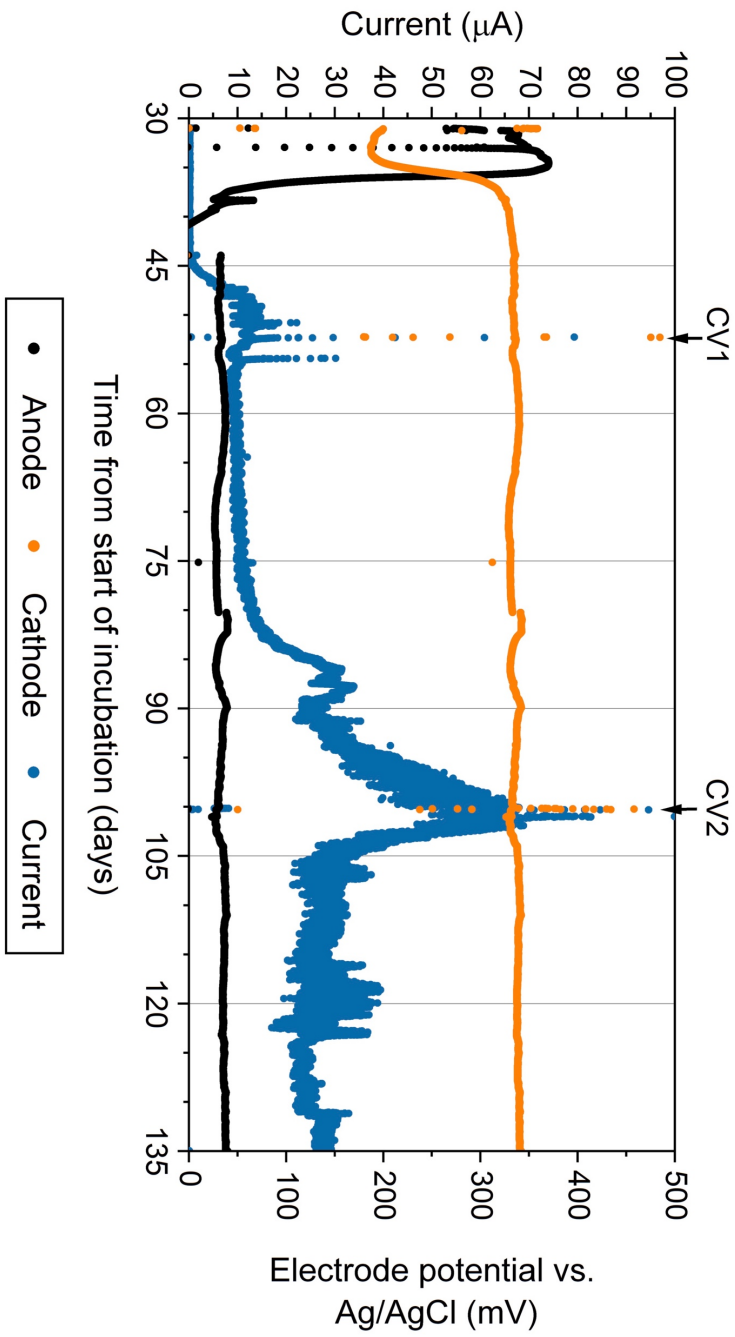
560

Fig. 4


a.
2 µm
b.
2 µm
c.
5 µm
d.
10 µm
e.
"Candidatus Electrothrix marina A5" (KR912342)
"Candidatus Electrothrix marina A3" (KR912341)
"Candidatus Electrothrix marina A2" (KR912340)
I
OTU2 (Isolated Bacterial Filaments)
OTU54 (Intertidal Mudflat Sediments)
OTU1 (Oyster Farm Sediments)
OTU1 (Isolated Bacterial Filaments)
OTU1 (Intertidal Mudflat Sediments)
"Candidatus Electrothrix aarhusiensis MCF" (KR912338)
II
"Candidatus Electrothrix japonica TB" (KR912349)
III
OTU5 (Isolated Bacterial Filaments)
OTU45 (Intertidal Mudflat Sediments)
"Candidatus Electrothrix communis A1" (KR912339)
"Candidatus Electrothrix communis US4" (KR912347)
"Candidatus Electrothrix communis US5" (KR912348)
"Candidatus Electrothrix communis US2" (KR912346)
"Candidatus Electrothrix communis US1" (KR912345)
"Candidatus Electrothrix communis N3" (KR912344)
"Candidatus Electrothrix communis N2" (KR912343)
IV
OTU93 (Oyster Farm Sediments)
OTU107 (Oyster Farm Sediments)
"Candidatus Electronema nielsenii F5" (KP728465)
"Candidatus Electronema nielsenii F2" (KP728462)
V
"Candidatus Electronema palustris F4" (KP728464)
"Candidatus Electronema palustris F3" (KP728463)
VI
Escherichia coli (A14565)
Desulfobulbus propionicus (AY548789)
10% nucleotide divergence
"Candidatus Electrothrix"
"Candidatus Electronema"
Desulfobulbaceae


CV1
CV2
Current (μA)
Electrode potential vs. Ag/AgCl (mV)
Time from start of incubation (days)
Anode
Cathode
Current

565 **Fig. 5**


Current (μA)
Time from start of incubation (days)
Electrode potential vs. Ag/AgCl (mV)
CV1
CV2
Anode
Cathode
Current

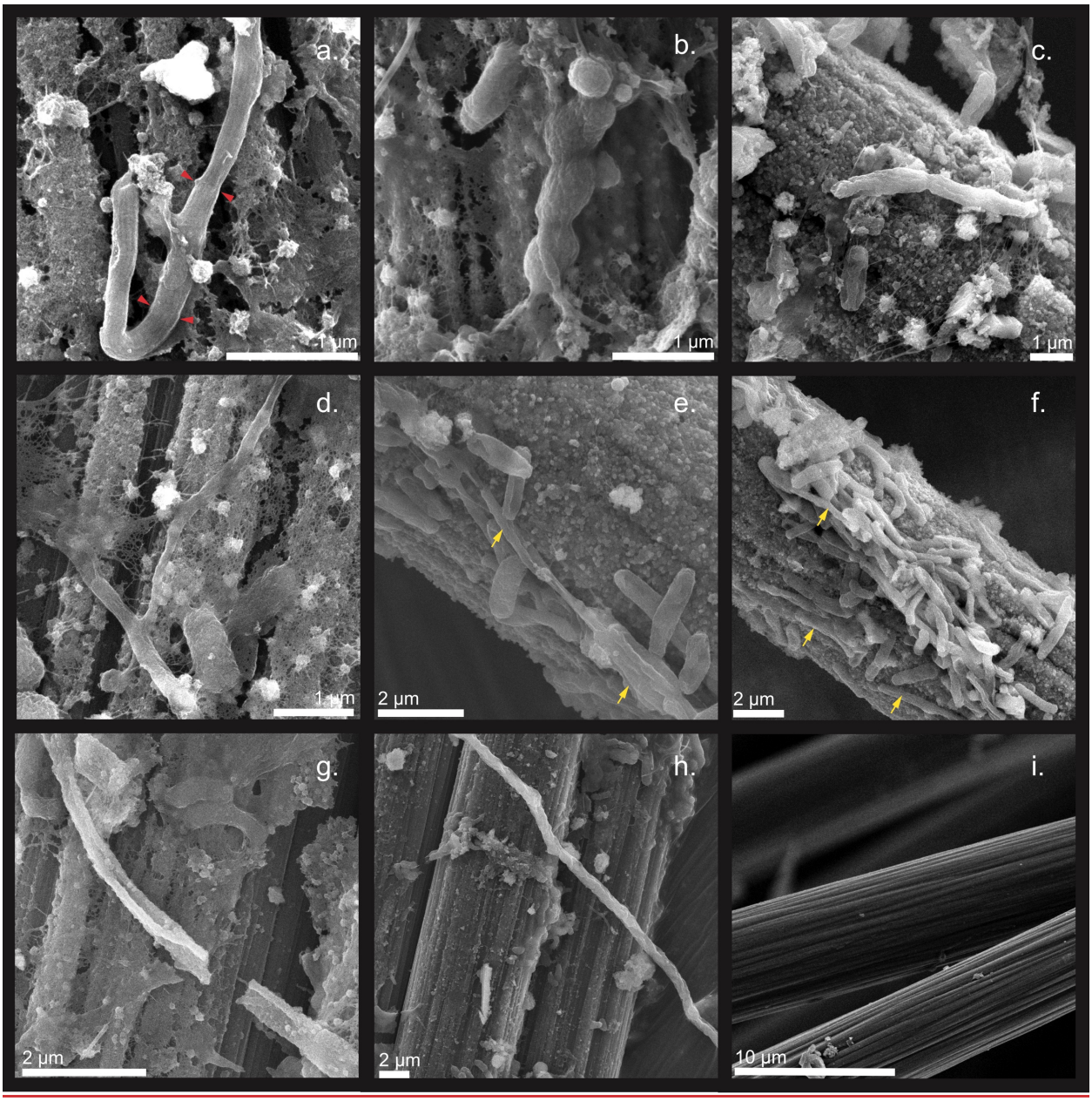
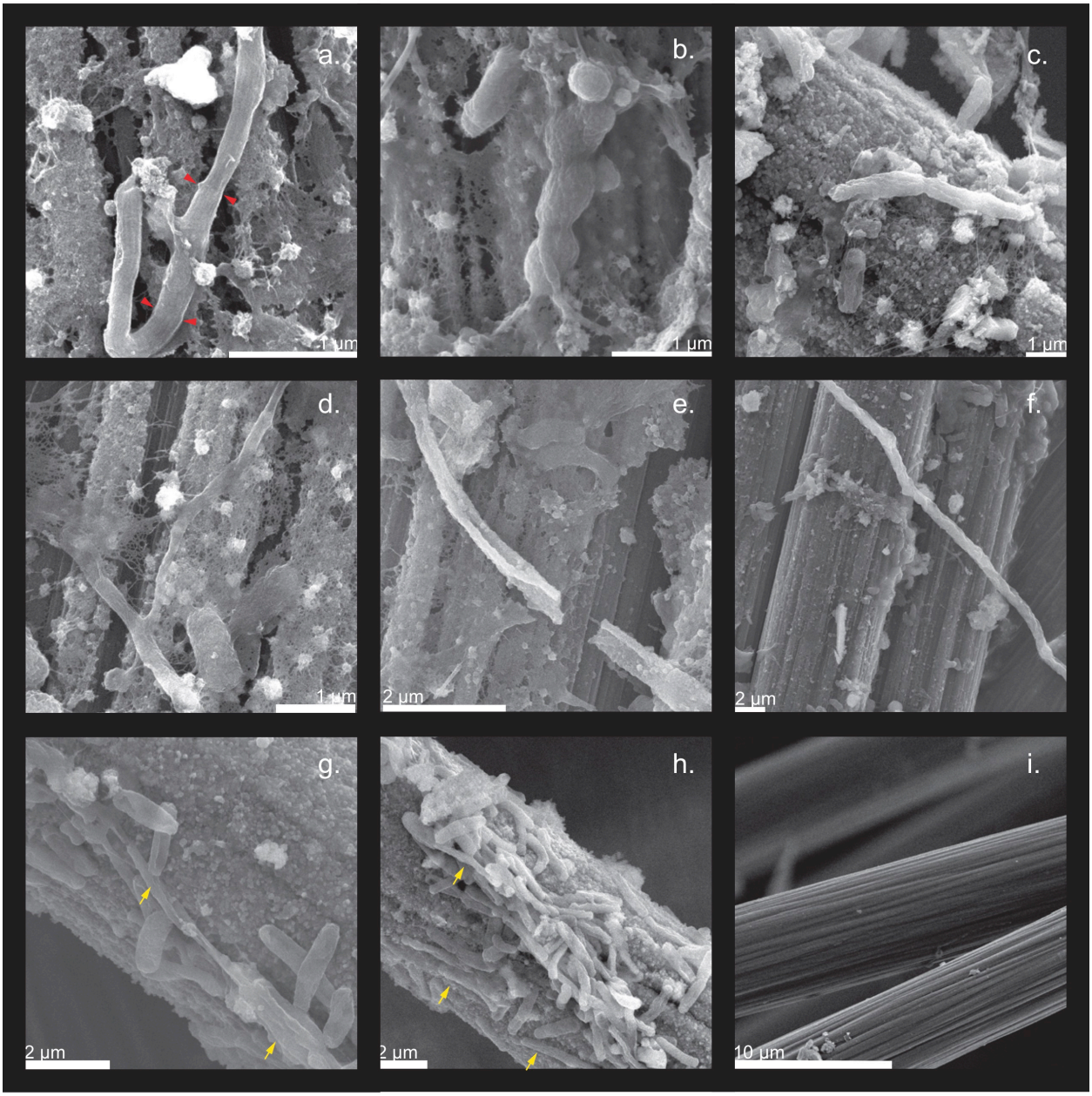
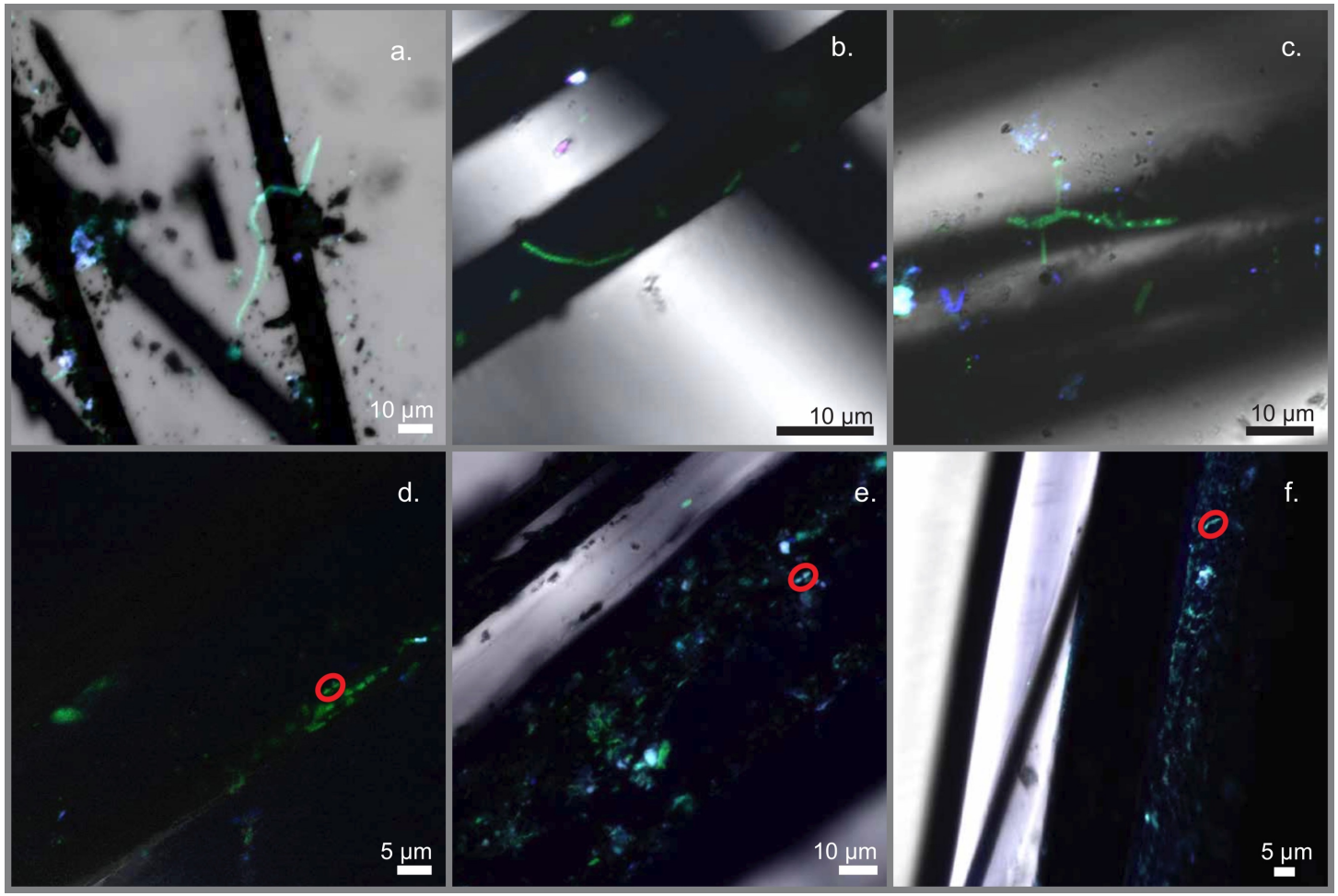

a.
1 µm
b.
1 µm
c.
1 µm
d.
1 µm
e.
2 µm
f.
2 µm
g.
2 µm
h.
2 µm
i.
10 µm

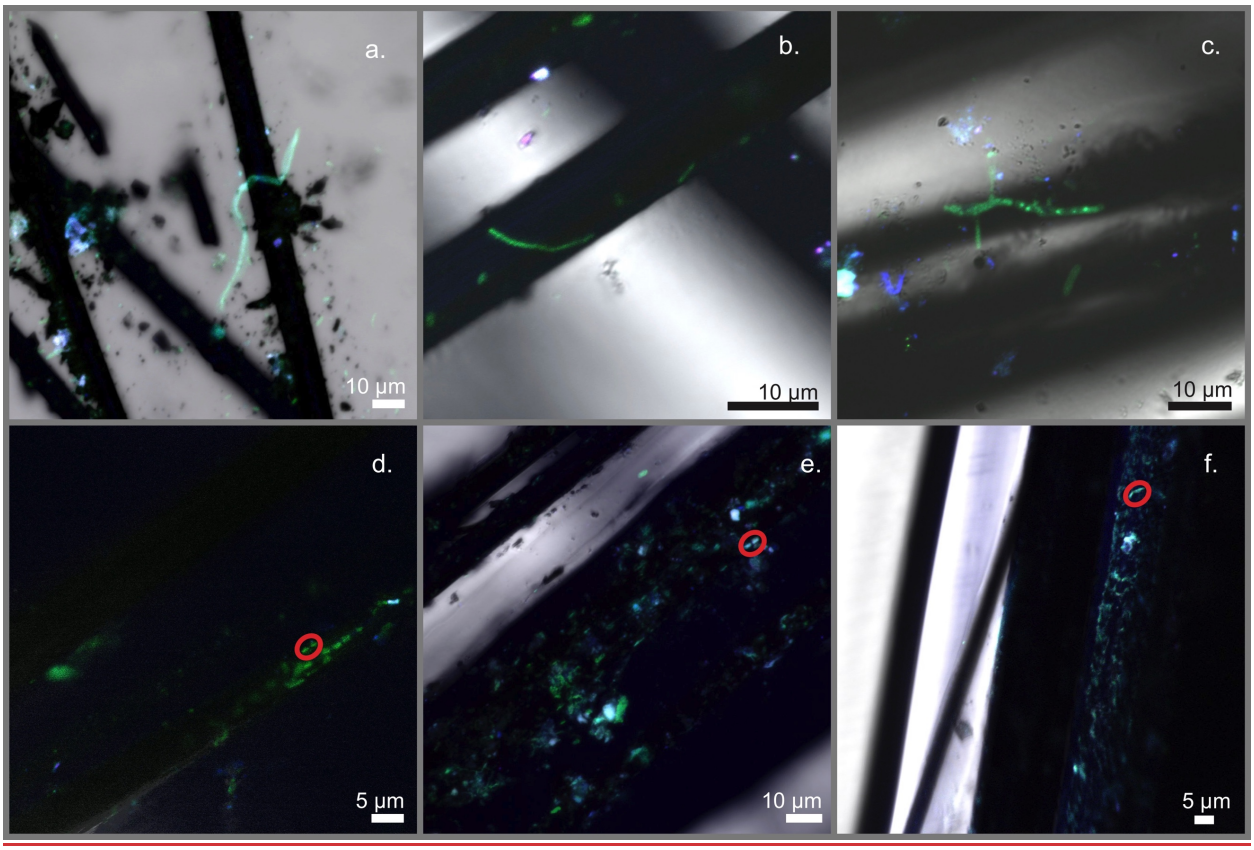
Fig. 6



570

~~Fig. 7~~


a.
10 µm
b.
10 µm
c.
10 µm
d.
5 µm
e.
10 µm
f.
5 µm


a.
10 µm
b.
10 µm
c.
10 µm
d.
5 µm
e.
10 µm
f.
5 µm