

Comment from Reviewer #2:

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? 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 conclusions. The techniques were used in the sediment studies, why where they not applied on in the experiment? 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. 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.

Response: 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 than 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.

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 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.

Thirdly, we thank the reviewer for pointing out our mistakes when sorting references. We did mean to cite Risgaard-Petersen 2012, 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 S⁰, H₂, and organic acids like pyruvate, lactate, and propionate.

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.

Changes in manuscript: 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. 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. 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. 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.