Response to Referee #2

General comments

The manuscript addresses bio mineralization associated with cable bacteria, a newly discovered group of filamentous bacteria within the *Desulfobulbaceae* family that performs electrogenic sulfide oxidation. Using a series of advanced microscopic techniques, the authors investigate the minerals formed within cells in the filaments or on the exterior of cable bacteria harvested from sediment-based enrichment culture. The authors identify the presence of polyphosphates within the cells, the presence of external coatings composed of EPS and (probably) clay minerals; and the presence of external encrustations of iron oxy hydroxides. The findings are discussed primarily in relation to the eco-physiology of cable bacteria, which makes the paper relevant for the community of researchers dealing with these aspects. However, the work also addresses more general aspects of biomineralization, through the focus on model organisms, which through its peculiar metabolism has significant influence on many geochemical pathways. Moreover, this organism has been shown to be abundant in many aquatic sediments and therefore I think that the study would be relevant and interesting also for readers of BG that are not directly associated to cable bacteria research. In general, I find the manuscript well prepared, with (mostly) proper citing (see my point 3) and credit to related work. To my knowledge, the methodology used is sound and I find no reason to doubt the results and the interpretations of raw data. I therefore recommend publication of this manuscript.

Answer to general comments

We would like to thank the author for his/her attention to detail and the positive recommendation Each of his/her suggestions is discussed individually below.

Comment 1

When discussing the encrustation the authors refer to the LPS of gram-negative bacteria, as the cable bacteria are gram-negative bacteria. My question to this end is what is known about the composition of the outer membrane of cable bacteria i.e. the common membrane that encapsulates all individual cells in the filament? Is there any evidence that this membrane is composed from LPS? Note that we can easily image that the individual cells in the filament has both an inner and an outer membrane composed as for gram-negative prokaryotes and that the common outer membrane is composed differently than from that? Until more knowledge about the composition of the outer membrane is known, I do not think that the authors cannot make firm conclusions about the relationship between iron precipitations and the membrane properties and I encourage the authors to tone it down.

Answer to comment 1

At the moment not much is known about the chemical structure of the common membrane of cable bacteria and we do not have any evidence that this membrane is composed of LPS. So this needs to be investigated. However, in our manuscript, no claims or conclusions are being made about the relationship between iron precipitation and membrane properties (such as the presence of LPS). We do, however, present a hypothesis that might explain the precipitation of iron minerals containing Ca, Mg and Si that was observed and we believe it is a valid hypothesis in this context.

To clarify to the readers that no research has been done on the chemical composition of the outer membrane, a sentence will be added to the paragraph that starts at L.476:

"Although the structure and composition of the common outer membrane shared by all cells in a cable bacterium filament is not known, gram-negative bacteria typically possess an outer membrane that forms an asymmetric lipid-protein bilayer which incorporates phospholipids, lipopolysaccharides (LPS) and proteins, and separates the external environment from the periplasm."

We will temper our claim by not mentioning phosphoryl and carboxyl surface groups in the final paragraph of our discussion. It will be rewritten as follows:

"It appears that the metabolism of cable bacteria results in a cascade of reactions that eventually results in the uncontrolled mineralization of filaments that are present in the oxic zone. The cell surface provides a nucleation site and template for mineral formation, and the increase of the pH in the oxic zone as a result of the electrogenic metabolism of cable bacteria, favors Fe-mineral precipitation and growth. Since the mineral precipation does not appear to be controlled by the cable bacteria, it forms an example of biologically induced mineralization. The formation of a mineral crust on a cell surface could potentially limit cell metabolism and may eventually lead to cell death (Konhauser, 1998a; Schädler et al., 2009). However, the extent to which this affects cable bacteria is currently unknown and so the impact of encrustation on cable bacteria metabolism needs to be resolved."

Comment 2

In the discussion on the mechanics behind the formation of iron (oxy) hydrates encrustations on the cable bacteria the work of Otte et al. 2018 is used as a model for explanation of crust formation. This model assumes direct electron transfer between cable bacteria and iron oxidizers present in anoxic sediment strata and as a consequence formation of iron (oxy) hydrates in the absence of oxygen. Perhaps this can occur but is really documented sufficiently well to be used as an explanation for the observation that some cable bacteria are covered with iron (oxy) hydrates? I do not think so. More over as I read the methods section, cable bacteria for this analysis were collected from the oxic zone of the sediments and there you do not need anything more than well-known geochemistry to explain the formation of iron (oxy) hydrates. So I suggest that the authors tone down the more exotic explanations and choose the most simple model: that the iron (oxy) hydrates are formed through well-known reactions between O2 and Fe2+ in the oxic zone.

Answer to comment 2

We agree fully with the referee on this point. We do not think there is enough evidence for interspecies electron transfer from cable bacteria to iron oxidizers. Effectively, our reference to Otte et al (2018) was intended to signal the co-existence of cable bacteria and iron-oxidizing bacteria, but not intended to support their model of interspecies electron transfer.

We mention first (l. 509 – l. 513):

"Iron (oxyhydr)oxides are widespread and form in any environment where Fe^{2+}-bearing waters come into contact with O_2 (Konhauser and Riding, 2012). In electrogenic sediments, the formation of an iron oxide crust has been observed in both laboratory experiments (Risgaard-Petersen et al., 2012; Rao et al., 2016) as well as in-situ (Seitaj et al., 2015; Sulu-Gambari et al., 2016). It is an example of biologically induced mineralization (Lowenstam and Weiner, 1989) resulting from the metabolic activity of the cable bacteria and the subsequent availability and re-oxidation of the Fe^{2+} ions in the oxic zone."

Next we discuss whether the oxidation of Fe^{2+} in the **oxic zone** is biotic or abiotic for which we use the co-existence of cable bacteria and iron-oxidizing bacteria found by Otte et al. (2018) as well as the presence of stalks of *Gallionella spp*. as an argument to hypothesize that the oxidation of iron in the oxic zone is most likely biotic. We do not mention any of the models discussed by Otte et al. (2018).

To clarify this, we will rewrite this paragraph as follows:

“At pH values above 8, the oxidation rate is fast, but no longer varies with the pH. The rate of oxidation is both thermodynamically and kinetically enhanced by adsorption of dissolved iron species to hydrous oxide surfaces (Morgan and Lahav, 2007). For the Fe^{2+} oxidation to be biotic, Fe^{2+} oxidizing bacteria need to outcompete the abiotic reaction. The twisted stalks of the Fe^{2+} oxidizing *Gallionella* spp. have been found in samples of encrusted cable bacteria (Fig. 8d) showing that, despite the high pH values in the oxic zone, Fe^{2+} oxidizing bacteria (partly) outcompete the abiotic reaction. There is also direct evidence for the co-existence of active cable bacteria and Fe^{2+} oxidizing and Fe^{3+} reducing bacteria in sediments representative of typical marine environments (Otte et al., 2018). Whenever cable bacteria were abundantly present (0.1%-4.5%), both Fe^{2+} oxidizing and Fe^{3+} reducing bacteria were homogeneously distributed throughout the sediment and their presence was therefore decoupled from the traditional geochemical gradients (Otte et al., 2018). After oxidation, ferric iron (hydr)oxides are expected to precipitate more or less instantly at the alkaline pH values in the oxic zone due to the low solubility of Fe^{3+} under these conditions. The alkaline pH value in the oxic zone is the result of the separation of two redox half-reactions in electrogenic sulfur oxidation by cable bacteria (Fig. 1). Precipitation most likely occurs directly where the Fe^{3+} is formed and because of the proximity of cells, Fe^{3+} ions, Fe^{3+} complexes, Fe^{3+} colloids and Fe^{3+} minerals are expected to adsorb to prokaryotic cell surfaces that are generally effective sorption interfaces for metal ions (Beveridge, 1999; Ferris et al., 1987; Fortin et al., 1997) as well as negatively charged silicate ions (Schultze-Lam et al., 1996).”

Comment 3

There are some references to unpublished work (e.g. Cornelissen. subm.) and I suggest that these are taken out of the manuscript. In my view the information the Cornelissen. et al. subm. Paper, as cited in the manuscript does not contribute to an understanding of the data as it apparently deals with the internal structure of the cable bacteria. Encrustation (the topic of the paragraph) is related to external structure – i.e. the outer membrane. Please also be aware that all information related to this is sufficiently well described in the Pfeffer et al 2012 paper, and that the Meysman 2018 paper, which also is cited along the line of description of the cellular structures (l.453) does appear in the reference list. Here only Meysman 2017 appears and this is a review that does not add more information to the topic, than already described in the primary literature.

Answer to comment 3

The paper submitted by Cornelissen et al. has now been published in Frontiers in Microbiology, so the reference will be changed in the finalized version of this manuscript. It deals with the structure and morphology of cell envelope, both internal and external, It expands the knowledge that was presented by Pfeffer et al in 2012, by providing a quantitative model of outer surface morphology by means of extensive atomic force microscopy measurements (for different types of cable bacteria). Therefore we believe that a reference to “Cornelissen et al.” is justified. We will change all references to “Cornelissen et al.” to “Pfeffer et al. and Cornelissen et al.”.

The reference to Meysman 2018 will be removed as well as the incorrect reference to Meysman 2017.