

Interactive comment on “Microbial Community Function in Electroactive Biofilm-based Constructed Wetlands” by Carlos A. Ramírez-Vargas et al.

Anonymous Referee #2

Received and published: 5 February 2019

This manuscript examines the carbon substrate usage of different coke-filled water treatment laboratory columns (so called METlands), as a model for electrically conductive constructed wetlands for water treatment. The authors examine two different putatively electrically conductive coke substrates and the effect of columns with or without added plants. The study samples these columns at five depths and uses this sample to inoculate Biolog Ecoplates, 96-well plates where each well is loaded with an individual carbon substrate and a tetrazolium dye to indicate metabolic activity. Plates were incubated aerobically for two days and then analyzed. I cannot recommend this paper for publication in Biogeosciences for a number of reasons.

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1) This experimental set up is problematic in that it introduces a strong culture-bias: only organisms capable of being cultured in the provided media at ambient oxygen concentrations will grow and be assayed for their carbon utilization. Given that their experimental columns are suboxic (~ 4 mg/L) and that their entire premise is that the electrical conductivity of these columns stimulates activity, it is really not clear to me what the significance of their findings using an aerobic culture-based assay will be. Is this type of analysis done in other studies? Absolutely. However, I would argue that it's biogeochemical relevance to in situ processes is minimal and does not merit publication in a journal focused on biogeochemical transformations

2) The focus on this paper is very much on identifying phenotypic differences between these different water treatment systems, and much less on the biogeochemical significance of this engineered system. As such, this paper seems like it would find a better audience in a more applied or engineering-focused journal than in Biogeosciences.

3) The written English throughout is in need of revision and does not meet publication standards in its current form. It would benefit significantly from the assistance of a professional editor.

4) Regrettably, I am unable to find important cited paper p2. Ln 35: Esteve-Nuñez, A., 2015. Electricity-generating bacteria. *Bioelectrogenesis: sustainable biotechnology*. International Innovation, 181, 109-111. The webpage for the journal is no longer available: <http://www.internationalinnovation.com/thank-you/> and I am unable to find any record of this journal in our library system. I suggest removing this citation or providing a link to where it could be found. Their other citation regarding METlands, Aguirre-Sierra et al. 2016, was readily available from Environmental Science Water Research & Technology and would be a better reference to use here as it describes the behavior of the system well.

It is somewhat unclear to me, though, how or whether the authors confirm the coke substrates they use are in fact electrically conductive, and whether this electrical con-

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ductivity is directly responsible for the observed microbial phenotypes. However, as the engineering and electrochemistry of this system is outside my field, I leave this matter to the other reviewers' and editor's discretion. I instead focused on chemical and microbial aspects of the paper.

Comments for improving manuscript: In general, the authors' use of acronyms is distracting. While certainly a few are appropriate for brevity and may well be the convention in their more engineering fields, for many readers the constant rechecking of different acronyms detracts from their overall flow. When using acronyms, be sure it has been defined previously. Examples of acronyms that, in my view, detract more than they add are listed here: CLPP, CSUP, EAB

I have no idea what is being plotted in Figure 1a. What are each is the significance of plotting an aggregate of the response by carbon use guild as a function of depth? I have read p. 6 ln 3 – 8 and p.7 ln 18-21 multiple times trying to understand what was done here, but it remains unclear. Why pool by guild as opposed to plotting each carbon source separately and coloring points by guild? Why is the depth the variable being examined? How were samples “pooled” across columns?

Clarify if the points in PCA of Fig 1b are each individual samples collected from the 12 columns at the 5 depths

Minor Issues: p.1 ln 13-14: sentence is grammatically unclear p.1 ln 22: sentence is grammatically unclear p.1 ln 23: incomplete sentence, revise p.2 ln 2: Define COD & BOD p.2 ln 3: EAB not defined p2. ln25: “anoxic/aerobic” is confusing in this context. Clarify whether you mean it can be both and under which conditions? p.3.ln 7: “Most of these microorganisms” not “most of this microorganism” p3. ln 11: reference formatting errors; “genera” not “genus” p.3. ln 30 – 35: sentence is awkwardly phrased, consider revising. After this point, I stopped marking typographical and grammatical issues – suggest that the services of a professional editor or writer would be of use to the authors.

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