Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2018-428-AC2, 2019

Biogeosciences
Discussions

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

Carlos A. Ramírez-Vargas et al.

c.a.ramirez@bios.au.dk

Received and published: 25 February 2019

Dear Referee 2, thank you very much for positive comments and suggestions to improve our manuscript. Below you will find our the reply to them. 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. R// Dear reviewer, thank you very much for your comments and suggestions to improve our manuscript. With the explanations given below, we intent to demonstrate that the manuscript can be published in Biogesocience.

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 (âĹij4 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 R// The microbial functionality characterization based on community level physiological profile (CLPP) analysis, has been reported for constructed wetlands in several peer review papers (Osem et. al. 2007; Weber et al., 2007; Faulwetter et al., 2009; Weber and Legge, 2009; Zhang et. al., 2019 Button et. al. ,2015; Button et. al. ,2016; Lv et. al., 2017; Zhang et. al., 2018), and none of them have reported any anomaly regarding the incubation under room temperature, atmospheric conditions nor oxygen saturation conditions. Even though the eco-plates used in the study are incubated at such conditions, it is important to remark that most of the bacteria existing inside constructed wetlands, including electroactive bacteria, are facultative and not exclusively anaerobic, therefore they can develop without problem in the EcoPlates. Also, it is important to mention that inside water-saturated constructed wetlands is common to find gradients of oxygen concentration, as well as micro-environments with aerobic conditions (specially under the presence of plants), that serve as shelter for development of facultative bacteria, therefore is common to find microbial consortia of aerobic-facultative-anaerobic

bacteria.

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. R// As authors, we disagree with this statement. It is important to clarify that the focus of this study was to assess the impact of different electro-conductive materials on the microbial community function of planted and non-planted METland set-up fed with real wastewater, not phenotypic differences between systems. The microbial functionality was assessed based on carbon source utilization patterns (CSUP), derived microbial metabolic indexes (average well color development – AWCD; richness; diversity), and carbon utilization. It is important to ratify that the our manuscript matches with the aims and scope of Biogeosciences (BG) journal: “BG is an international scientific journal dedicated to the publication and discussion of research articles, [. . .] on all aspects of the interactions between the biological, chemical, and physical processes in terrestrial or extraterrestrial life with the geosphere, hydrosphere, and atmosphere. The objective of the journal is to cut across the boundaries of established sciences and achieve an interdisciplinary view of these interactions. Experimental, conceptual, and modeling approaches are welcome”. Besides, our manuscript is embedded in the subject areas of "biodiversity and ecosystem function", "environmental microbiology", and the "interactions between microbes, organic matter sediments, and rocks".

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. R// Thank you very much for your suggestion. The written English in the manuscript is under review, and under correction by an English 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. R// Indeed the mention paper is not accessible online, given that the mention online journal is no longer available. Therefore, the reference was replaced by Aguirre-Sierra et. al. 2016 and Ramirez et. al. (2018), that explained in a detailed way the METland operation.

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 conductivity 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. R// PK-A and PK-LSN are electro-conductive materials, fact that was verified in laboratory test and reported in Ramirez et. al. (2019). In Table 2, are summarized the characteristics of the materials used in the experiment. On it is include the parameter “Resistivity”. This parameter denote the degree of resistivity of the tested materials in Ω-m. The values of both electro-conductive materials (PK-A and PK-LSN) are very low, therefore indicating that they have a high electrical conductivity. To clarify the link between the electroconductivity and the development of more specialize bacteria communities, the reference Ramirez et. al. (2019) was included in the document. In the mentioned study, apart of assessing the removal rates of organic matter and nutrients and the derived removal rate kinetics, also includes the measurement of electric potentials and the estimation of ionic/electron fluxes, as indicator of the presence of electroactive microbial communities in the same tested materials. In that study, it can be seen how the material PK-A has the highest ionic/electron flux, and the highest performance in terms of organic matter removal, with a high correlation

between both parameters. This information is complementary to the findings done by Aguirre-Sierra et al. (2016), Li et al. (2016), J. Wang et al. (2017), F. Xu et al. (2018) that report biofilms mainly composed by genera with high proportion of electroactive bacteria (Page 3, lines 8-12), as well as by findings reported by several authors in Page 9 Lines 3-13.

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. R// Thank you very much for your comment. The "EAB" acronym was change for "electroactive bacteria" in Page 3, Line 1. That change was already asked by the editor in a first review. The changes were done, but it seems that the acronym that you mentioned we forgot to change it. The other acronyms like CLPP (community level physiological profile), CSUP (carbon source utilization pattern), average well color development (AWCD), are wide accepted and use for the mentioned methods in literature specialized in the topic. They are developed in the abstract as well as in the introduction sections. 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? R// Fig.1 is the graphical result of the Principal Component Analysis done to the data collected for the samples of all the systems. As explained in the section 2.6 Statistical analysis (Pag. 7 line 6 to 11), the "PCA is a multivariate analysis method that ease the study of multidimensional datasets of quantitative variables (carbon guild utilization), by projecting them in a 2-dimensional chart (factors). The resulting PCA chart allows to visualize the objects under study (tested systems),

and depending on their position on the charts, let to establish their relationship with the quantitative variables. The correlation between variables under study can be determined by the angles formed among them. Acute angles indicate that the variables are correlated, right angles indicate that the variables are not correlated, and obtuse angles indicate that the variables are inversely correlated". In summary, both graphs depicted in Fig.1 allow to understand how are the preferences of the microbial communities of the different systems for the consumption of different carbon guilds. Figure 1a and Figure 1b are complementary graphs. Fig. 1a depicts the carbon guilds as main variables and level (inside the tested system) as a complementary variable; Fig 1b represent all the samples collected in the columns, and are included the centroids as summary of the information in each column. The interpretation of Fig.1a and 1b and their relation is explained in section 3.1. The variable level was included as complementary variable in the analysis, since it was expected to occur different consumption patterns of the carbon guilds along the flow pathway, as is reported in section "3.3 Effect of vertical profiles on carbon source utilization" To ease the dimensionality of the data and subsequent analysis and interpretation, the 31 carbon sources were grouped in 5 different carbon guilds, as it has been reported in different studies CLPP in constructed wetlands (Osem et. al. 2007; Weber and Legge, 2009; Button et. al. ,2015; Button et. al. ,2016; Lv et. al., 2017; Zhang et. al., 2018).

Clarify if the points in PCA of Fig 1b are each individual samples collected from the 12 columns at the 5 depths R// Yes, each dot in Fig. 1b accounts for each individual sample collected in the 16 tested columns (6 different systems; see section "2.1 Experimental set-up") at 5 different depths (section "2.2 Sample collection and biofilm detachment and CLPP inoculation").

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, R// By suggestion of one of the reviewers, the abstract was modified, therefore the grammar mistakes that you mention were corrected.

Revise p.2 ln 2: Define COD & BOD p.2 ln 3: EAB not defined R// Chemical oxygen demand (COD) and biological oxygen demand (BOD5) were defined; EAB was change for “electroactive bacteria”.

P2. Ln25: “anoxic/aerobic” is confusing in this context. Clarify whether you mean it can be both and under which conditions? R// In constructed wetlands is common to find aerobic/anoxic/anaerobic microenvironments, depending on the performance state of the wetland. On the upper part of the system (close to the atmosphere), the aerobic environments can happen due to the diffusion of oxygen from the atmosphere, as well as due to the release of oxygen from the plant rhizosphere. Whereas the anoxic environments can prevail if there are low oxygen concentrations and active nitrification processes inside the system. This information has been broadly explain in literature specialized in constructed wetlands design.

P3.ln 7: “Most of these microorganisms” not “most of this microorganism” R// The change was done in the manuscript.

P3. Ln 11: reference formatting errors; “genera” not “genus” R// The change was done in the manuscript. P3. ln 30 – 35: sentence is awkwardly phrased, consider revising. R// The sentence was rewritten as follows: “The most common plate used for CLPP analysis is the Biolog® EcoPlate. This plate contains 31 relevant environmental carbon sources, that ease the differentiation of soil microbial communities, as well as known to be derived of plant root exudates (Insam and Goberna, 2004). Carbon sources of the Biolog® EcoPlate can be grouped by composition into carbohydrates, polymers, carboxylic acids, amines/amides, and amino acids (Weber and Legge, 2009). This grouping represent a large degree of compounds, and depending on their consumption by microorganisms, can simplify the understanding of the functionality of the microbial communities involved in the removal of pollutants in wastewater treatment systems such as in constructed wetlands (Button et al., 2016).”

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. R// Thank you very much for your suggestion. The manuscript is under review and correction by an English professional editor.

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-428, 2018.