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

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Dear Referee 1, thank you very much for positive comments and suggestions to improve our manuscript. Below you will our the reply to them. 1. Abstract. The abstract is perhaps too much generic. More detailed results about the results about the function of the microbial communities have to be included. R// Indeed the abstract in the current state is too generic, therefore it was modified including the most relevant results of the study. The abstract was modified as follows: "Abstract. The performance enhancement of constructed wetlands can be achieved through the coupling with microbial electrochemical technologies (MET). MET is a setup designed to mimic metabolic electrons

exchange with insoluble donors and acceptors with the aid of electroactive bacteria and external electrical circuits. An alternative MET that dispenses of electrodes and circuits but uses an electro-conductive biofilter is called Microbial Electrochemical-based Constructed Wetland (METland). The biodegradation rates of METlands are higher than those of horizontal flow constructed wetlands, however given its novelty there are still uncertainties related to the removal of pollutants, including their microbial activity. The genetic characterization of microbial communities of a METland is desirable, but is time and resource consuming, then a characterization alternative can be based on functional analysis of the microbial communities. Community-level physiological profile (CLPP) is a useful method to evaluate the functional diversity of microbial communities based on the carbon source utilization pattern (CSUP), and derived indexes (average well color development - AWCD, richness and diversity). Therefore, this study was focused on the characterization of the microbial community function of laboratory scale METland based on CLPP analysis. The study included the analysis of two carbon-based electro-conductive materials, (calcined petroleum coke from crushed electrodes – PK-A; calcined petroleum coke with low sulphur and nitrogen content – PK-LSN), colonized with mixed biofilms (including electroactive bacteria) in planted and non-planted set-ups. Variations on the microbial metabolic activity of tested systems were identified and it seems to be related to the characteristics of the material, rather than the presence/absence of plants. In general PK-A systems showed lower values of AWCD, richness and diversity, compared to sand and PK-LSN systems. This suggest that PK-A systems, provided favorable conditions for the development of relatively homogeneous microbial biofilms. These homogeneous biofilms showed a higher consumption of "carbohydrates" and "carboxylic and acetic acids" compared to more heterogeneous biofilms of sand and PK-LSN systems. CSUP showed differences along the flow pathway of tested systems, with a higher consumption of "carbohydrates" and "carboxylic & acetic acid", by microbial communities at inlet zones of tested systems, whereas more complex sources like "polymers", showed a higher consumption at outlet section. Also, were established some correlations between the utilization of carbon sources and

the removal of pollutants. Denitrification seems to be associated to the consumption of “amines/amides”, “amino acids”, and “polymers”, which were consumed at higher rates at the outlet section of the systems. Biofilms inclined to consume “polymers”, were involved in a high extend in the removal of BOD5 and TOC, whereas those prone to consume “carbohydrates” were involved in the removal of COD as well as TOC.” The obtained results provide useful insight into the spatial dynamics of the microbial activity of METland systems.”

2. Introduction. A more detailed explanation on next sentence “The genetic characterization of microbial communities of constructed wetlands is desirable, however all microorganisms inside the systems must be identified, task that is time and resource consuming, and may not be of much interest from the engineering point of view” has to be included. Genetic characterization can be a source of important information on the microbial communities diversity and therefore, about the abundance of different functional groups. Even with recent Illumina sequencing techniques, a fast results can be obtained. On the other hand, microbial molecular analyses with PCR real time of functional genes give today, relevant information about the general performance of any CW. More information and references about that aspects have to be included in that part. R// It is desirable to carry out a genetic characterization of the microbial communities inside the tested systems. However, the intention of our study was to implement an economic, quick and reliable method to characterize of the microbial communities in terms of functional diversity and activity in terms of carbon sources used inside the tested METlands. We propose to re-write the paragraph in the following way: “The complete genetic characterization of microbial communities of constructed wetlands is desirable, however this task is time and resource consuming, requires specialized expertise and may not be useful from the engineering point of view (Weber et al., 2008). From the design and operation context, a functional characterization of the microbial communities in a constructed wetland can be more useful, since they provide information related to the types of compounds that may be treated, and possible, utilization rates (Weber 2011)”.

3. Materials and methods. Which water flow was used? R// The systems were operated at a hydraulic load rate (HLR) of 104 L m-2 d-1. The information is going to be included in the section methods as well as in Table 2.

4. Results and Discussion. Review the correct use of verbal forms in English. R// The grammatical structure of manuscript is under review and modification where necessary. Besides, the manuscript is going to be reviewed and corrected by an English professional editor.

Rewrite this sentence: Differences on the microbial community functionality between planted and non-planted systems, studies analyzing the change in Microbial community structure in aerated constructed wetlands (Osem et al., 2007) have been reported, treating tannery wastewaters (Calheiros et al., 2009), as well as in full scale constructed wetlands treating domestic wastewater (Zhang et al., 2010). R// Thank you very much for your recommendation. This was a typo in the writing process. The sentence was rewritten as follows: Differences on the microbial community functionality between planted and non-planted systems have been reported. Examples of this differences has been reported for aerated constructed wetlands (Osem et al., 2007), for constructed wetlands treating tannery wastewater (Calheiros et al., 2009), as well as for full scale constructed wetlands treating domestic wastewater (Zhang et al., 2010).

The section 3.3 and 3.4 has to be improved, especially in its written English. Rewrite this sentence “Easy degradable carbon sources, such as carbohydrates are preferred by microorganism as primary source of energy, and usually are consume at early stages of the flow pathway inside systems (Salomo et al., 2009).” Improve this sentence: “In the case of polymers, it the opposite occurred, with relative low consumption in the inlet section and higher consumption at the outlet section of the tested systems”. R// The grammatical structure of manuscript is under review and modification where necessary. Besides, the manuscript is going to be reviewed and corrected by an English professional editor. As suggested, the mentioned sentences were rewritten as follows: “Easy degradable carbon sources, such as carbohydrates, are preferred by

microorganism as primary source of energy, therefore usually are consumed early in the treatment stage, at the water inlet section of constructed wetlands (Salomo et al., 2009)". "In the case of polymers, the consumption showed a different pattern, with relative low consumption at the water inlet section, with relative high consumption at the water outlet section of the tested systems".

Improve this sentence, "In parallel to the microbial metabolic activity, were measured the effluent water quality parameters (pH, electrical conductivity, temperature, dissolved oxygen and Redox potential), and were estimated the removal rates of organic matter (BOD5, TOC and COD), nutrients (NO3-N, NH4-N, TN and PO4-P) and TSS. . .". R// The sentence was rewritten as follows: "In parallel to the characterization of the microbial metabolic activity, in-situ water measurements namely, pH, electrical conductivity, temperature, dissolved oxygen and redox potential were measured along with the lab parameters. Likewise, the removal rates of organic matter (BOD5, TOC and COD), nutrients (NO3-N, NH4-N, TN and PO4-P) and TSS were calculated".

Rewrite and improve this sentence: "Given that the main removal pathway of NO3-N is denitrification a carbon source is required (Dotro et al., 2017)". R// The sentence was rewritten as follows: "The low removal rates of NO3-N can be explained by the low nitrification rates occurring in the system, and the relatively low concentration of organic matter (expressed as BOD5) is insufficient to sustain denitrification. Both, nitrification and organic matter availability are indispensable for the occurrence of an effective denitrification process (Dotro et al., 2017)".

5. Conclusions. The conclusions paragraph has to be improved in its written English. R// The grammatical structure of the conclusions was reviewed and corrected. The paragraph was rewritten as follows: "To the best of our knowledge there are not peer-reviewed publications reporting the analysis of microbial metabolic function in electroactive biofilm-based constructed wetlands. The assessment of the microbial metabolic function was done in a fast and reliable way with CLPP analysis, using BIOLOGTM EcoPlates. In the present study differences were identified in terms of the

metabolic activity indexes of systems using electro-conductive materials in comparison with control systems. PK-A filled systems showed lower values of AWCD, richness and diversity, in comparison with sand and PK-LSN filled systems. This suggests a positive impact of the material in the evolution of the microbial communities inside the electro-conductive systems. This means that the electro-conductive properties of PK-A systems, provided favorable conditions for the development of relatively homogeneous microbial biofilms. This is similar to what has been reported in microbial characterization studies of carbon-based electrodes operating for long periods of time (Aguirre-Sierra et al.,2016; J. Wang et al., 2017). Judging by the values of AWCD, richness and diversity, the planted systems had a slightly higher impact on the microbial metabolic function of tested systems, however, the registered differences were not significant between planted and non-planted system. The carbon guild utilization showed a general trend in all tested systems, with high consumption of "carbohydrates" and "carboxylic and acetic acids", followed by "polymers" and "amino acids", and in lower proportion by "amines and amides". Additionally, the carbon guild utilization showed differences along the flow pathway inside the systems, as well as among them. The trends in the consumption of carbon guilds, had an impact on the values of microbial metabolic indexes. The systems with relatively higher consumption of "amino acids", "amines/amides" and "polymers" (PK-LSN/P, PK-LSN/NP and Sand/NP), seems to have more heterogeneous microbial communities in comparison with systems with higher consumption of "carboxylic and acetic acids" and "carbohydrates" (PK-A/P, PK-A/NP and Sand/P). The consumption of carbon guilds is associated with the removal efficiency of the tested systems. NO3-N transformation seems to be associated to the consumption of "amines/amides", "amino acids", and "polymers", which were consumed at higher rates at the outlet section of the systems. The microbial communities inclined to consume "polymers", were involved in a high extend in the removal of BOD5 and TOC. Whereas the microbial communities prone to consume "carbohydrates" (in a higher proportion at the inlet zone of the systems), were involved in the removal of COD as well as TOC."

Specific technical comments Page 3. Line 11. Include this reference in the previous references. (Aguirre-Sierra et al., 2016). R// The inclusion of the reference was done.

Page 8, line 2. Add of. Promote the growth of microbial communities. Page 9. 27. Delete in. “The uniformity in the in carbon guild. . .”. Page 10. Line 18. Add s. “utilization of all carbon sources”.âĂĺ R// The changes were done in the manuscript.

Page 10. Line 19. Add s. “microorganisms”. R// The change was done in the manuscript.

Page 10, Line 34. Add from. “electrical conductivity varied from. . .”. R// The change was done in the manuscript.

Page 12, line 3. Change “The low TN removal is the results. . .”, by “The low TN removal is the result. . .”. R// The change was done in the manuscript.

Page 14, line 12. Change testes. R// The change was done in the manuscript.

This paper is really interesting for all researchers that are working today on the study of constructed wetlands. There are really few publications reporting the microbial metabolic activity function in an electroactive biofilm-based constructed wetlands. However, the authors should improve the written English, especially in the results and discussion and conclusions sections. My recommendation is to accept the manuscript after a minor revision, and obviously, taking care about the final quality of the English in the last version of the paper. R// Thank you very much for your positive comments and suggestions. Based on the suggested changes we will improve our manuscript and we will submit it to be reviewed and corrected by an English professional editor.

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