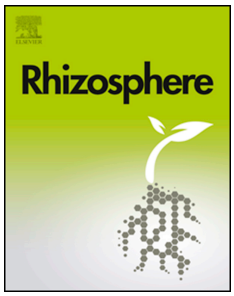

PCB decomposition promoted by sugarcane bagasse organic waste

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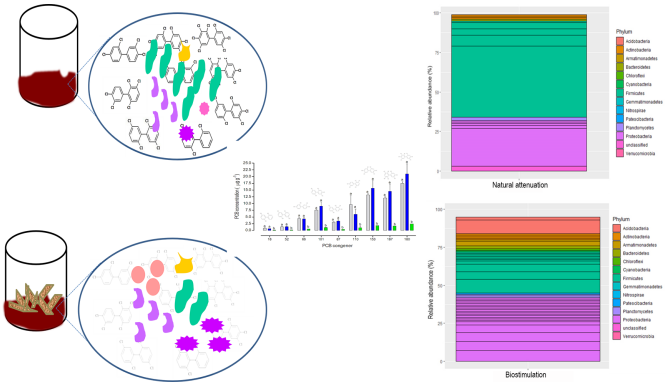

Sugarcane bagasse, a waste resulting from the extraction of sugarcane juice by the agroindustry, demonstrated to be an effective treatment of polychlorinated biphenyls (PCBs) contaminated soils [1]. This residue might be a support for cell immobilization and lignocellulosic substrate to microorganisms involved in the bioremediation of contaminated soils and, enhances the porosity and aeration of the soil with the consequent aerobic conditions to improve the biostimulation process [1, 2].

Genetic technologies were developed to analyze the entire microbial community and identify the dominant microorganisms in PCBs-contaminated soils [3], but little is known about the changes at the microbial community scale in biostimulation processes and at high concentrations of the pollutant. In our present study, we aimed to explore the dominant microorganisms under biostimulation treatment with sugarcane bagasse in high concentrations of PCBs. We hypothesized that sugarcane bagasse addition to PCB-contaminated soil may positively affect the bacterial composition with biodegradation capacity to remove this pollutant.

Non-polluted soil was collected from Posadas, (Misiones, Argentina), which was spiked with PCBs contaminated transformer oil with different chlorination grades. The PCB congeners and concentrations in soil were: PCB 18 (0.863 ± 0.096 μg g^{-1}), PCB 52 (1.349 ± 0.060 μg g^{-1}), PCB 66 (4.412 ± 0.701 μg g^{-1}), PCB 87 (3.103 ± 0.131 μg g^{-1}), PCB 101 (7.531 ± 0.209 μg g^{-1}), PCB 110 (9.628 ± 6.839 μg g^{-1}), PCB 153 (13.157 ± 0.634 μg g^{-1}), PCB 180 (17.469 ± 1.182 μg g^{-1}), PCB 187 (12.035 ± 0.900 μg g^{-1}), to reach a total PCB concentration of 69.547 ± 9.799 μg g^{-1}. The moisture content was adjusted to 60% (w w^{-1}) of their respective water-holding capacities and, arranged in biostimulation treatment with sugarcane bagasse as previously described [1]. Non-amended soil (containing only sterile distilled water and PCBs) was used to verify natural attenuation. The experiment was performed in triplicate under non-sterile conditions and incubated 90 d in darkness at 25 ± 1 °C, sterilized deionized water was added periodically to keep 75% (w w^{-1}) moisture content constant. Arochlors concentrations were obtained using a Shimadzu QP-2010 chromatograph with a

mass detector (GC-MS), equipped with a ZB-5Msi capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness) [1].

Genomic DNA from soil was extracted using NucleoSpin® soil kit (Biocientífica SA, ARGENTINA) following the manufacturer's recommendations and sent to Macrogen Inc. (Seoul, Republic of Korea) for PCR, amplicon library construction and sequencing. Primers Bakt_341F (5'-CCTACGGGNGGCWGCAG515F-3') y Bakt_805R (3'-GACTACHVGGGTATCTAATCC-5') were used to amplify the variable V3-V4 regions of the 16S rRNA gene. Mothur v.1.22.2 was used to denoise, trim, filter and align sequences, find chimeras, and assign sequences to OTUs (at 97% and 95% similarity). First, quality-filtered sequences were separated by primer and barcode and then trimmed. Chimeras identified with the 'UCHIME' algorithm were also removed [4]. The filtered reads were also used for generating a distance matrix and then clustered into OTUs (defined at 3% cutoff) by average neighbor linkage. Then, OTUs were classified using the SILVA seed database v. 132. Soil bacterial composition relative abundance graphics were performed in RStudio Version 1.2.5033 with the Phyloseq package. Bacterial alpha-diversity was determined by species richness (Chao1) and diversity (Shannon) indexes.

The concentration of the different congeners was significantly reduced in the biostimulation treatment with sugarcane bagasse (88 %) compared to the natural attenuation treatment and the control soil ($p < 0.01$). The removal of PCB congeners in biostimulation treatment was independent of their chlorination grade ($p > 0.01$) (Fig. 1), in contrast with other findings [5, 6]. The addition of sterilized sugarcane bagasse to PCB-contaminated soil increased significantly the contents of organic carbon and oxidable organic matter, and decreased available phosphorus content significantly, while pH was similar to both samples [1].

Figure 1. PCBs congener concentration in control soil at 0 d (gray bars), soil treated by natural attenuation (blue bars) and biostimulation (green bars) at 90 d. Concentration is expressed as µg in g OC of soil. Means with different letters are significantly different ($p < 0.01$).

The Chao1 index showed significant differences between biostimulation and natural attenuation, 141 and 20 respectively ($p < 0.01$). Shannon index was significantly higher at biostimulation treatment compared to natural attenuation, with values of 3.4 and 1.2 respectively ($p < 0.01$). The presence of pollutants usually decreases microbial diversity, due to the selection pressure that favors microorganisms with the ability to tolerate and/or degrade such pollutants [7]. In this sense, the addition of sugarcane bagasse for biostimulation may have also created an impact on the soil biota, resulting in higher diversity indexes than those shown by the natural attenuation and it is agrees with the results of other biostimulation treatments of PCB-contaminated soils [5, 8].

In addition, the increase in bacterial community could be related to physicochemical parameters like organic carbon and oxidable organic matter which influences bacterial metabolic processes and the mineralization of organic matter and possibly of the PCBs (as C source). It was proposed that the high content of organic carbon promoted the growth of microorganisms [3, 6]. Additionally, soil dehydrogenase activity (DHA) was significantly higher in the biostimulation compared to natural attenuation, suggesting that PCBs or their metabolites were likely used as substrates by the microbial community [1]. Similar to our findings, it was found that the DHA has enhanced in sugarcane bagasse-derived biochar in cadmium and chromium-contaminated soil compared with soil without biochar application [9].

Taxonomic identification of the OTUs revealed a total 91 bacterial genera, belonging to 13 different phyla. In biostimulation treatment, bacterial phyla were dominated by *Proteobacteria* and to a lesser extent by *Firmicutes, Acidobacteria, Actinobacteria, Bacteroidetes, Armatimonadetes, and Patescibacteria* (Fig. 2A). In concordance with other studies, biostimulation by the addition of inducers can lead to changes in the bacterial community structure [10]. Our results are in accordance with previous studies reporting *Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes and Firmicutes* is directly associated with the improvement of PCBs degradation [8, 10, 11, 12]. The main surviving genera in biostimulated soil were *Acidibacter*, *Acidipila*, *Acidipilia*, *Acidothermus*,

Actinomadura, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Ammoniphilus*, *Aneurinibacillus*, *Arenimonas*, *Sphingobacterium*, *Sphingomonas*, *Solirubrobacter*, *Singulisphaera*, *Shimazuella*, *Steroidobacter*, *Streptomyces*, *Streptosporangium*, *Bryobacter*, and *Burkholderia-Caballeronia-Paraburkholderia* (Fig. 2B), probably due to their specialized metabolism capable of overcome the selective pressure by the high PCB concentration [3]. *Arenimonas*, *Sphingomonas*, *Burkholderia*, *Streptomyces*, *Paraburkholderia* are linked with PCB degradation [13, 14, 15, 16, 17, 18, 19, 20].

Figure 2. Soil bacterial composition relative abundance at the (A) phylum level and (B) genus level in natural attenuation and biostimulation treated with sugarcane bagasse at 90 d ($p < 0.01$). A - N - P - R: Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium.

There were significant changes in the bacterial community structure caused by the bioremediation process, clearly indicating that PCBs polluted soil biostimulated with sugarcane bagasse contains unique PCBs-degrading bacterial communities to remove high concentrations of nine PCB congeners at different chlorination grades. However, further research is needed to explore the underlying mechanism of how this bacterial community participates on PCBs removal, which may contribute to finding appropriate PCBs bioremediation for soil.

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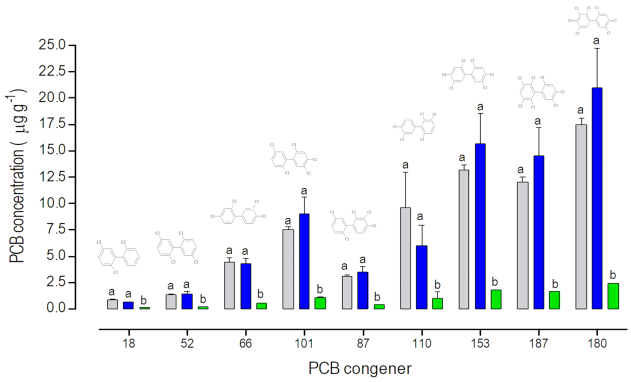

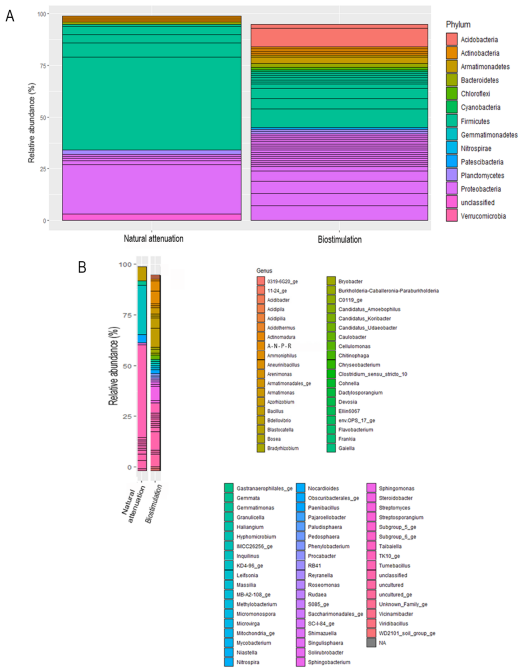

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: