Metagenomic Characterization of a Bioreactor with Polyhydroxyalkanoates and Biochar as Biomaterials to Prompt Reductive Dechlorination

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Background/Objectives. The development of new biotechnologies for the remediation of chlorinated solvents contaminated sites attracts research activities, in particular when the use of bio-based materials is proposed. Bio-based materials are capable of stimulating and supporting the reductive dechlorination (RD) with valuable implications for the development of novel bioremediation treatments in the circular economy. Some studies have demonstrated the effectiveness of the polyhydroxybutyrate (PHB), a bio-synthesized polyester completely biodegradable in the environment, as a slow-release electron donor suggesting that PHB can successfully sustain the RD also for in situ application in bioengineering systems. Also, organic waste-derived biochar (BC) has received attention as it can accelerate the electron transfer in several bioprocesses. BC is a carbon-rich bio-material BC, produced by the pyrolysis of diverse biomass species and commonly used for contaminants' adsorption and recently emerged as a good candidate for mediating electron transfer in contaminants biotransformation processes. Recently, bioreactors that employ PHB or biochar for remediation applications have been tested at laboratory scale. However, the composition and functions of the microbial communities established when PHB or biochar are used in a biological reactor for RD has been poorly investigated. In this study we describe the composition and metabolic features of the microbial community established in a column bioreactor that couples PHB and biochar as bio-based materials to prompt the RD of TCE-contaminated groundwater. The PHB and BC reactive zones were described via 16S rRNA gene and metagenome sequencing to investigate the central mechanisms occurring in the biofilm growing on the biomaterials used for the system.

Approach/Activities. Powder samples were collected from PHB and BC reactive areas of the column system after five months of the reactor operations, where TCE dechlorination was observed. *Dehalococcoides mccartyi* (Dhc) and reductive dehalogenase genes (tceA, bvcA, vcrA) have been quantified by digital droplet PCR (ddPCR). 16S rRNA gene amplicon sequencing has been performed for the microbial community characterization and a metagenomic analysis has been conducted to highlight the main functional features occurring on the PHB and BC reactive zones.

Results/Lessons Learned. In line with the kinetic performances of the reactor, Dhc established both in the PHB (1.46E+06 16S rRNA gene copies/g) and in the BC (5.51E+08 16S rRNA gene copies/g) reactive zones, suggesting for the first time BC as a vehicle for biological RD. Additionally, based on 16S rRNA gene amplicon sequencing, members of the *Clostridiaceae* (Clostridium, 48% of total ASVs) and *Victivallaceae* (15% of total ASVs) families, primarily involved in the fermentation processes, dominated the PHB zone, while Dhc represented 14% of total ASVs observed on the BC biofilm. In this study we report for the first time the growth of Dhc on the BC, and the establishment of the RD process associated with this biomaterial, primarily used as a sorbent for chlorinated ethenes. Our findings suggested that the BC's potential to prompt RD is appealing for the development of novel sustainable bioremediation technologies and the comprehension of the role of Dhc is crucial. The results of the genomic-centric analysis highlighted the main metabolic features of the PHB and BC biofilms of the column system and will be discussed in the presentations.