Department of Chemistry, Life Sciences and Environmental Sustainability

University of Parma - XXXIII cycle

Title of PhD research: "Biochar as sustainable nano-fertilizer"

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Summary of the second year of PhD research

Biochar is a carbon-rich structure obtained from wood material, agricultural by products, and vegetative biomass treated with heat technologies under limited or absent oxygen supply. This material has many applications such as waste reduction, energy production, water resource protection, and immobilization of contaminants in soil. Moreover, biochar has been increasingly proposed as a soil amendment for its demonstrated agronomic and environmental benefits. My project focuses on the possibility of using biochar as an innovative soil amendment, the evaluation of its life cycle assessment (LCA), and its sustainability in agricultural management.

I have three different samples of biochar, all deriving from broadleaf of different area of Emilian Appennines. They are all produced by gasification but at different temperatures and by employing different industrial plants.

Biochar in soil could be a source of contamination: a possibility that need to be evaluated by determining its physical chemical properties, as well as its biological and mutagenic ones, and its interactions with plant tissues.

During my first year, I focused on the determination of the physical chemical and biological characteristics of biochars' structures and, during my second year of PhD, I assessed the potential mutagenic effect of biochar trough Ames test. The latter is a short-term bacterial reverse mutation assay specifically designed to detect genetic damage leading to gene mutations induced by toxic compounds. The test employs several histidine dependent Salmonella strains each carrying different mutations in various genes of the histidine operon. These mutations act as a hot spots for mutagens that cause DNA damages via different mechanisms. When the Salmonella strains are grown on a minimal media agar plate with only traces of histidine, only those bacteria that revert to histidine independence (His+) are able to form colonies. Ames test was performed using PBS (water soluble) and DMSO (water insoluble) extracts, along with biochar as crystallin solid from the three sources of char. Four doses of each material were tested. After the count of reverted colonies, I evaluated the mutagenic index that is the ratio between the number of reverted colonies in treated plates and the number of reverted colonies in negative control plates. The Ames test on biochar extracts or on solid biochar revealed that there is no mutagenic compound within biochar.

Established this, I then focused on the functionalization of biochar. I worked with a collection of Plant Growth Promoting Rhizobacteria (PGPR): ten different microorganisms, bacteria and fungi, with different roles in the nitrogen cycle (nitrogen-fixating or nitrogen-reducing). The idea is to find consortia (a mix of microorganisms), happy to live together and that can improve the reactions of the normal N-cycle. First, I

established the relationship between all the chosen microorganisms via competition test and, according to the results I thought at all the possible *consortia* among microorganisms to perform the functionalization of biochar. Then, I evaluated if the microorganisms are able to colonize the biochar surface. The quantification of growth on the biochar surface was investigated via spectrophotometric analysis using XTT, a dye that gives info on the metabolism capacity of cells. XTT assay was conducted studying all three biochar samples and observing which microbes better live on biochar and how colonization changes over time. According to these experiments, I found the microbial species that better colonize biochar and which is the required time of their growth.

Moreover, colonization was observed both with fluorescence and electron microscopy. To prepare the samples for the fluorescence microscopy, I added a fluorescence dye, syto9, to the functionalizes biochar. Cells appear attached to biochar, possibly forming a biofilm. At the electron microscope, I saw also growth on the surface of the biochar and also into the nanopore structures. *Consortia* of microorganisms were also observed on biochar.

Finally, I worked on finding a possible packaging for long term storage of functionalized biochar. Preliminary results suggest that covering biochar with alginate is a good solution for storage and delivery.

To conclude, the aim of my PhD project is to study biochar from different points of view and it will continue with a whole evaluation of sustainability, Life Cycle Assessment (LCA) and its economic feasibility.