https://doi.org/10.1051/e3sconf/202016902013

Evaluation of the biological activity of meadow chernozem soils after the application of biochars with different pyrolysis temperatures in a model experiment

Vladislav Zinchenko[1*], *Andrey* Gorovtsov [1,2], *Svetlana* Sushkova [1], *Svetlana* Antonenko [1], *Elena* Fedorenko [1], *Pavel* Pogonyshev[1], and *Anatoly* Barakhov [1]

[1] *Southern Federal University, Rostov-on-Don, Russia*
[2]*Federal Rostov Agricultural Scientific Center, settl. Rassvet, Russia*

Abstract. The paper aims to study the effect of fresh and aged biochar with different pyrolysis temperatures and metal resistant bacteria on the numbers of microorganisms and the dehydrogenase activity of meadow chernozem soils. The use of fresh biochar leads to an increase in the number of soil bacteria and suppression of dehydrogenase activity; after ageing biochar by incubation in the soil, dehydrogenase activity increases significantly.

1 Introduction

Biochar is a carbon sorbent that is made by pyrolysis of organic residues. A key aspect of creating biochar is the raw materials and the pyrolysis temperature. For example, the temperature of pyrolysis directly affects the physical properties of the biochar: specific surface area, pore sizes, etc.

Biochar creates new structures for microorganisms that ensure soil aeration and the use of mineral nutrition elements. On the other hand, in the process of pyrolysis, a large number of substances can be produced that can exert an inhibitory effect on the soil and microorganisms living in it. One of the most studied and common groups of pyrolysis products are volatile organic compounds (VOCs). It is known that the content of these substances in biochar does not depend primarily on raw materials, but rather on the conditions for biochar production [1]. VOCs in the biochar are often represented by methanol, acetone, benzene, sorbed gases. Various aliphatic and aromatic acids, phenols are often also present [2, 3]. The negative influence of VOCs on microbial communities and plants is well established. That is why soil microbial communities undergo significant changes in the first months after the biochar application.

A variety of approaches are used for the remediation of contaminated soils, including the use of microorganisms as one of the agents for the remediation of contaminated soils [4]. The activity of introduced microbial cultures leads to the conversion of pollutants into forms inaccessible to the plants and accelerates the decay of organic pollutants, reducing soil toxicity and increasing its suitability for agricultural use.

* Corresponding author: zinj007@gmail.com

The aim of this study was to study the effect of biochars obtained at different pyrolysis temperatures and strains of metal-resistant bacteria on the biological parameters of soils.

2 Object and research methods

The soils of the Seversky Donets River floodplain have been studied. The model experiment was laid to study the effects of biochar and microorganism strains application on the biological parameters of these soils. In the experimental variants, biochar produced at a final pyrolysis temperature of 500 C° (biochar-500) and 700 C° (biochar-700) was uniformly mixed with soil at a dosage of 2.5% by weight. The biochar was produced from oilseed waste - sunflower husks. For the preparation of biochar, pyrolysis was carried out in a muffle furnace in a special thick-walled metal vessel (retort) under limited oxygen conditions. The heating was carried out stepwise; when the final pyrolysis temperature was reached, the oven was turned off and left for complete cooling. Spores of metal-resistant bacteria belonging to the *Bacillus* genus were introduced into the soil in an amount of 10^{10} CFU / kg of soil. Inoculation of the strains was implemented as follows: the biomass of bacteria was grown on a solid nutrient medium for 3 days, then the biomass was washed into the test tubes with water. The number of cells for inoculation was determined by densitometry. Calibration curves were prepared for each strain prior to the experiment using densitometry in combination with the plate counts on a solid nutrient medium. Suspensions of three strains were mixed proportionally to ensure an equal share of each strain in the final inoculum. The amount of water in the inoculum was calculated to adjust the soil moisture to 60% of the maximum moisture capacity. The sieved air-dried soil was introduced into the pot layer by layer, and each layer was wetted with a suspension of bacterial strains. This allowed us to achieve the most uniform distribution of bacterial cells throughout the soil. Experimental variants with the biochars only and the control variants were placed in the same 2-liter pots and brought up to 60% of the total moisture capacity with water instead of inoculum. Spring barley (*Hordeum vulgare* L.) was grown in the pots. After 30 days of vegetation, the plants were harvested and soil samples were taken for analysis. This was followed by a period of maintaining optimal soil moisture without plants for 90 days, then the sampling procedure was repeated.

The numbers of copiotrophic bacteria were determined on nutrient agar, prototrophic bacteria capable to assimilate inorganic nitrogen source were enumerated on starch-ammonia agar. To determine the soil dehydrogenase activity, the amount of red triphenylformazane formed by dehydrogenation of colorless triphenyltetrazolium chloride was measured at wavelength 490 nm after extraction with ethanol.

3 Results and discussion

According to the data obtained by plate counts, it becomes evident that in the sample with the application of fresh biochar-500, the number of microorganisms was 1.54 times higher than in control. The increase in the number of microorganisms can be explained by the colonization of new ecological niches created by the pore space of the biochar. The application of fresh biochar-700 didn’t cause any significant differences from the control. Such trends were observed for both copiotrophic and prototrophic bacteria. The use of microorganism strains increased the numbers of copiotrophic and the protorophic bacteria by 29.3% and 36.8% respectively. (Table 1).

Table 1. The effect of biochar with different pyrolysis temperatures on the number of microorganisms in the soil.

Experimental variant	**The number of bacteria, million CFU / g of absolutely dry soil**	
	Copiotrophic	**Prototrophic**
Control	37.86±1.48	47.43±4.16
Bacteria	48.99±2.24	64.93±1.4
Biochar 500	58.56±1.21	62.84±6.17
Biochar 700	39.56±2.45	39.56±6.41

The study also evaluated the activity of microorganisms and the level of cellular respiration by measuring soil dehydrogenase activity.

According to the results of the analysis of dehydrogenase activity immediately after plant harvesting, it becomes clear that, despite the increase in the number of microorganisms, the activity of dehydrogenase in the experimental variants with the addition of microorganisms, fresh biochar 500 and 700 was significantly lower than in the control, by 41.8%, 18.3%, and 77% respectively. (Fig. 1).

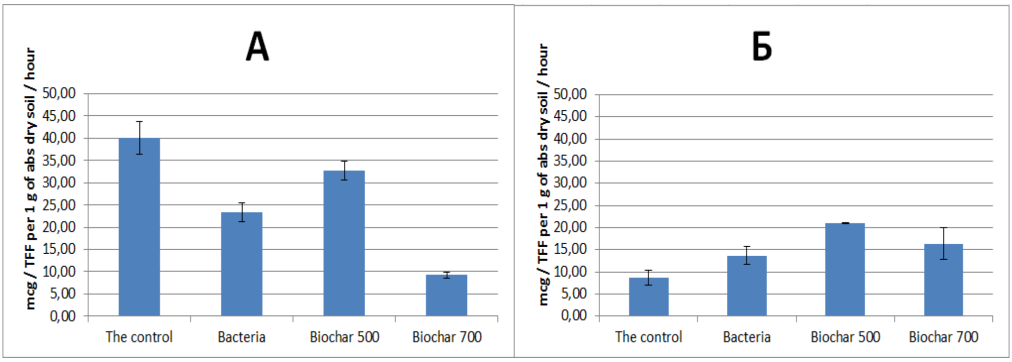

Fig. 1 Dehydrogenase activity of the soil immediately after harvesting the plants (A) and after a 3-month incubation without plants (B)

A significant decrease in dehydrogenase activity indicates the inhibition of oxidative metabolism. The cause of the inhibition was probably various volatile organic substances, as well as polycyclic aromatic hydrocarbons (PAHs) accumulated during pyrolysis. The reason for the inhibition of dehydrogenase activity in the variant with the application of bacteria was probably due to antagonistic biotic interactions between soil bacteria and newly introduced strains. After a 3-month period of incubation of biochar in soil, while maintaining optimal moisture without plants opposite trends were observed. The analysis showed a decrease in dehydrogenase activity in the control sample and in the sample with the introduction of microorganism strains, which is explained by the lack of stimulation from the root system of the plants. However, after the period of incubation without plants, all remediation variants exceeded the control in the level of dehydrogenase activity. In the samples with the application of biochar 500, the dehydrogenase activity also slightly decreased in comparison with the results immediately after plant harvesting. However, it remained at a level 2.4 times higher than the control, and for the sample with the application of biochar 700, an increase in dehydrogenase activity can be noted. In both cases, the destruction of toxic volatile organic substances that had a negative effect on the microbial communities of soils, as well as the colonization of the pore space of the biochar can explain the observed results. Similar data are also available in the literature: the application of biochar increases the metabolic activity of soil microbial communities in drought conditions, and the "aged" biochar turned out to be more effective than fresh one [5]. In addition, biochar can retain nutrients through adsorption and makes them available to the microbiota that colonizes it. The initial substrates from which biochar is made, such as wood, straw, composts, sewage sludge, etc. always contain a significant amount of ash mineral elements that remain in the biochar after pyrolysis. Carbon begins to transform into a gaseous state at 100 ºC, nitrogen at 200 ºC, sulfur at 375 ºC, phosphorus and potassium only at 700-800 ºC [6]. Nutrient elements can then slowly diffuse into the soil and are used by plants and microorganisms. It has been shown that a significant proportion of potassium and phosphorus contained in biochar granules ends up in soil solution [7]. The stability of the bonds between biochar granules and nutrients is directly related to the pH of the soil solution, and with its change (i.e. due to root exudation), the process of sorption and desorption of nutrients can occur. The introduction of mineral elements with wood ash has a pronounced positive effect on soil microbial communities [8]. A similar effect can be expected in the case of the introduction of biochar rich in ash elements. An indirect confirmation of this is the data [9], which showed an increase in the activity of phosphate and sulfate mobilizing bacteria when biochar is introduced into the soil.

4 Conclusion

After analyzing all the data obtained during the study, we can conclude that the biochar creates additional niches for microorganisms and stimulates the biological activity of soils; however, it is recommended to use aged biochar that has undergone preliminary incubation to prevent temporary negative effects from pyrolysis products. Biochar produced at a final pyrolysis temperature of 500 ℃ is more effective and less toxic than biochar with a final pyrolysis temperature of 700 ℃. This is probably due to the physicochemical properties of biochars – pore size, specific absorption surface, and the amount of toxic pyrolysis products accumulated during the manufacturing process.

The study was supported by a grant from the Russian Science Foundation 19-74-10046.

References

1. K.A. Spokas et al., Chemosphere, **85**, №. 5, P. 869-882, (2011)
2. W. Buss, O. Mašek, Env.Sci.Pollut.Res., **23,** №. 19, P. 19580-19589, (2016)
3. M. Ghidotti, et al., ACS Sust. Chem. Engin., **5,** №. 1, P. 510-517, (2017)
4. X. Chen, & V. Achal, Biostimulation of carbonate precipitation process in soil for copper immobilization. *J. Hazard. Mater.*, *368*, 705-713. (2019).
5. L. Paetsch et al., Scientific reports, **8**, №. 1, P. 1-11, (2018)
6. T.H. DeLuca et al., Biochar for environmental management: science, technology and implementation, **2**, P. 421-454. (2015)
7. T. Limwikran, et al., Geoderma, **312**, P.139-150. (2018).
8. S. Mahmood et al., FEMS Micr. Ecol., **43(1)**, P.121-131. (2003)
9. A. Fox et al., FEMS microbiology ecology, **90(1)**, P. 78-91.(2014)