SZENT ISTVÁN EGYETEM

PH.D. THESIS

BIOCHAR AND BIOEFFECTOR COMBINATIONS AFFECTED ON BIOLOGICAL CHARACTERISTICS OF SANDY SOILS

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Budapest
2018

PhD School

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1. BACKGROUND AND OBJECTIVES

Biomass production is considered for the main biological-ecological role of the soils, which through its own physical, chemical and biological characteristics are responsible for the qualitative and quantitative requirements of food production (BIRÓ et al. 2010). It is about 79% of the of Hungarian territory (~7.4 million hectares) from which about ~5.3 million hectares are currently under agricultural cultivation (KSH, 2017). Therefore, to preserve of the soil fertility is being an important economic issue nowadays. There are more than 100 kinds of biological bioeffector products marketed by industrial partners in Hungary. Their use is aimed at restoring the degradation of soil fertility, the absorption of macro- and microelements and the optimization of decomposition processes of soil organic matter. Their impact, however is currently influence due to the climate change processes and the continuous anthropogenic activities, for example by soil acidification, and accumulation of chemicals and heavy metals (KÖDÖBÖCZ et al. 2005). Biochar is a promising tool of worldwide research to mitigate these environmental and anthropogenic risks. These products have been made by controlled industrial processes from various biomass wastes of using reductive, oxygen-free technology, called pyrolysis. The final outcome, the biochar is being a highly porous carbon, which surface layer and area is relatively high, can reach the 5000 m^2/g biomass in general. The biochar industrial products are very stable and almost recalcitrant for extended periods, without significant structural changes and therefore might be able to catalysing key-important effects on several soil physical-chemical parameters (KOCSIS et al. 2018). It means that, while the nutrient content of biochars are able to support the plants and the soil biota, still the biochar might adsorb the soil nutrients, which are becoming non-available for the plants at certain periods in the low-quality soils, investigated. Other adventure of this technology is the compatibility with the organic farming systems and providing sustainable production techniques. Due to this properties it is also an advantage, that of using biochar products, the mechanical fertilizer application can be reduced or totally diminished

Considering the effects to the soil biological properties by different bioeffector and biochar products the following objectives are assigned to the PhD study:

1. Are there any limits of biochar application, especially at the low quality sandy soils, with main focus for the biological parameters?

2. Measure, how can the abiotic and biotic parameters influence the plant-microbe relations in biochar-effected soils?

3. Study, how two different plant hosts, such as the tomato (*Solanum lycopersicum)* and the corn *(Zea mays*) how to respond to different biochar doses? Which differences will be found between the two plants (in rhizosphere compositions, and in nutrient uptake)?

4. Measure, the physico-chemical- also the microbiological properties of soils, highlighting the Plant Growth Promoting Rhizobacteria (PGPR) and the potential soil-borne pathogens.

5. Study of the combination effects of biochar and the PGPR bacteria with main focus on the synergism, among environmental stress-circumstances (i.e. nutrient deficiencies and drought).

6. To get information about the optimization of biochar and PGPR bioeffective technologies.

2. MATERIALS AND METHODS

2.1. Experimental details

Effect of plant-coal biochar on low quality (H~1.5%) sandy soil (Arenosol) was studied of using the Experimental Farm of Szent István University (Soroksár, Hungary), in 2015 and 2016. The biochar was prepared from wood chips by thermal pyrolysis at about T~650°C. The primary purpose of its application was to improve the water management properties and nutrients adsorption of sandy soil. I also expected improvement in some of the measured biological parameters. The study was conducted in pot and in plot experiments. In the second year, a Plant Growth Promoting Rhizobacteria (PGPR) was used in combination with the biochar application. I examined the effect of bacterial biofertiliser inoculation in the biochar-treated soil in the field-experiment and preceding it in pot experiments.

2.2. Treatments of experiments

The research was conducted in two parts and in two steps. In the pot and plot experiments tomato (*Solanum Lycopersicum* L. var. Mobil) was used in the first year (2015), while in the second year (2016) I have tested the growth rate and the quality of maize (*Zea mays* L.) FAO 370 DKC 4490 hybrid crop. In the plot experiments, two different (4 to 10 t/ha) biochar doses were applied in mixing the doses into the upper 20 cm of soil layers. The individual doses were tested alone or in combination with bioeffective inoculums.

In the pot experiments, the following biochar doses were applied beside the 0 (control) pots: 0,5-; 1-; 5 and 10 m/m%, of using 8 replicates per treatment. At the beginning of the bioeffector treatments, the seeds were inoculated with 5 cm^3 of inoculums at 1.5×10^8 cells/cm^3 titre containing a mesophilic siderophore-forming soil bacterium (bioeffector, BE) strain.

2.3. Measured parameters

In the light room pot experiment was performed with 24 °C daytime (14 hours, 14000 LUX) and 18 °C night-time (10 hours) temperature. Water content of the soil was set for 60% of the total field capacity.
The **microbial enzymatic activities** of the rhizosphere soil were monitored: 1) by the dehydrogenase (DHA) and 2) by fluorescein diacetate (FDA) methods.

Among the physiological groups of **countable microorganisms**, the 1) mesophylic aerobs, 2) the facultative anaerobs, 3) the *Pseudomonas* genus and the 4) microscopic fungi (yeasts and micromycetes) were measured by Colony Farming Unit (CFU) and Most Probable Number (MPN) methods.

Beside the quantitative yield parameters, the dissolved dry matter content (Brix^{o}) and the colour value of the tomato fruits was also examined. Amount of nutrient-elements in the shoot biomass was measured by atomic absorption spectrophotometer (AAS).

2.4. Statistical analysis

For the evaluation of the results, the one-way ANOVA test was applicable to compare means among treatments. Normality assumption was proven by Kolmogorov-Smirnov test ($p>0.05$; $p=0.200$) or Shapiro-Wilk test ($p>0.05$; $p=0.244$), and the homogeneity of variances was checked by Levene's test. Estimation was investigated by Tukey HSD post hoc test or Games-Howell post hoc tests. SPSS statistics was used to all statistical analyses, and significant differences were set at a 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Risk of biochar application in soil-plant systems

3.1.1. *The PAH content of biochar*

Four types of industrial biochar products were tested for the PAHs 19 content and quality composition. Results of HPLC analysis are shown in Table 1. It was find that all of the tested biochar samples were containing high rates of PAH compounds, each of them exceeded the limit value of the 1 mg/kg level. There is a Hungarian standard and a decision of the Hungarian Ministry of Agriculture and Rural Development (36/2006.V.18.FVM) about the limits of biochar. Furthermore, the Hungarian soil conservation and protection law (129/2007) has also stated that caution is needed at any biochar products when used potentially in soils. The PAHs content in biochar-treated soils cannot exceed the level of 1 mg/kg on a dry soil basis.

Table 1. Various characteristics of used biochar samples, originating from natural and industrial sources.

	Sample 1	Sample 2	Sample 3	Sample 4
Characteristics	Plant coal Boksa-type	Wood chips (100%)	Separated cow manure/wood chips (80:20%)	Separated cow manure (100%)
Burning temperature at preparation (°C)	450–550	600–650	650–750	650–750
pH (H2O)	09.aug	8.81	9.66	16.szept
Total dissolved solids (mg/kg)	375	1750	2125	1425
Application form	Forestry	Industry	Agriculture	Agriculture
PAH compounds (μg/g)				
Anthracene	0.0948	0.7938	0.1209	0.0909
Benzo A anthracene	1.6928	0.2864	0.3276	n.d.
Benzo B fluoranthene	n.d.	0.2086	n.d.	n.d.
Benzo A pyrene	n.d.	0.2098	n.d.	n.d.
Chrysene	0.4377	0.6112	7.3454	0.1632
Fluoranthene	5.3783	1.1874	2.4044	0.8587
Fluorene	0.7191	1.3658	0.4437	1.5768
Phenathrene	0.1720	n.d.	n.d.	0.3012
Pyrene	0.8298	n.d.	n.d.	0.0871
SUM	9.3246	4.6630	10.6419	3.0780

Content of some poly aromatic hydrocarbon (PAH) compounds, measured by HPLC method.

3.1.2. The nutrient absorption capacity in time

According to literary data, the biochar can absorb the available nutrients on its surfaces. It can provide some risk especially in sandy soils, and mainly among drought conditions. For studying these processes, the storing capacity and the release of the boksa-type of biochar was studied among natural conditions. The soil-nutrient-content was assessed after 20, 30 and 80 years of the biochar application. We have found, that after 20-80-years of biochar persistence periods, the coal surface structures are releasing the stored macro- and micro-elements into the soil, and this process can provide slow and continuous nutrient supply in the tested soil–plant systems (KOCSIS et al., 2018). Further informations are on Table 2 and 3, where the correlation among different measured soil-physical and chemical parameters are shown.

3.2. Benefits of biochar in soil-plant systems

3.2.1. Effect on soil physical and chemical properties

Pearson's correlation analysis was done to study the interrelation among several soil-physical chemical characteristics in biochar treated soils. Strong correlation was found between the capacity of the available nutrients (NPP) and the Cation Exchange Capacity (CEC) of the assessed soil samples (Table 2). The total nitrogen content also correlated with the organic matter content (OM) and the inorganic nitrogen content (Table 3). These relationships were always significant. Considering this mechanism, it is considered as benefits, that in biochar-added soils, less nutrients will be lost and leached into the below-ground subsoil water layers.

Table 2. Results of Pearson's correlation coefficient analysis between cation exchange capacity (CEC) and the available nutrients (N, P, K) in soil, treated by plant coal biochar.

	NO_3–NH_4^+	**P_2O_5**	**K_2O**	**CEC**
NO_3–NH_4^+	1	0.617*	0.833**	0.660*
P_2O_5	0.617*	1	0.859**	0.640*
K_2O	0.833**	0.859**	1	0.695*
CEC	0.660*	0.640*	0.695*	1

Table 3. Results of Pearson's correlation analysis between the measured N (total or only the inorganic) and the soil organic matter (SOM) content, in plant coal amended soils.

	Total N	Loss On Ignition (LOI)	NO_3–NH_4^+
Total N	1	0.866**,*	0.833**,*
Loss On Ignition (LOI)	0.866**,*	1	0.845**
NO_3–NH_4^+	0.833**,*	0.845**	1

*Correlation is significant at 0.05 levels
**Correlation is significant at 0.01 levels

3.2.2. Effect on soil biological properties

Examining the obligate- and facultative anaerobic physiological groups, we have found that, the abundance of those bacteria decreased in the soil, after the experiment was set up. During the experiment, the aerobic layer of the soil shifted horizontally to deeper, thus reducing the substrate reduction by hydrolysis (presence of obligate aerobic and optional anaerobic organisms). By studying the indicator of the effect of biochar on edaphon, the relation between dehydrogenase (DHA) and fluorescein diacetate (FDA) enzymes was studied with several soil chemical and soil biological variables (Table 4). Activity of DHA was strongly correlated with the biomass of all aerobic, fluorescein pigment producing *Pseudomonas* and the filamentous fungal biomass from the soil. Positive correlation was developed also with the potassium and magnesium concentrations in the plants. In addition, the DHA and FDA enzyme activity was closely related to the amount of *Pseudomonas* biomass in the soil, and there was a positive relation between FDA activity and the magnesium concentration of plants. It can be stated therefore, that the two enzymes (DHA, FDA) activities can demonstrate the positive effect on the soil enzyme activity by biochar application. Comparing of the 2 assessed enzymes, the FDA method was found to be suitable for modelling the extracellular enzymatic processes, while the DHA is potentially applicable for study on the inside biological processes. Both enzymes could be in positive correlation with the microbial abundance, investigated.

Table 4. Evaluation of biochar application by ANOVA test – statistical analysis

	DHA	FDA	Aerob	Anaerob	*Pseudomonas*	Fungus	Nitrogen	Phosphorus	Potassium	Mg	Mn	Zn
DHA	1											
FDA	,786**	1										
Aerob	,845**	0,576	1									
Anaerob	-0,56	-0,519	-,582*	1								
Pseudomonas	,881**	,765**	,788**	-,797**	1							
Fungus	,826**	,818**	,648*	-,823**	,960**	1						
Nitrogen	0,409	0,497	,591*	-0,183	0,237	0,168	1					
Phosphorus	0,343	0,424	0,409	0,139	0,119	0,042	,807**	1				
Potassium	,804**	0,521	,833**	-,824**	,917**	,828**	0,23	,089**	1			
Mg	,798**	,591*	,814**	-,884**	,935**	,879**	0,264	0,1	,988**	1		
Mn	-0,007	-0,233	0,328	0,34	-0,272	-0,441	,603*	,598*	-0,093	-0,146	1	
Zn	0,353	0,071	0,517	0,142	0,022	-0,132	,587*	0,533	0,105	0,051	,761**	1

*Correlation is significant at 0.05 levels

**Correlation is significant at 0.01 levels

3.4. Impact of biochar and bioeffector combinations

Beneficial, plant growth promoting rhizobacterium strains were isolated from the biochar-added soils, on the basis of their siderophore-producing ability. Their taxonomic identification was performed by 16S rRNA gene fragment analysis. The most efficient siderophore- producing isolate was considered as the potentially efficient bioeffector (BE) strain. The selected BE was found to be the member of *Alcaligenes* sp. genus (100%). Identification of species level was not successful because the isolate showed more than 98% identity with other species. When analyzing the anabolic and catabolic processes in the soil at the 60% flowering stage of the testplants (as the most intensive plant root-symbiotic microbe connection) significant difference ($p<0.05$) was found between the untreated control and the 4t/ha biochar+BE and the 10t/ha biochar+BE treatments.

3.4.1. Combined effects on the yield quantity

Based on the dry biomass weight, some biochar doses had a positive effect on tomato shoots and root weight in a pot experiment. From the untreated control group, I recorded a significantly higher shoot+root weight due to the 0.5- and 2.5m/m% biochar treatments. In combination with biochar and bioeffectors (B+BE), the differences were smaller in the treatments in comparison with single biochar treatment. Similarly, to the weight of the shoot-biomass, yield improvement was found to be parallel with the increasing doses of biochar. In this way the 10t/ha biochar+BE combination resulted significantly higher yields, compared to the control plots, in the tomato experiment (2015). In the 2016 maize field experiment, I found a significant difference between the control group, 10t/ha biochar and the 10t/ha biochar+BE treatments. There was no significant difference, however between biochar and the biochar+BE treatments among the field conditions. The *Alcaligenes* sp. inoculums cannot develope positive effect for the growth of the maize.

3.4.2. Combined effects on tomato quality

Beside the examination of the tomato yield, the nutritional properties were also examined. In order to determine the effect of various biochar doses and its combinations with the BE (bioeffector) strain, the Water-soluble dry matter content (Brix^{o}) and the colour intensity of the tomato fruits were investigated. Generally, the higher of the dry matter content of the fruits, the greater of the Brix value was found as in the study of SZALÓKI-DORKÓ, 2016. Significantly lower Brix values were measured between biochar+BE and the biochar, without biofector treatments (Figure 1).

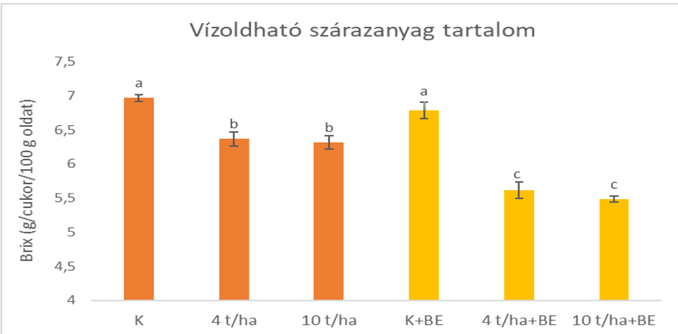

Figure 1. Water-soluble dry matter content of tomato fruits (Brix^{o}) due to different biochar doses in plots, Soroksár, Hungary, 2016.

In plot experiment, the measured yield per plant was closely related with the water-soluble dry matter content of the tomato fruits (Table 5). This means that, it is relatively easy to increase the yield weight of the tomato by increasing the water content with the biochar addition, however, the Brix synthesis is being of more complex process, resulting, that the larger fruits have lower dry matter content. This negative correlation in the non-irrigated field experiment indicates the effective water retention capacity of the biochar, which can be attributed to the plant. In general, the average acidity of tomato yields ranged from 4.27 to 4.40 pH. The results of the harvested and crushed berry's extract colour were constantly changing during the experiment. Generally, at the end of the maturation process, the colour of the samples became increasingly intensive and darker with maturity progression (L*). I investigated the luminance values by Pearson's correlation analysis to find correlation between the L * value, the yield volume and the water-soluble dry matter content of the yield. I have found that the luminosity factor has a positive linear relationship with the amount of crop and close negative linear relation with the water-soluble total dry matter content of the extract (°Brix) (Table 5).

Table 5. Correlation of biochar treatments and bioeffector combinations for some properties of tomato. The statistical evaluation in field experiment. Soroksár, 2015.

	termés	brix	L*	a*	b*	hajtás+gyökér	DHA
termés	1						
°Brix	-,964**	1					
L*	,894*	-,840*	1				
a*	-,201	,391	-,198	1			
b*	-,401	,441	-,559	,773	1		
hajtás+gyökér	,949**	-,897*	,855*	-,083	-,337	1	
DHA	,769*	-,580	,792*	,246	-,295	,811*	1

** p<0,01 (kétoldali próba)
* p<0,05 (kétoldali próba)

4. NEW SCIENTIFIC RESULTS

1. It was found, that biochar can adsorb the nutrients of arenosols and luvisols, which is slowly released after long periods. From natural boksa-type of charcoal, the nutrient release was found after 20-30 year-affecting periods. With adequate water supply, the biochar can provide continuous nutrient-supply for the crops and leaching of nutrients is being also reduced.

2. All of the tested biochar samples were containing high rates of PAH compounds, each of them exceeded the permissible limit value of the 1 mg/kg products directed by the Hungarian Ministry of Agriculture and Rural Development (36/2006.V.18.FVM). Hungarian soil conservation and protection law (129/2007) has also stated that caution is needed at any biochar products when used in soils, not exceeding the level of 1 mg/kg on a dry soil basis.

3. Regarding the pot experiments, 1m/m% biochar doses have resulted the greatest increase in the nutrient uptake of tomato and maize. Furthermore, significantly higher dehydrogenase and fluorescein diacetate enzymatic activities was found on low quality sandy soil. Intensity of enzymatic processes in soil was positively correlated with the aerobic, facultative anaerobic, *Pseudomonas* sp. and total fungal biomass, furthermore with the potassium and magnesium content of test-plants.

4. Differences was found between the plants and the pot- and field experiments. Increasing doses of biochar had positive linear relation with the biomass-production of both plants. There was synergistic positive effect found of biochar+bioeffector combined inoculums. *Alcaligenes* sp. isolate, however had direct positive effect only for the growth of tomato among the field conditions.

5. The plant coal biochar improved the water retention capacity of the low quality sandy soil, thus positively correlating with the average fruit sizes of the tomatoes.

6. The plant-growth-promoting, PGPR bacterium was isolated and identified from biochar-treated soil. The isolate is belonging to the *Alcaligenes* genus, which can have positive effect in combination with the used biochar on the yield of tomato.

5. CONCLUSIONS AND SUGGESTIONS

Based on the results of this study, the main risks of the biochar products of the various industrial technologies are covering two main directions:
1) The risk of the polycyclic aromatic hydrocarbons (PAH),
2) the risk of adsorbing nutrients in the low quality soils, which might diminish the proper nutrient availability of crops in arable soils.

Most of the compounds, originated from the raw materials are concentrated in the products, during the pyrolysis processes. It should be also noted, that all the negative biochar quality is being in relation with the insufficient heat dissipation. There is a Hungarian standard and a decision of the Hungarian Ministry of Agriculture and Rural Development (36/2006.V.18.FVM) about the limits of biochar. Furthermore, the Hungarian soil conservation and protection law (129/2007) has also stated that caution is needed at any biochar products when used them potentially in the soils. The PAHs content in biochar treated soils cannot exceed the permissible levels of 1mg/kg on a dry soil basis. The biochar increases the adsorption capacity of soils, which improves the cation exchange capacity (CEC), therefore increased nutrient-water absorption had been also observed.

With the increasing biochar doses, the soil pH shifted into the alkaline direction, which can prevent the plants to absorb some macro-, meso- and micro-nutrients, more particularly the phosphorus, manganese, zinc…etc. These properties of the soils are considered to be of key important issue for the horticulture and agriculture.
Considering the Dehydrogenase- (DHA) and Fluorescein diacetate (FDA) assessment methods, the DHA activity was more effective, it had been in positive linear correlation with total aerobic and anaerobic bacteria, *Pseudomonas* genus and microscopic fungi and with the potassium and magnesium nutrients in both of plant measurements.

Based on this study, the lower biochar doses could be suggested to replace by effective PGPR soil bacterial inoculums at certain soils and at certain soil-plant conditions.

The expected effects of biochar application are very diverse, as the biochar itself has a great variability in composition, in quality, in the production methods and in many other properties. Therefore, generalization of the results is not really recommended. Effects may only be valid and applicable under certain environmental conditions.

7. PUBLICATIONS

Lectured and referred scientific full-length papers in relation with the topic of thesis:

Kocsis T, Biró B, Ulmer Á, Szántó M, Kotroczó Zs (2018): Time-lapse effect of ancient plant coal biochar on some soil agrochemical parameters and soil characteristics. *Environmental Science and Pollution*, 25 (2): 990-999. **IF: 2.876**

Dudás A, Szalai ZM, Vidéki E, Wass-Matics H, **Kocsis T**, Végvári Gy, Kotroczó Zs, Biró B (2017): Sporeforming bacillus bioeffector for healthier fruit quality of tomato in pots and field. *Applied Ecology, Environmental Research,* 15 (4): 1399-1418. **IF: 0,68**

Dudás A, Kotroczó Zs, Vidéki E, Wass-Matics H, **Kocsis T**, Szalai ZM, Végvári Gy, Biró B (2017): Fruit quality of tomato affected by single and combined bioeffectors in organically system. *Pakistan Journal Agricultural Sciences,* 54 (4): 847-856. **IF:0,61**

Kocsis T, Biró B (2015): Bioszén hatása a talaj-növény-mikróba rendszerre: előnyök és aggályok. *Agrokémia és Talajtan,* 64 (1): 257-272.

Kocsis T, Biró B, Mátrai G, Ulmer Á, Kotroczó Zs (2016): Növényi eredetű bioszén tartamhatása a talaj szervesanyag-tartalmára és agrokémiai tulajdonságaira. *Kertgazdaság,* 48 (1): 89-96.

Kocsis T, Kotroczó Zs, Biró B (2017): Bioszén dózisok és bioeffektor baktériumoltás hatása homoktalajon tenyészedény kísérletben. *Talajvédelem* különszám, pp. 53-60.

Kotroczó Zs, Biró B, **Kocsis T**, Veres Zs, Tóth JA, Fekete I (2017): Hosszú távú szerves anyag manipuláció hatása a talaj biológiai aktivitására. *Talajvédelem* különszám, pp. 73-83.

Biró B, Domonkos M, **Kocsis T**, Juhos K, Szalai Z, Végvári Gy (2015): Két mikrobiális oltóanyag hatása tehéntrágya alapú komposztok és a talajok várható minőségi tulajdonságaira *Talajvédelem* különszám, pp. 9-18.

Full-length publication in conference proceedings (ISBN, ISSN or other, authenticated papers)

Kocsis T, Wass-Matics H, Kotroczó Zs, Biró B (2015): A bioszén kedvező hatása a talaj pszikrofil- és mezofil csíraszámára. In: Futó Zoltán (szerk.): *A hulladékgazdálkodás legújabb fejlesztési lehetőségei.* 126 p. (ISBN:978-963-269-464-1)