

# **EFFECT OF BIO-COAL OVER THE ESHERICHIA COLI AND LYSTERIA INNOCUA FROM TREATED SOILS**

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## **ABSTRACT**

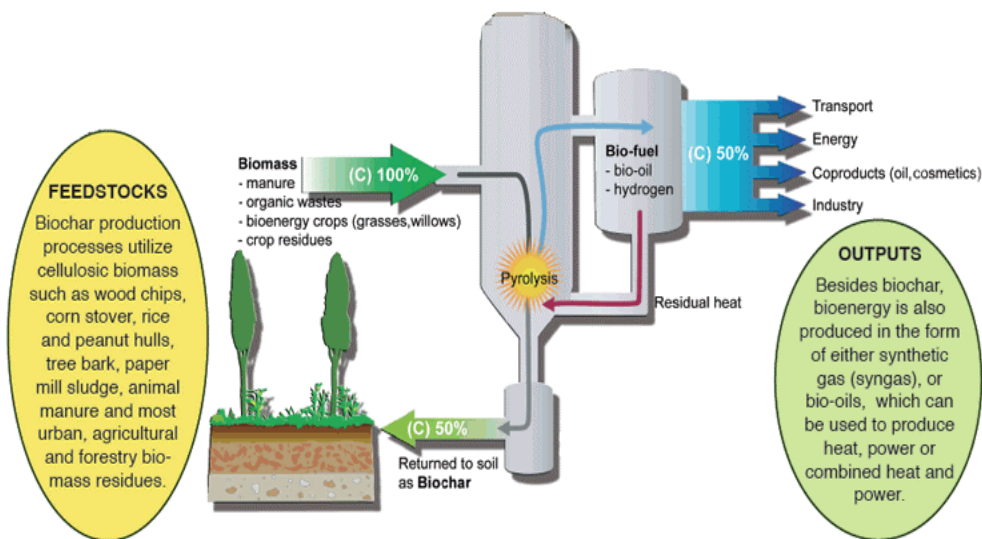
*Soils used in intense agriculture are prone for degradation. Farmers try to reduce the soil degradation with different treatments and amendments based on organic waste materials. Organic amendments are heterogenous mixtures and induced in soils several infections with microorganisms. Bio-coals are cheap source that can be considered a solution in replacement of organic amendments to provide safe soils. The study is a preliminary study and has in view the effect of soils treated with bio-coal over the ESHERICHIA COLI and LYSTERIA INNOCUA development. Next studies will follow the effect of soils treated with bio-coal over the plants growing up in selected soils.*

## **INTRODUCTION**

Bio-coal is a class of low-rank coal which is naturally abundant and deposited near the surface of the earth, enabling it to be mined more economically compared to other high-rank coals (Mahdy, 2011; Qi et al., 2011; Kashiwagi et al., 2015). There are different types of bio-coal which are characteristically distinct based on the origin and level of coalification of the parent material. In addition, bio-coal has a high moisture content which ranges from 30 to 70% (Krawczykowska and Marciniak-Kowalska, 2012). Much of this exists as free water which evaporates rapidly in dry conditions, leaving hygroscopic water that is bespoke to individual bio-coal (Krol-Domańska and Smolinska, 2012). Victorian bio-coal from Australia, for example, can have as high as 66% moisture content, which makes bulk transportation and storage difficult. Drying bio-coal prior to utilisation is an essential

requirement to increase heat capacity, more especially as it was mainly used as fuel in power plants or raw material in the chemical industry until recently (Lu et al., 2019). There are high levels of organic compounds in bio-coal and this has drawn a lot of interest in its potential use as a soil amendment. The bio-coal has a high composition of humic (10–90% d.w.) and fulvic acids (Krol-Domańska and Smolinska, 2012; Saha et al., 2016; Anemana et al., 2019).

## MATERIAL AND METHOD



**Figure 1. Bio-coal production**

**Sample preparation of *Escherichia coli* and *Listeria innocua*:** For the multiplication of the E-coli and Listeria culture we used a Tryptone Glucose Extract (TGE) liquid culture medium. Preparation of nutritional broth TGE Trypton 0.5%; Glucose 1%; Yeast extract 0.25%. Measure the necessary quantities of weights, place them in a sterile stopper bottle, make up with distilled water, then sterilize the broth in autoclave. After the mixture has cooled, the inoculation stage with bacterial cultures follows. The inoculated broth was kept in the incubator for 24 hours, temperature 30 degrees Celsius. The configuration of the experiment in terms of soil and bio-coal it was carried out according to the following table.

Table 1

**Experimental model**

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Blank sample	800 G OF DRY SOIL	800 G OF DRY SOIL	800 G OF DRY SOIL	800 G OF DRY SOIL	800 G OF DRY SOIL
Treatment with <i>ESCHERICHIA COLI</i>	800 G OF DRY SOIL	800 G OF DRY SOIL	800 G OF DRY SOIL	800 G OF DRY SOIL	800 G OF DRY SOIL
Treatment with <i>LISTERIA INNOCUA</i>	800 G OF DRY SOIL	800 G OF DRY SOIL	800 G OF DRY SOIL	800 G OF DRY SOIL	800 G OF DRY SOIL
Combined treatment	800 G OF DRY SOIL	800 G OF DRY SOIL	800 G OF DRY SOIL	800 G OF DRY SOIL	800 G OF DRY SOIL
Sample Blank + Bio-coal	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL
Treatment with <i>E. COLI</i> + Bio-coal	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL
Treatment with <i>LISTERIA INNOCUA</i> + Bio-coal	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL
Treatment with COMBINAT + BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL	720 G OF DRY SOIL + 80 G BIO-COAL

**Preparation of soil samples:** take a few grams of soil from each container with the same composition (1-5 ,6-10, 11-15, ... 36-40), homogenize, and place in a sampling bag.

**Preparation of culture media:** For *Escherichia coli* we chose the Harlequinn culture medium (HAL008), which is a selective

environment. Composition: Tripton 2%; Bile salts 0.15%; X-glucoronide 0,0075 %; Magenta-beta-galactose 0,01 %; Agar 1.5% For the preparation of the nutrient medium we weighed 36.6 grams of dry medium , dissolved in 1 L of distilled water , after which we sterilized in the autoclave 15 min, 121 degrees Celsius .

To identify the Bacteria *Listeria innocua* we used Compass nutrient medium, of which we weighed 71.9 grams of dehydrated medium, dissolved in 1 L of distilled water, after which we sterilized in the autoclave 15 min, 121 degrees Celsius.

After cooling the environment I added 10 mL of "supplement ( BS071).

## RESULTS AND DISCUSSIONS

Table 2

### Soil sample in day 7

	HAL008	COMPASS
Blank	2*10 <sup>2</sup>	1.09*10 <sup>4</sup>
Treatment with <i>ESCHERICHIA COLI</i>	1.6*10 <sup>9</sup>	-
Treatment with <i>LISTERIA INNOCUA</i>	-	1.19*10 <sup>5</sup>
Combined treatment	8.39*10 <sup>9</sup>	1.39*10 <sup>7</sup>
Blank + Bio-coal	0	4.3*10 <sup>4</sup>
Treatment with <i>E-COLI</i> + Bio-coal	10.3*10 <sup>9</sup>	-
Treatment with <i>LISTERIA INNOCUA</i> + Bio-coal	-	5.5 * 10 <sup>7</sup>
Combined treatment + Bio-coal	9.8*10 <sup>8</sup>	3.03*10 <sup>6</sup>

Samples recorded after 7 days present a decrease of bacteria population in all the samples treated with bio-coal.

Presence of bio-coal in soil relieved an antiseptic effect over the microorganism contamination.

After 63 days the antiseptic role of bio-coal can be observed in tables 3 and 4. Soils with bio-coal present a low number of colonies forming units **CFU** than the samples without bio-coal.

The effect increases in time. In day 63 the **CFU** decrease in all samples treated with bio-coal.

Table 3

**Soil sample in day 63**

	<b>HAL008</b>	<b>COMPASS</b>
Blank	1*10 <sup>2</sup>	4.12*10 <sup>4</sup>
Treatment with <i>ESCHERICHIA COLI</i>	8.5*10 <sup>5</sup>	-
Treatment with <i>LISTERIA INNOCUA</i>	-	1.6*10 <sup>4</sup>
Combined treatment	4.9*10 <sup>5</sup>	2*10 <sup>5</sup>
Blank + Bio-coal	0	3*10 <sup>4</sup>
Treatment with <i>E-COLI</i> + Bio-coal	7*10 <sup>4</sup>	-
Treatment with <i>LISTERIA INNOCUA</i> + Bio-coal	-	2.9 * 10 <sup>5</sup>
Combined treatment + Bio-coal	2.9*10 <sup>4</sup>	1.5*10 <sup>4</sup>

Table 4

**Total number of colonies forming units**

<b>CFU</b>	<b>Day 7</b>	<b>Day 63</b>
Blank + Bio-coal +Listeria	43000	30000
Blank+ Bio-coal+E-Coli	0	0
Blank+E-Coli	200	100
Blank + Listeria	10900	41200
Combined treatment + Bio-coal+E-Coli	9800000000	29000
Combined treatment + Bio-coal+Listeria	3030000	15000
Combined treatment +E-Coli	839000000000	490000
Combined treatment + Listeria	13900000	200000
Treatment E-Coli	1600000000	850000
Treatment E-Coli + Bio-coal	103000000000	70000
Treatment Listeria	119000	16000
Treatment Listeria + Bio-coal	55000000	290000
<b>TOTAL</b>	<b>2134210310000</b>	<b>2031300</b>

In samples with combined treatment decrease of bacteria is significant that in samples without treatment.

**CONCLUSIONS**

The positive effect of bio-coal on soil and plants can be proved by the fact that the samples enriched with bio-coal evolved much faster, they were much more resistant, there was no need for replanting, as in the case of samples planted in the soil.

The soil's capacity to retain water has increased significantly in the case of bio-coal containers, positively influencing the growth of plants, due to their constant humidity, their weight did not show a significant

decrease (about 100-120 g of evaporated water in 3-4 days) as in the case of containers filled with a lower quality soil, where the weight decreased significantly from one watering to another (about 170-200 g of evaporated water in 3-4 days).

In the case of samples enriched with biofuel, mature plants had a higher number of leaves, they were much fleshier and richer.

Bio-coal has a positive effect on the soil, providing a possibility to restore the normal quality of eroded soils or increase the productivity of poor quality soils, even in areas with fluctuating precipitation, with a higher yield of production, in sustainable conditions of agriculture.

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