

Article

Biochars Originating from Different Biomass and Pyrolysis Process Reveal to Have Different Microbial Characterization: Implications for Practice

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Abstract: Sustainable technologies are increasingly promoted in various production areas. Protection of natural resources, as well as rational waste management, may lead to better optimization of technologies. Biochar, a product of pyrolysis of organic residues has found wide applications in waste management, agriculture, energy and construction industry. In the present study biochar samples produced in Poland and in Brazil were analysed for microbial content using three substrates: Plate Count Agar, Malt Agar, and Potato Agar. Both qualitative and quantitative measurements were done. Microscopic analysis of the biochar structure was also performed. We found that microbial cultures in both biochars represented a wide range of biodiversity of microorganisms genera and species. We demonstrate that the biochar samples differ depending on the botanical origin as well as on the production technology. Structure of the tested samples also varied depending on the botanical origin. Sample 1-PL (pine) was characterised by a compact and regular structure, while sample 2-PL (oak) showed porous and irregular structure. Sample from Brazil (1-BR) showed a more delicate structure than Polish biochars. Obtained properties may suggest a range of implications for practice.

Keywords: biochar; microbiological analysis; structure; implications for practice

1. Introduction

Biochar, carbonous substrate originating from the process of pyrolysis [1] has been demonstrated to have various potential benefits such as increasing agricultural yields [2–4], soil and sediment remediation [5] or improving animals health in livestock farming [6]. There has been also a recent interest in using biochar in construction [7] with potential benefits for diminishing humidity inside buildings, thermal isolation and fungus development control [8–12]. Biochar or microporous carbon is also used for humidity control, a property that has been extensively researched by Nakano et al. (1996) and Abe et al. (1995). Humidity control by microporous carbon can reduce asthma and

dermatitis occurrences by controlling the growth of mould and ticks [10–13]. However, there is little research that looks into a range of biochars, identifying and quantifying microbial colonies. Although the number and the type of microbial colonies in biochar have various implications, including crop interactions and safety, the microbial composition remains an under-investigated aspect of biochar research. Of particular interest, are the bacteria from family Bacillus. They are common throughout a range of environments and tolerate acid and alkaline conditions. On the account of the possibility to create endospores they can survive in extreme environments and are commonly isolated from unfresh and spoiling food products. Majority of the Bacillus species are safe for people with the exception of *B. anthracis* and *B. cereus*. Due to their widespread persistence and resistance, these bacteria are used in commercial production of enzymes, antibiotics, insecticides, vitamins and metabolites such as hyaluronic acid (used for example in cosmetics) [14,15]. Enzymes such as α -amylases, able to hydrolyse α -1,4-glycoside bonds in starch, originating from Bacillus sp. are commonly used in food, chemical and textile industry as well as for the production of pharmaceuticals [16–18].

Another important characterization of biochar is their fungus content, such as *Alternaria*, *Aspergillus*, *Candida*, *Cladosporium*, *Penicillium* and *Rhizopus*. Although favouring humid environments, *Aspergillus* and *Penicillium*, are able to survive in dry conditions. A range of fungus metabolites are antibiotics, such as the metabolites of *Penicillium puberulum*. An important feature of fungus is their mycotoxin production (such as aflatoxins), on the account of their cancerogenic and teratogenic properties, even in small concentrations [19,20]. They are degraded in an alkaline environment and under UV radiation [21–25]. The implications of microbial and fungus content in biochar can be far-reaching. Even though some of these organisms may remain inactive due to its limitations in accessibility, the presence of microbes and fungus may have both positive and negative implications, and their use will vastly depend on destination. Biochars rich in desired bacteria may be used as a substrate in in-vitro production whereas those with limited undesired organisms may be used for construction or in the food industry. However, the microbial characterization is not yet common place and the properties of different biochars from different biomass and different pyrolysis processes have been rarely reported while the microbial community composition associated with biochar is poorly understood [26,27].

Here we present the qualitative and quantitative comparison of two dissimilar biochars, focusing on the microbial content, and discuss the implications for the use of these biochars in practice. The biochars were produced in different pyrolysis processes: biochar from Brazil was produced in home-made stove adopted usually by the farmers in their on-site production for their agricultural use or for charcoal sale. Biochar from Poland was industrially produced from a large-scale energy plant. Our results have important implications for the use of biochar in practice and are the first that discuss in comprehensive manner biochars from dramatically different production processes and different biomass with different potential applications.

The aim of the study was to compare Brazilian and Polish biochar samples in terms of the microbial content and the structure of the surface. In addition, an attempt was made to assess the properties of test samples in terms of their potential use in practice.

2. Materials and Methods

The research material consisted of two types of biochar coming from Brazil and Poland:

1. biochar from Brazil obtained from *Gliricidia* plant (1-BR),
2. biochar from Poland obtained from Pine woodchips (1-PL),
3. biochar from Poland obtained from Oak woodchips (2-PL).

Biochar samples provided for the research were placed in sterile conditions. Similarly, throughout the study, extensive precautions were put in place to limit microbial contamination.

1. Brazilian biochar characterization

The Brazilian biochar was produced in home-made drum stove from *Gliricidia sepium* at the temperature of approximately 350°C. *Gliricidia* is a commonly used plant in organic farming for

nitrogen-fixing. It is also used as ‘living fence’ at agricultural farms and pasturelands. Despite its benefits, it grows fast and may provide excessive shadow, limiting plant growth. In certain circumstances, there is, therefore, a need for cutting off the upperground branches that do not have an alternative use. Moreover, at the site where the biochar was produced (Brazilian Agricultural Research Station, Embrapa Agrobiologia, Seropedica km 47), *Gliricidia* become an invasive species that disseminated rapidly entering native forest fragments. The source of biomass for biochar production in Brazil, therefore, did not compete with alternative biomass uses, as at times criticised in literature [28]. Branches of *Gliricidia* were dried for two weeks before pyrolyzation. Cut and dry biomass was put into the stove, fired up and closed with drum cup and isolated with a layer of sand. The pyrolyzation time was 24 hours. After that time biochar was cooling for another 24 h (with open stove) and sieved at 4 mm (Figure 1).



Figure 1. Steps of production: Rio de Janeiro state where biochar was produced, *Gliricidia* tree, dry *Gliricidia* before being cut at put into the stove, drum stove, biochar (before being sieved), table with biochar properties [4].

2. Polish biochar characterization

Biochar was produced according to Fluid SA company technology. The process consisted in thermal refining of plant biomass and other post-production biomass residues through their autothermal roasting at the temperature 260°C in reduction atmosphere and without the use of additional energy, catalysts, and chemical additives.

Carbonising products consisted mainly of biochar (from 65% to 70% of energy compared to the energy of the feed) and process gases (from 20% to 35% of energy compared to the energy of the feed). During the process, a significant increase of the carbon element (C) in relation to the biomass of the feed was recorded (1.5 to 2.0 times) as well as an increase of energy density, on average by 4.0 times, reduction of the amount of hydrogen (H), on average by 2.5 times, and reduction of the amount of oxygen (O₂), on average by 3.0 times. Polish samples of biochar were produced from pine woodchips (1-PL) and oak woodchips (2-PL).

The research was carried out at the Innovation Research Center (CBNI) and Regional Center of Agriculture, Environmental and Innovative Technology (EAT) Pope John Paul II State School of Higher Education in Białá Podlaska, Poland.

2.1. Microbiological Analysis of Biochar Samples Consisted of Qualitative and Quantitative Determination of Bacteria and Fungi Species

For the research three standardized substrates were used in the assessment process:

- The total number of microorganisms cultivated on PCA substrate (PLATE COUNT LAB-AGAR™) in temperature of 30°C and time period 72 h.
- The total number of fungi colonies cultivated on PDA substrate (POTATO DEXTROSE LAB-AGAR™) in temperature of 30°C and time period 72 h, after that time the temperature was decreased to 21°C for the next 72 h.
- The total number of fungi colonies cultivated on MA substrate (MALT EXTRACT LAB-AGAR™) in a temperature of 24°C and time period 144 h.

The substrates used had been purchased from BIOMAXIMA S.A. The dilution method PN-EN ISO 7218 [29] was used with the dilutions of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} .

The qualitative and quantitative assessment was done on the basis of microbial flora species composition using macroscopic and microscopic methods, as well as taxonomic keys and atlases. It was expressed in CFU/g units [30–35].

2.2. Microscopic examination of the structure.

Examination of the biochar sample structure was done using a research microscope Nikon Eclipse E-200 with fluorescence attachment and SCA image analysis.

3. Results

In the sample, 1-BR cultured on PCA substrate dominated strains of *Bacillus* sp. (the average number of colonies from UNC to 204 colonies for dilutions ranging from 1 to 10^{-5}). The plates were overgrown on the entire surface with the colonies of *Bacillus* sp. On two plates confluent growth was observed, which made it impossible to perform a quantitative count (Table 1).

Table 1. Microbiological analysis of the culture from the biochar sample from Brazil (1-BR) cultivated on PCA (PLATE COUNT LAB-AGAR™) substrate (CFU/g).

	I	II	III	Average Number of Colonies
	Bsp. (UNC)	Bsp. (UNC)	Bsp. (UNC)	UNC
Dilution	10^{-1} Bsp (UNC)	Bsp (UNC)	Bsp (UNC)	UNC
	10^{-2} Bsp. (214)	Bsp. (194)	Bsp (UNC)	204.0
	10^{-3} Bsp. (36)	Bsp. (62)	Bsp (UNC)	49.0
	10^{-4} Bsp. (3)	Bsp. (6)	Bsp. (23)	10.7
	10^{-5} Bsp. (2)	0	Bsp. (4)	2.0

I, II, III – repetitions; (UNC) – in brackets the uncountable colonies; (NC) – in brackets the number of colonies (NC); Bsp. – *Bacillus* sp.

On malt substrate (Table 2) strains of *Bacillus* sp. were identified for the dilution of 1 and 10^{-1} . Furthermore, in the second repetition for the dilution of 10^{-1} , the following strains were isolated: *Aspergillus versicolor* (UNC), *Aspergillus flavus* (UNC), *Aspergillus fumigatus* (UNC), *Aspergillus niger* (3), *Aspergillus candidus* (2), *Aspergillus nidulus* (7), *Penicillium* sp. (UNC), *Rhizopus* sp. (2), while during the third repetition: *Aspergillus versicolor* (UNC), *Aspergillus fumigatus* (8), *Aspergillus niger* (8), and *Penicillium* sp. (UNC). For the dilution of 10^{-2} , in the first repetition, besides *Bacillus* sp. the following were isolated: *Aspergillus versicolor* (UNC), *Aspergillus nidulus* (1), *Aspergillus flavus* (1), *Aspergillus niger* (5), *Rhizopus* sp. (UNC), in the second repetition: *Aspergillus versicolor* (18), *Aspergillus fumigatus* (1), *Penicillium* sp. (8), while in the third repetition: *Aspergillus versicolor* (3), and an overgrowth of *Bacillus* sp. on the entire surface plate was recorded. In the case of dilution of 10^{-3} , on average 7.3 colonies of *Aspergillus* sp., *Aspergillus versicolor*, *Aspergillus flavus*, and *Penicillium* sp. were isolated, as well as *Bacillus* sp., in the case of which confluent growth was observed.

Table 2. Microbiological analysis of the culture from the biochar sample from Brazil (1-BR) cultivated on MALT substrate (CFU/g).

	I	II	III	Average Number of Colonies
	1	Bsp (*)	Bsp (*)	-
			Aver (UNC)	
			Afl(UNC)	
			Afum (UNC)	Aver (UNC)
			Anig (3)	Afum (8)
	10 ⁻¹	Bsp (*)	Acan (2)	Anig (8)
			Anid (7)	Psp (UNC)
			Psp. (UNC)	UNC
			R (2)	
			Σ(UNC)	
Dilution				
		Aver(UNC)	Aver (18)	Aver (36)
		Afl(1)	Afum (1)	Asp. (1)
		Anig (5)	Psp (8)	Psp (6)
	10 ⁻²	Anid (1)		R (2)
		R (UNC)		36.0
		Bsp (*)		
		Σ(UNC)	Σ(27)	Σ(45)
		Aver (6)	Aver (5)	Aver (5)
		Asp. (1)	Afl (1)	Psp (1)
	10 ⁻³	Bsp (*)	Asp. (1)	
			Psp (1)	7.3
		Σ(7)	Σ(8)	Σ(7)
	10 ⁻⁴	Bsp (*)	Aver (2)	Aver (1)
			Σ(2)	Σ(1)
	10 ⁻⁵	0	0	0

I, II, III – repetitions ; (UNC) – in brackets the uncountable colonies; (NC) – in brackets the number of colonies (NC); Σ (NC) – total numbers of colonies; Aver-Aspergillus versicolor; Afl- Aspergillus flavus; Afum - Aspergillus fumigatus; Anig - Aspergillus niger; Acan - Aspergillus candidus; Anid - Aspergillus nidulus; Asp.- Aspergillus sp; Psp. - Penicillium sp; R - Rhizopus sp; Bsp (*) - plate overgrown by Bacillus sp.

For the dilution of 10⁻⁴, in the first repetition, Bacillus sp. was isolated, characterised by a confluent growth, while in the second and third repetition Aspergillus versicolor species were recorded with the average number of colonies equal to 1.0. After averaging the readings the average number of fungi colonies ranged from UNC to 45. On POTATO substrate (Table 3) for the dilution of 1, no colonies of microorganisms were recorded.

Table 3. Microbiological analysis of the culture from the biochar sample from Brazil (1-BR) cultivated on POTATO substrate [CFU/g].

Dilution	I		II		III		Average Number of Colonies
	1	0	0	0	0	0	0
10 ⁻¹	Bsp (*)		Anig (2) Afl (2) Asp. (2) Bsp (*) Σ(6)	Aver (UNC) Acan(2) Anig (1) Afl (UNC) Σ(UNC)			3.0
10 ⁻²	Bsp (*)		Aver (2) Acan(1) Afl (1) Σ(4)	Aver (3) Bsp (*) Σ(3)			2.3
10 ⁻³	Bsp (*)		Bsp (*)	Aver (6) Acan(1) Anig (1) Afum (1) Afl (1) Psp (1) Bsp (*) Σ(11)			3.7
10 ⁻⁴	Bsp (*)		Bsp (*)	Psp (1) Bsp (*) Σ(1)			0.3
10 ⁻⁵	0		0	0			0

I, II, III – repetitions; (UNC)—in brackets the uncountable colonies; (NC)—in brackets the number of colonies (NC); Σ (NC)—total numbers of colonies; Aver—*Aspergillus versicolor*; Afl—*Aspergillus flavus*; Afum—*Aspergillus fumigatus*; Anig—*Aspergillus niger*; Acan—*Aspergillus candidus*; Anid—*Aspergillus nidulus*; Asp.—*Aspergillus* sp; Psp. - *Penicillium* sp; R—*Rhizopus* sp; Bsp (*)—plate overgrown by *Bacillus* sp.

In the case of dilutions from 10⁻¹ to 10⁻⁵, in all repetitions, strains of *Bacillus* sp. overgrown on the entire surface of the plate were observed, which made a quantitative count impossible to perform. Additionally, the following genera or species were isolated: *Aspergillus* sp., *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus versicolor*, *Aspergillus candidus*, *Aspergillus fumigatus*, as well as *Penicillium* sp. (the average number of colonies ranging from 0.3 for the dilution of 10⁻⁴ to 3.7 for the dilution of 10⁻³). However, for the dilution of 10⁻⁵, no colonies of microorganisms were isolated. In the case of sample no. 1-PL cultured on PCA substrate (Table 4) dominated strains of *Bacillus* sp.

Table 4. Microbiological analysis of the culture from the biochar sample from Poland (1-PL, 2-PL) cultivated on PCA substrate (CFU/g).

	I		II		III		Average Number of Colonies		
	Biochar	Pine	Oak	Pine	Oak	Pine	Oak	Pine	Oak
		1-PL	2-PL	1-PL	2-PL	1-PL	2-PL	1-PL	2-PL
Dilution	1	Bsp. (UNC)	0	Bsp. (UNC)	Afl (1)	Bsp. (UNC)	0	UNC	0
	10 ⁻¹	Bsp (421)	0	Bsp (443) Micro (1) Σ(444)	0	Bsp (771)	0	545.3	0
	10 ⁻²	Bsp. (61)	0	Bsp. (54)	0	Bsp (124)	0	79.7	0
	10 ⁻³	Bsp. (6)	0	Bsp. (3)	0	Bsp (10)	0	6.3	0
	10 ⁻⁴	0	0	0	0	Bsp. (1)	0	0.3	0
	10 ⁻⁵	0	0	0	0	0	0	0	0

I, II, III – repetitions; (UNC) – in brackets the uncountable colonies; (NC) – in brackets the number of colonies (NC); Σ (NC) – total numbers of colonies; Bsp. – *Bacillus* sp; Micro – *Micrococcus* sp; Afl – *Aspergillus flavus*.

The average number of colonies ranged between 545.5 for the dilution of 10⁻¹ and for the dilution of 1 also uncountable growth of *Micrococcus* sp. For the dilution of 10⁻⁵, no colonies of microorganisms were isolated. On MALT substrate (Table 5), for the dilution of 1, strains of *Aspergillus flavus* were isolated, and the average number of colonies count was 1.7.

Table 5. Microbiological analysis of the culture from the biochar sample from Poland (1-PL, 2-PL) cultivated on MALT substrate (CFU/g).

	I		II		III		Average number of colonies		
	Biochar	Pine	Oak	Pine	Oak	Pine	Oak	Pine	Oak
		1-PL	2-PL	1-PL	2-PL	1-PL	2-PL	1-PL	2-PL
Dilution	1	Afl (2)	0	0	0	Afl (1) # (2)	0	1.7	0
	10 ⁻¹	0	0	0	0	0	Psp. (1)	0	0.3
	10 ⁻²	0	0	0	0	0	0	0	0
	10 ⁻³	0	0	0	0	0	0	0	0
	10 ⁻⁴	0	0	0	0	0	0	0	0
	10 ⁻⁵	0	0	0	0	0	0	0	0

I, II, III – repetitions; (UNC) – in brackets the uncountable colonies; (NC) – in brackets the number of colonies (NC); Σ (NC) – total numbers of colonies; Afl- *Aspergillus flavus*; Psp. - *Penicillium* sp; # - unidentified fungi colony.

Within the range of dilutions between 10⁻¹ and 10⁻⁵, no colonies of microorganisms were isolated. On POTATO substrate (Table 6) strains of *Bacillus* sp. overgrown on the entire plate were observed (average number of colonies from UNC for the dilution of 1 to 1 colony for the dilution of 10⁻⁴). For the dilution of 10⁻⁵, no colonies of microorganisms were isolated. In the case of sample no. 2-PL on PCA substrate for the dilution of 1, only in the second of the three repetitions, 1 colony of *Aspergillus flavus* was isolated. On the plates, with the dilutions ranging from 10⁻¹ to 10⁻⁵, no colonies of microorganisms were isolated. On the MALT substrate for the dilution of 10⁻¹ 1 colony of

Penicillium sp. was isolated. Meanwhile, for the dilutions ranging from 10^{-2} to 10^{-5} no colonies of microorganisms were isolated. On POTATO substrate, for the dilution of 10^{-1} , 1 colony Aspergillus versicolor was isolated, while for the dilution of 10^{-2} 1 colony of unidentified fungi was recorded. On the plate, with the dilution of 10^{-3} , no microorganisms were recorded. In contrast, on the plate with the dilution of 10^{-4} one yeast colony was isolated. On the plate, with the dilution of 10^{-5} , no microorganisms were recorded. After the microbiological analysis of biochar samples, it was noted that sample 2-PL was the least microbiologically polluted. However, it should be stated that in Polish samples biologically different microorganisms were present. In the sample, 1-PL dominated strains of Bacillus sp. While in sample 2-PL dominated, though in small amount, strains of fungi species: Aspergillus flavus, Aspergillus versicolor, and Penicillium sp. In the case of biochar sample from Brazil, the same microorganisms were present as the ones isolated and identified in Polish samples.

Table 6. Microbiological analysis of the culture from the biochar sample from Poland (1-PL, 2-PL) cultivated on POTATO substrate (CFU/g).

	I		II		III		Average Number of Colonies		
	Biochar	Pine 1-PL	Oak 2-PL	Pine 1-PL	Oak 2-PL	Pine 1-PL	Oak 2-PL	Pine 1-PL	Oak 2-PL
Dilution	1	Bsp (*)	0	Bsp (*)	0	Bsp (*)	0	0	0
	10^{-1}	Bsp (*>100)	0	Bsp (*>100)	Aver (1)	Afl (1)	0	0.3	0.3
	10^{-2}	Bsp (*32)	# (1)	Bsp (*43)	0	Bsp (*54)	0	0.3	0
	10^{-3}	Bsp (*1)	0	Bsp (*5)	0	Bsp (*9)	0	0	0
	10^{-4}	0	0	Bsp (*1)	0	0	## (1)	0.3	0
	10^{-5}	0	0	0	0	0	0	0	0

I, II, III – repetitions; (UNC) – in brackets the uncountable colonies; (NC) – in brackets the number of colonies (NC); Σ (NC) – total numbers of colonies; Aver-Aspergillus versicolor; Afl - Aspergillus flavus; Bsp (*) - plate overgrown by Bacillus sp; # - unidentified fungi colony; ## - unidentified yeast colony.

The microscopic analysis, carried out using research microscope Nikon Eclipse E-200 with fluorescence attachment and SCA image analysis, shows the structure of biochar 1-BR (Figure. 2) as irregular, but this structure is not as dense as in the case of biochar 2-PL (oak) (Figure. 4). The structure of biochar sample 1-BR (Brazil) is undulating and porous. However, this sample seems to have the most irregular structure when compared with samples 1-PL (pine) (Figure. 3) and 2-PL (oak).

Oak (sample 2-PL), being hardwood, in the microscopic images shows a hard and porous structure, while pine (sample 1-PL) has regular, slightly porous, and delicate structure.

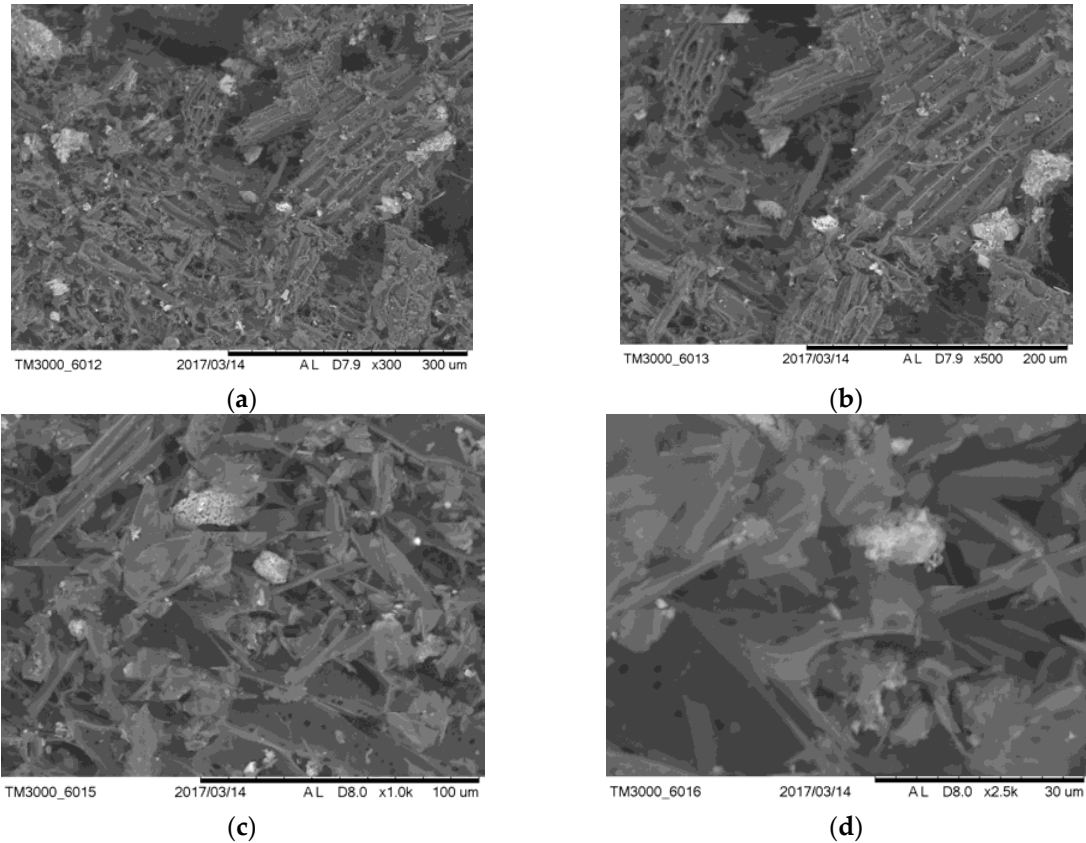


Figure 2. Microscopic images of biochar from Brazil (1-BR). (a) magnification 300x (b) magnification 500x, (c) magnification 1000x, (d) magnification 2500x.

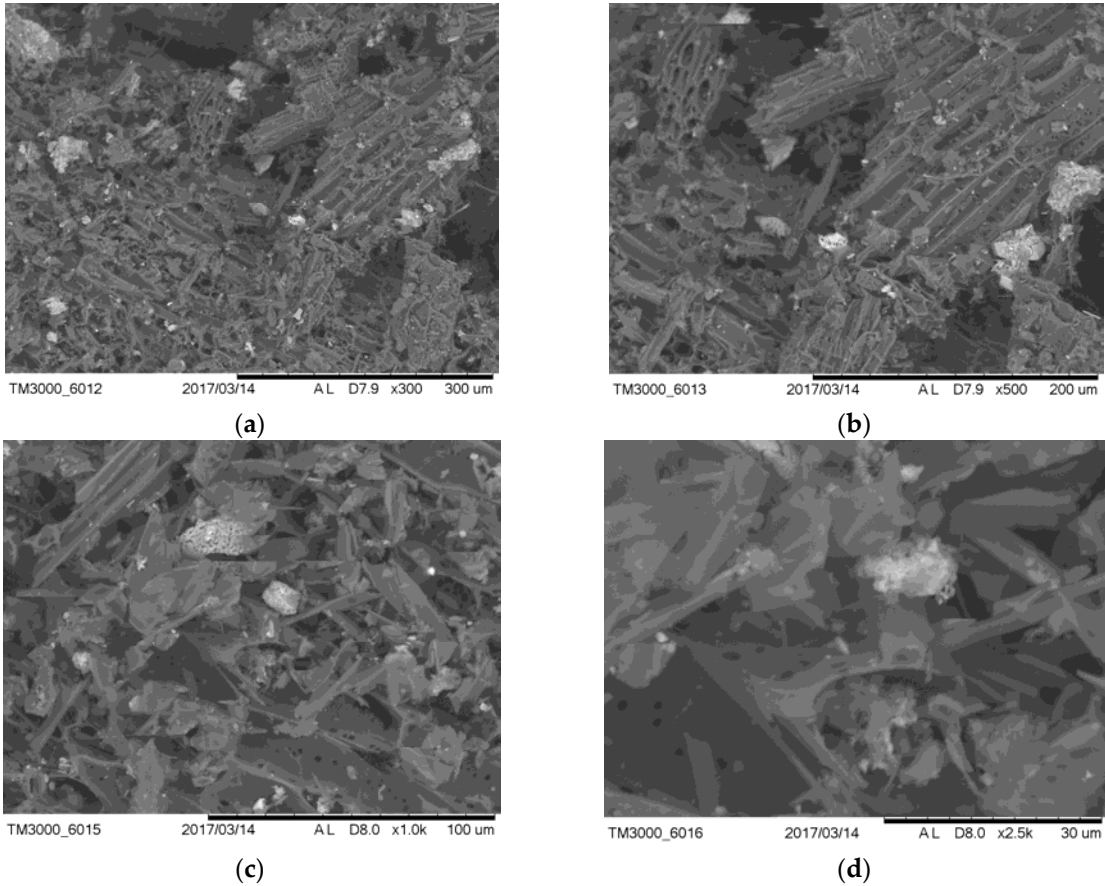


Figure 3. Macroscopic images of biochar from Poland (1- PL). (a) magnification 300x (b) magnification 500x, (c) magnification 1000x, (d) magnification 2500x

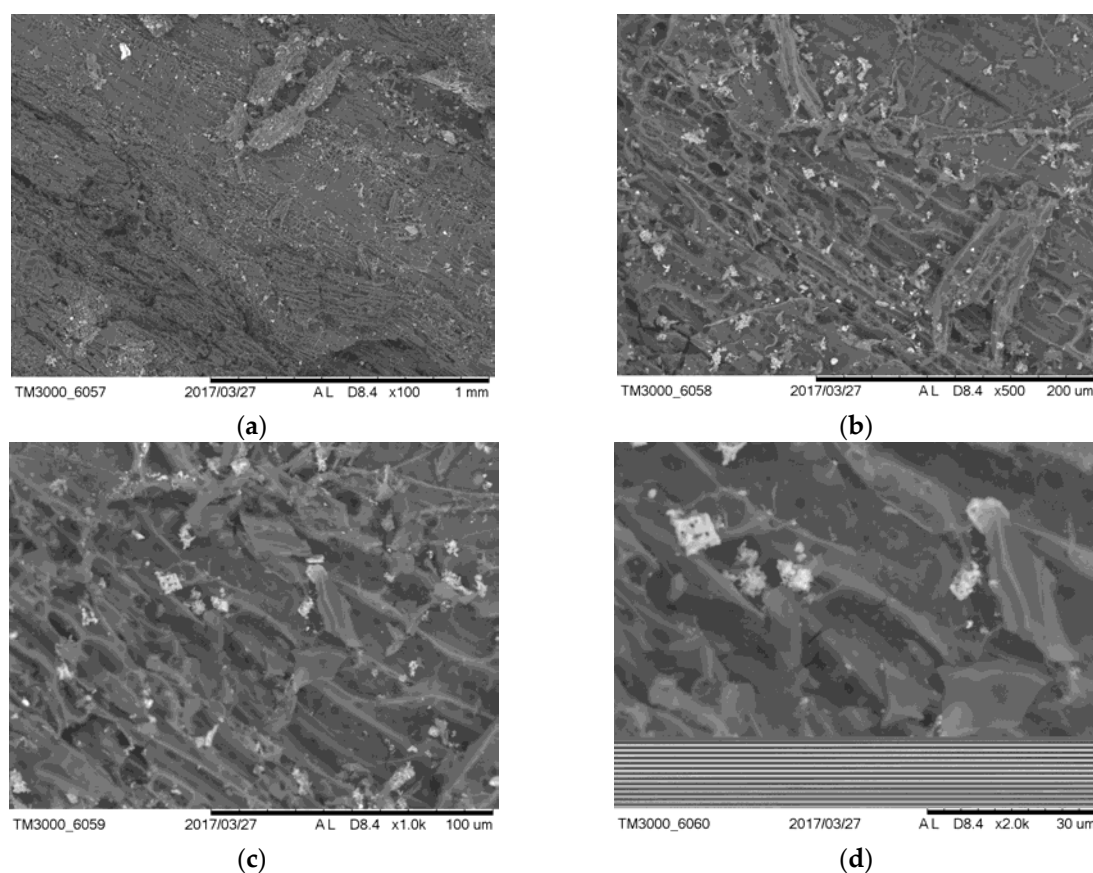


Figure 4. Microscopic images of biochar from Poland (2-PL). (a) magnification 100x. (b) magnification 500x, (c) magnification 1000x, (d) magnification 2000x.

4. Discussion

The microbiological analysis of the three biochar samples demonstrated that samples 1-PL and 2-PL were less microbiologically contaminated than sample 1-BR. In the case of sample 1-BR, similar microorganisms were isolated as from samples 1-PL and 2-PL. In sample 1-BR, among others, the following were present: *Bacillus* sp. *Aspergillus* sp. *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus versicolor*, *Aspergillus candidus*, *Aspergillus fumigatus*, as well as *Penicillium* sp. and *Rhizopus* sp. Such a broad range of microorganisms was isolated neither from sample 1-PL nor 2-PL. At the same time, there is a certain similarity between the biochar from Poland and Brazil, namely the strains isolated from Brazilian biochar were present in two types of biochar from Poland, originating from two botanically different sources. When analysing the two samples from Poland opposite observations should be made, namely that biologically different microorganisms were present in these two samples. In sample 1-PL (pine) strains *Bacillus* sp. dominated. In sample 2-PL (oak) dominated, though in small amounts, strains of fungi: *Aspergillus flavus*, *Aspergillus versicolor*, as well as *Penicillium* sp. In the sample from Brazil strains *Bacillus* sp., *Aspergillus* sp., and *Rhizopus* sp. were the most numerous ones. After the microbiological and microscopic analysis, it can be concluded that the two samples have very interesting characteristics that can be used depending on the purpose. It was observed that sample 2-PL was less microbiologically contaminated. However, it should be stated that in the tested samples biologically different microorganisms were identified. In the sample 1-PL strains *Bacillus* sp. dominated. In the sample 2-PL dominated, thought in small amount, strains of fungi: *Aspergillus flavus*, *Aspergillus versicolor*, as well as *Penicillium* sp. Additionally, differences were observed in the microscopic images, where sample 1-PL (pine) was characterised by a compact and regular structure, while

sample 2-PL (oak) had irregular and porous structure. At the same time, the significantly differing structure was observed in the case of biochar 1-BR (Brazil). It was more delicate than the other test samples. The porous structure and specific surface of biochar are its most important physical properties and they are responsible for the course of various processes in the soil. Biochar has high internal porosity, which affects the water absorption, sorption capacity, and retention of nutrients in the soil. Therefore, it improves physicochemical properties of the soil, facilitates the use of nutrients by plants, and prevents nutrients leaching. Biochar may improve soil structure (water-air properties) [36,37]. Presence of certain genera and species in the test samples may be associated with the generic traits of the raw materials from which they were derived. In addition, the manner, in which test samples were produced, might also be important. The biochar from Poland came from controlled, patented, and repeatable production. On the other hand, the biochar from Brazil was produced in a simple way reflecting the possibility to be repeated by the farmers, wherein not every production parameter can be controlled. This type of dependency is directly proportional to the microbiology of test samples. Summing up, all the samples have very interesting properties that may be used depending on the intended purpose. Implications for practice are now widely used in many research centres around the world. Herrman et al. (2019) concluded that the addition of biochar from rubber tree caused a raise of the soil pH value as well as its nutrient content. In the research cited higher sensitivity to the activity of applied biochar was recorded in the case of fungi occurring in the soil as compared to bacteria present there [38]. Similar studies were performed in Bulgaria [39] using biochar in the cultivation of wheat and maize in crop rotation. The stimulating effect of biochar on soil microflora was observed, in particular on the number of bacteria. The research was considered to be a promising method for preserving soil fertility. Azis et al. (2019) concluded that in the case of soil on which wheat is cultivated addition of biochar, in experimentally determined amount, improves the physicochemical properties of the soil and soil microflora, which in turn has a positive effect on the productivity of the soil and increased yields [40]. In other studies, Hardy et al. investigated the long-term application of biochar in order to improve the quality of soil microorganisms. For this purpose, specialized models imitating soil conditions were designed. Biochar was shown to modify the quantity and quality of soil microorganisms, however, in order to properly design a successful modification of the soil using biochar its properties should be considered in conjunction with the soil conditions [41]. Unfortunately, in the available literature of the subject, there is no similar research with which one could compare the results obtained in this work. Based on the definition of sustainable development, strategies of environmental protection should consist of continuous, integrated and anticipatory actions undertaken in production processes. Such actions lead to an increase in production efficiency and reduce risks to humans and the natural environment. Sustainable production aims at the manufacture of goods which uses processes limiting environmental pollution. In this context, biochar production based on waste-free use of organic biomass has the traits of sustainable development.

5. Conclusions

1. In the biochar sample from Brazil (1-BR), both bacteria: *Bacillus* sp., and fungi: *Penicillium* sp., *Aspergillus versicolor*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus candidus*, *Aspergillus nidulus*, and *Rhizopus* sp. were observed and identified.

2. In biochar sample from pine woodchips (1-PL) colonies of *Bacillus* sp. and *Micrococcus* sp. bacteria were observed and identified.

3. In biochar sample from oak woodchips (2-PL) fungi: *Aspergillus flavus*, *Aspergillus versicolor*, and *Penicillium* Sp. were observed and identified.

4. On the basis of microscopic images, diverse structures of the samples were recorded. Sample 1-PL (pine) is characterised by a compact and regular structure, while sample 2-PL (oak) shows porous and irregular structure. Sample from Brazil (1-BR) is characterised by a structure more delicate than these of the remaining test samples.

5. Samples coming from controlled production (1-PL, 2-PL) were less microbiologically contaminated than biochar sample from Brazil (1-BR).

6. The results obtained can be used as relevant utilitarian data for implications for practice.

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