Review

The Importance of the Targeted Design of Biochar Physicochemical Properties in Microbial Inoculation for Improved Agricultural Productivity—A Review

Angelika Gryta [1], **Kamil Skic** [1], **Agnieszka Adamczuk** [1], **Anna Skic** [2], **Magdalena Marciniak** [1], **Grzegorz Józefaciuk** [1] **and Patrycja Boguta** [1,*]

[1] Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4 Str., 20-290 Lublin, Poland; angryta@ipan.lublin.pl (A.G.); k.skic@ipan.lublin.pl (K.S.); a.adamczuk@ipan.lublin.pl (A.A.); m.marciniak@ipan.lublin.pl (M.M.); g.jozefaciuk@ipan.lublin.pl (G.J.)
[2] Department of Mechanical Engineering and Automation, University of Life Sciences in Lublin, 28 Głęboka Str., 20-612 Lublin, Poland; anna.skic@up.lublin.pl
*   Correspondence: p.boguta@ipan.lublin.pl; Tel.: +48-(81)-744-50-61

Abstract: Biochar has great potential as a soil conditioner and as a carrier of beneficial microorganisms that support the removal of pollutants, influence the circulation of nutrients, and support plant growth. This review summarizes and discusses factors shaping the physicochemical properties of biochar, including feedstock, pyrolysis conditions, and accompanying processes used as post-pyrolysis modification to improve the functionality of biochar. Key physical and chemical properties such as high porosity and specific surface area, nutrient content, pH, and biochar functional groups are discussed in detail to show biochar's potential as a carrier for microorganisms. This review also discusses and summarizes biological indicators that allow for assessing the quality and efficiency of the microbiological modifiers. Finally, this paper presents the benefits and limitations of biochar application to agriculture and provides recommendations for future research to improve the quality and expand the applicability of biochar-based inoculants.

Keywords: engineered biochar; modified biochar; microbial inoculants for agriculture; biochar-based carriers for microorganisms

1. Introduction

The current world population is 7.9 billion, which is double that of 1975 (4 billion) [1]. Considering an expected population increase to between 9.6 and 12.3 billion by 2100 [2], the intensification of work fulfilling the population demands and maintaining the standard of living is strategically important. Population growth entails, in particular, a rapidly growing demand for food, water, energy, and materials [3,4]. At the same time, special emphasis is placed on developing strategies promoting sustainable activities, including management and utilization of biomass wastes of agricultural, wood, food, municipal, or sewage origin. The European Environmental Agency reports that approximately 88 million tons of food (173 kg per person) is wasted in the European Union per year [5]. Wood waste accounts for around 50.2 million tons [6], while wasted crops reach 700 million tons per year in Europe [7,8].

Agricultural wastes generate particularly large disposal problems and governance issues. The current state of their processing is not satisfactory. The management is largely based on leaving the biomass in the field for natural decomposing, composting, landfilling, or open burning, resulting in greenhouse (CO_2, CH_4, N_2O) and pollution gas (H_2S, SO_2, NH_3) emissions [9], surface/groundwater pollution, and pathogen spread [4]. The smaller part is applied for renewable energy and fuel production [10]. Even smaller

amounts are converted into value-added products for special applications, e.g., medicine or food packaging [11], extraction of nanocrystalline cellulose [12], or production of activated carbons [13]. The appropriate way of managing waste biomass based on a circular economy strategy entails drastic changes, converting the value chains into a loop that recycles higher rates of biomass to value-added applications [14]. This is also required to achieve the key important target of the EU for net-zero greenhouse gas emissions by 2050 [15].

Among numerous agricultural or wood biomass processing technologies, the pyrolysis process seems to be an effective way for the utilization of waste. The superiority of this technique can mostly be justified by cost efficiency and the possibility of using a wide range of large-volume substrates to produce biochars—the carbon-based material possessing a large surface area, high chemical stability, and regeneration capacity [9,16]. Biochars can be widely used in various branches of industry [9,17–21]. However, due to biochar's natural origin, porous structure, the content of functional groups, organic carbon, and nutrient elements, its greatest potential lies in improving the agronomic value of soils and as a component of effective fertilizers [22,23]. Many studies have shown the positive effect of biochar on carbon sequestration [21]; CO_2, N_2O, and CH_4 emission reduction [24]; toxic metals remediation [18]; or antibiotic, pesticide, or other personal care products' immobilization [25]. However, not all results show a similar scale or even a similar direction of biochar addition effects [26–28]. For example, Brewer et al. [29] indicated an example of biochar with an abundance of nanopores being not well accessible to plants. Weber and Quicker [30] noticed that a large surface area can be less relevant for the adsorption of some gases.

Recently, a great interest has also been directed toward the simultaneous use of biochar and microorganisms, pointing out a promising, synergic effect of such a combination [31]. It may be particularly important for the formulation of new biopreparations to improve agricultural soil quality and increase crop yield. Unfortunately, due to the high diversity of biochar properties, non-uniform findings on the interaction of biochars with microorganisms are reported in the literature [32]. The ambiguous results of previous research and the above conclusions related to the prospects for the synergistic effect of biochar and microorganisms show that extended studies are necessary to better design and control the biochar properties. Different studies revealed that pyrolysis conditions, as well as pre-pyrolysis or post-pyrolysis processing, can have a key influence on the physical, chemical, and physicochemical properties of the final product and its effect on the soil. Such biochar designing and modifications may introduce changes aimed at creating a favorable environment for the development of beneficial microorganisms.

This review attempts to provide a critical overview of biochar modification procedures to allow optimization of the desired properties of the final product. Due to the innovative nature of microbial modification of biochars, this subject is presented in more detail. The overall aim of this review is to demonstrate the importance of the targeted design of biochar physicochemical properties in microbial inoculation. Detailed goals include (a) assessment of methods for shaping the properties of biochar before, during, and after pyrolysis, (b) analysis of the optimal properties of biochar as a carrier and habitat for beneficial microorganisms used to improve agricultural productivity and soil quality, (c) overview of indicators useful for assessing the impact of biochar on microorganisms, (d) critical assessment of possible implications related to the use of biochar and biochar-based products for the environment and agriculture, and (e) highlighting the perspectives and further research needed in this research area.

2. Effect of Various Modification Strategies and Major Factors on Biochar Properties

Depending on the type of process and the kind of materials used, the physical, chemical, and biological methods can be roughly distinguished in the technology of biochar engineering [18]. Another more detailed division underlies modification

techniques and moments of decisions and actions aimed at obtaining a product with desired characteristics. Considering this second division, the first type of modifications can be performed at the pre-pyrolysis stage. It covers a selection of optimal kinds of feedstock and their moisture, density, or particle size. The second type of modification concerns the control of pyrolysis parameters, i.e., residence time, pyrolysis temperature, heating rate, pressure, and type of carrier gas or catalyst. The third extremely promising group is post-pyrolysis processing. These processes are still undergoing dynamic development and include both relatively simple methods, such as acid/alkali treatment or physical activation [18], but also more complex ones, such as microbial engineering or modifications with nano-particles. Figure 1 shows the biochar modification strategies considering the above criteria.
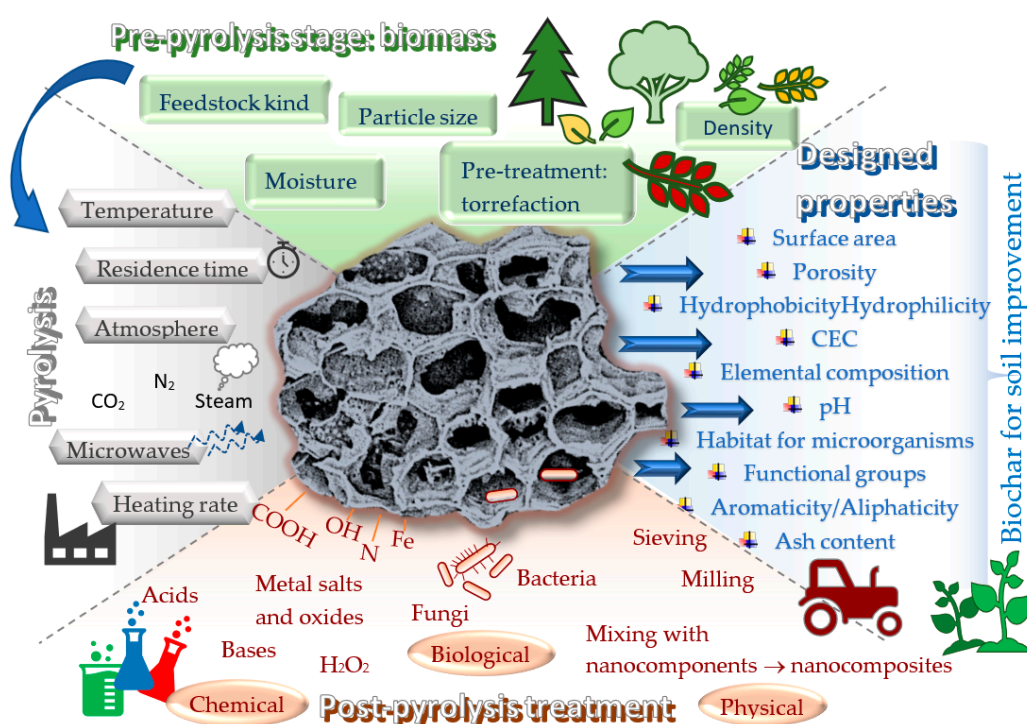

Figure 1. Strategies for the biochar modification before, during, and after pyrolysis stage.

Developing an optimal biochar modification strategy is key to improving the quality of agricultural soils, plant growth, development, and yield. The literature indicates that the application of biochar with specific, targeted properties can support and drive agricultural sustainability and yield by improving organic carbon, oxygen, moisture, and nutrient levels [18,23]. Fixation of contaminants by the biochar can reduce their uptake and accumulation in cultivated plants. At the same time, biochar addition to soils contributes to improving carbon sequestration and reducing emissions of carbon compounds into the atmosphere. Moreover, the reuse of processed agricultural waste in agriculture leads to returning nutrients to the soil, thus contributing to the development of a circular economy [18]. Optimizing biochar properties through targeted modification can further improve these effects.

2.1. Prospects for Designing Biochar Properties at the Pre-Pyrolysis Stage

The type of biomass is the first factor affecting biochar parameters. Studies by Wang et al. [33] indicate that the content of cellulose, hemicellulose, and lignin compounds in feedstock is critical for this step. Thermogravimetric measurements show that hemicellulose decomposes at 220–315 °C due to its low thermal stability. Unbranched cellulose is more thermally stable, being decomposed between 280 and 400 °C, while

degradation of the three-dimensional structure of lignin covers a wider range of temperatures (160–900 °C) due to a more complex polyphenolic propane structure [4,34]. Therefore, parameters of the thermal degradation of the biomass can directly determine biochar characteristics. For example, Byrne and Nagle [35] reported that carbonization of wood biomass can elevate the mechanical strength of the product by 28% as compared to the precursor. Biomass of high lignin content favors the biochar formation with higher carbon content and larger specific surface area but lower nitrogen and ash contents [33,36]. More hemicellulosic biomass like switchgrass will be a better precursor for biochars of larger pore volumes than those derived from wood due to thinner pore walls and an abundance of internal channels [36]. However, in some cases, high pyrolysis temperatures can reverse the above occurrences [33].

The ash content of biochar depends on the composition of the feedstock. The most important inorganic constituents of biomass, like silica and metal oxides, can have a significant impact on biochar features [37]. Larger concentrations of alkaline cations and higher pH of biomass can result in higher pH of the product, particularly at higher pyrolysis temperatures [38]. The higher ash content of the feedstock reduces the efficiency of the biomass pretreatment and provides higher particulate matter emission [4]. Agricultural wastes of a global abundance, like crop straw, rice husk, peanut shells, corncobs, and many others, contain a huge diversity of cellulose, hemicellulose, lignin, and ash [39]. Therefore, selecting the optimal feedstock mixtures would be important in the production of biochars of targeted characteristics.

Preparation of the substrate for pyrolysis may also govern product properties. Biomass pretreated in the anaerobic digestion process generated biochar with higher anion and cation exchange capacity and higher surface charge compared with pristine biochar [18]. Reduction of the biomass particle size increased the C content, surface area, pore volume, and pH of biochar and decreased its yield, N, O, and ash content [40]. Titova and Baltrėnaitė [41] showed that feedstock density corresponded to the biochar density. Moisture content in biomass decreases heat transfer effectiveness during pyrolysis, thus increasing the amount of energy required [42] and decreasing the yield of the solid product [43]. Lower moisture contributed to a more polyaromatic and graphite-like structure of biochar obtained from maple bark, while its influence on the bulk density of the product was limited [44]. Studies by Liu et al. [45] show that water content in the feedstock turned into steam at high pyrolysis temperature and can react with char, developing a specific surface. There are also reports of beneficial biomass pretreatment through torrefaction (mild pyrolysis, roasting, or high-temperature drying). This process, conducted usually between 200 °C and 300 °C under an inert or reducing atmosphere, offers higher energy density, lower moisture, and volatile matter contents that result in improved grindability and better stability of biomass devoted for further pyrolysis [4]. This pretreatment can lead to the decomposition of some of the hydrogen- and oxygen-containing organic structures and the breaking of some hydrophilic bonds, which makes the biomass more hydrophobic. It is worth mentioning that torrefaction effectiveness is higher for biomass of lower than 10% humidity. Apart from pyrolysis, torrefaction can also be an effective pretreatment method for other thermochemical conversion processes like, e.g., gasification.

2.2. Designing Biochar Properties by Controlling Pyrolysis Conditions

Optimization of pyrolysis conditions can affect the biochar properties within very wide ranges. The pyrolysis temperature is crucial for the transformation of organic components of biomass having different thermal stability [46]. Different studies showed that biochars prepared at temperatures above 500 °C exhibit higher specific surface area, aromaticity, pH, ash content, and/or water holding capacity and lower variable surface charge, contents of volatile matter, surface functional groups, oxygen, and hydrogen than those obtained at lower temperatures [18,47,48]. Biochars produced at the highest pyrolysis temperatures (above ~700 °C) can even reveal 95% of C and below 5% of O, while the pyrolysis temperature's effect on N can be ambiguous [30]. According to Sato et al. [49],

low-temperature biochars containing higher H/C and O/C ratios are more relevant to improving soil quality. An increase in surface area with pyrolysis temperature accompanies an increase in the product porosity. The degradation of intramolecular and intermolecular chemical bonds of cellulose, hemicellulose, and lignin leads to the formation of low-molecular-weight volatile liquids and gases, which escape, creating numerous channel structures [50,51]. The formation of the cracks leads, however, to a decrease in the mechanical strength of the biochar. Therefore, low moisture and higher lignin content may favor the production of materials with higher compressive strength. Some reports show that the trend of increasing surface area and porosity with increasing temperature may not be very clear for some feedstock compositions. Lee et al. [52] indicate that lower values of surface area (12 m²/g) at pyrolysis temperatures of 450 °C could result from a higher content of inorganic components that fill or block the biochar micropores. An increase in pyrolysis temperature can also increase water retention. Marshall et al. [53] reported that biochars derived from grapevine cane at 700 °C can have significantly higher plant-available water content than biochars produced at 500 °C and 23% higher than a typical clay soil. In addition to the importance of high porosity and surface area for the sorption of pollutants such as heavy metals, antibiotics, or pesticides [48], extensive microporous structure may be beneficial for microorganism colonization and organic matter storage [47,54,55].

The pyrolysis residence time is not only of purely economic importance related to energy inputs but can enhance the biomass transformation processes, as well. Ronsse et al. [51] reported that an increase in time of wood biomass pyrolysis at 300 °C from 10 to 60 min resulted in a decrease of volatile matter content from 78 to 42.6%, an increase in total C from 54.1 to 71.3%, and pH from 4.5 to 5.7. Weber and Quicker [30] highlighted that the significant effect of the residence time modulation on pH can cover the first 5–10 min.; therefore, its practical application can be limited. Moreover, surface area increase induced by residence time increase is not as effective as in the case of an increase in pyrolysis temperature [56]. Slow pyrolysis can help to reduce the thermal cracking of biomass and complete secondary pyrolysis reactions [57]. It enables the obtaining of higher surface areas and pore volumes due to the better diffusion of volatiles from the solid [58]. Long residence temperatures connected with low heating rates favor the generation of pyrolysis products of larger particle sizes [18]. Fast pyrolysis leads to a higher yield of liquid and gaseous products and oxygen-rich biochar [4,18].

The pyrolysis atmosphere can also regulate the biochar characteristics. Zhou et al. [18] noticed that vacuum or low pressure (between 0.05 and 0.20 MPa) prevents the steam volatilization of inorganic components. Zhang et al. [59] proposed ash removal by carbonization followed by CO_2-enhanced water leaching. They arrived at an ash removal rate of around 30% for peanut shell and poplar feedstock. Lee et al. [60] noticed that steam or CO_2 activation facilitates specific surface area development and porous structure formation. Steam facilitates the devolatilization and production of crystalline carbon [61]. Hydrothermal processing at mild temperatures and longer residence time could be a better solution for utilizing wet feedstock into hydrochar [62]. Microwave processing can reduce pyrolysis time and temperature through the easy and effective heat penetration of the internal part of the particles [63]. However, according to Rex et al. [9], poor microwave absorbers can generate plasma and hot spots. Such thermal disturbances can be weaker sides of the method.

Generally, it should be stated that obtaining biochar with the desired properties may depend on several different factors simultaneously regulating the pyrolysis process. Therefore, in the case of less influential factors such as residence time, the final effect cannot be determined clearly due to the simultaneous occurrence of other factors. Among the factors discussed in this section, the regulation of the pyrolysis temperature generates a clear, strong, and predictable effect. It has been commonly accepted that an increase in this parameter leads to an increase in porosity, specific surface area, pH, and ash content, as well as a decrease in negative surface charge, contents of volatile matter, and oxygen-containing functional groups.

2.3. Modifications of Biochar Properties through Post-Pyrolysis Processes

The interest in post-pyrolysis processing is particularly important for special applications of biochars. The most frequently used methods are based on physical or chemical modifications. Newer methods include nano- and microbiological engineering [64]. The beneficial effects of all the above modifications need strictly defined conditions; otherwise, the characteristics of the biochar may deteriorate. Physical techniques are believed to be more environmentally friendly and easy to process. The cheapest and simplest treatment seems to be water leaching, which helps reduce organic compounds, including potentially toxic chemicals condensed in the porous structure during the cooling stage [65]. The properties of biochar can also be modulated by particle size fractionation by sieving [66] or grinding [67]. Grinding in a ball mill may lead to the production of very fine particles (nanobiochar). In the latter technique, the biochar can be admixed with the other components that allow obtaining various nanocomposites of high surface area, pore volume, and contaminant adsorption efficiency equivalent to that of an activated carbon [67,68]. A certain challenge of ball milling is the difficulty of nanoparticles' application in real environmental conditions.

Chemical modifications are often characterized by more remarkable surface effects but can generate chemical wastes [69]. Post-processing with different acids, such as HNO_3, H_2SO_4, HCl, H_3PO_4, or organic acids, has the potential to generate better surface properties: higher porosity, surface area, and particularly higher content of carboxyl, phenolic, and lactone functional groups [61]. These features can be valuable in the immobilization of toxic chemicals like heavy metals, antibiotics, or phenols [70]. The change in the number of oxygen-containing groups or pore size distribution depends largely on the concentration of the modifying substance. Boguta et al. [71] reported a 15% and 9% increase in the number of acidic groups after biochar treatment with 0.5 M and 1.0 M H_2SO_4, respectively. The same groups increased by 15% and 4% after modification with 0.5 M and 1.0 M HNO_3. The highest acid concentration could cause partial degradation of the biochar skeleton containing acidic functional groups. Collapsing of the thinnest walls at the highest acid concentration might result in a decrease in surface area. Alkali treatments commonly utilizing NaOH and KOH as activating agents contribute to an increase in surface area, basic functional groups, and pH and a decrease in the population of the smallest pores [71,72]. Zhou et al. [18] reported that alkali modification can result in the formation of a positive charge on the surface which can be used in the sorption of anions. Enrichment in oxygen-containing functional groups and, thus, the increase in CEC and decrease in pH can also be achieved in treatment with increasing concentrations of H_2O_2 solutions [73]. Another type of modification is CO_2 or NH_3 treatment, which enhances porosity. The NH_3-treated biochars have higher nitrogen content and can be used for acidic gas capture [74].

Inorganic salts and metal oxides can be used for biochar modification both at the pyrolysis and post-pyrolysis stages to obtain materials with improved adsorption, catalytic, magnetic, and thermal properties [18,61,75,76]. Post-pyrolysis modification of biochar with metal nanoparticles, advantaging the synergic effect of both ingredients, can result in the production of highly efficient nanocomposites of high specific surface area. Such materials may have enhanced positive surface charge and be used for the sorption of anionic compounds. Among other examples, magnetic biochars, e.g., nanocomposites with zero-valent iron, have excellent regenerative and recycling properties. Improved surface properties, in particular the increased specific surface area of such nanocomposites, encourage further research using other nanoparticles like graphene, ZnS, chitosan, and carbon nanotubes [70]. A challenge of such materials may be their stability under various environmental conditions.

A brief overview of the literature dealing with biochar modification methods is presented in Table 1.

Table 1. Examples of biochar modification by various methods.

Modification	Modulated Factor	Information on Biochar	Effect on Biochar Properties	Ref.
		Pre-pyrolysis Modifications		
Feedstock composition	Different lignocellulosic biomass: spruce wood, corn stalk, corn cob	Pyrolysis: 600 °C, HR 10 °C min⁻¹ for 1 h	The corn cob and corn stalk biochar showed higher concentrations of inorganic elements, P (4550 and 3230 mg/kg), and K (28,410 and 21,150 mg/kg). Ash content was the lowest for woody biochar (1.5 wt%) and the highest for corn cob biochar (12.4 wt%). The S (N_2) was the highest for woody biochar (465 m²/g) and the lowest for corn cob (94 m²/g).	[33]
Feedstock moisture	Moisture: from 6 to 42 wt%	Feedstock: maple bark and softwood bark; vacuum pyrolysis: HR 10 K/min, 300–775 K.	With decreasing feedstock moisture content, oxygen concentration increased from 10 to 33% (stronger for softwood charcoal), the S decreased from 326 m²/g to 206 m²/g (softwood), and micropore volume decreased from 0.1 to 0.05 cm³/g (maple).	[44]
Feedstock moisture	Moisture: dry, wet, and soaked sludge	Feedstock: raw and dewatered sludge from MWTP, lignite; Pyrolysis: 60 min, HR 5–24 K/s (4–7 K/s at 873 K, 8–14 K/s at 1073 K, 14–22 K/s at 1273 K)	Higher moisture contributed to lower C content and increased O/C. No significant difference was found in the S between dry and soaked chars at 873 K. Bound water increased S from 114.09 m²/g to 128.98 m²/g at 1273 K.	[45]
Feedstock particle size	Biomass particle size: 10–50, 50–100, 100–200 mesh	Feedstock: rice husk; pyrolysis: 500 °C, RT 60 min, HR 10 C/min	Increase in particle size resulted in decrease in C content from 76.12 to 67.46% and in the S from 24.96 to 20.11 m²/g, as well as pore volume and pH of biochar, and in increase of biochar yield, N, O, and ash content.	[40]
Feedstock density	Feedstock density: tora willow: 314 kg/m³; artemisia dubia mugwort: 226 kg/m³; sewage sludge compost: 1043 kg/m³	Feedstock: willow, mugwort, sewage sludge compost; pyrolysis: 2 h, 450 °C and 700 °C, HR 10 °C/min	The highest density (986 and 1149 kg/m³) was found for biochars produced from the feedstock of the highest density (1043 kg/m3).	[41]
		Pyrolysis Modifications		
Pyrolysis temperature	PT: 300, 400, 500 °C	Feedstock: sunflower husks (SH), wood waste (WW); pyrolysis: 30 min.	Increasing PT of SH and WW caused increase in pH of biochars from 9.9 to 11.1 and from 8.1 to 10.1 and in the S from 71.7 to 85.6 m²/g and from 53.1 to 70.3 m²/g and decrease in carboxylic groups from 30 to 20 and from 40 to 30 cmol/kg.	[48]
Pyrolysis temperature and residence time	PT: 300, 450, 600, 750 °C RT: 10 and 60 min	Feedstock: wood, straw, green waste, dry algae	Increase in PT caused increase in ash, total C, and pH and decrease in volatile matter for biochars derived from all studied biomass and at two RT. Increase in RT caused decrease in volatile matter and increase in fixed and total C and pH for biochars derived from all studied	[51]

			biomasses. The S increased for all biomass at PT increase from 450 to 600 °C and decreased for woody biochar derived at PT 750 °C.	
Heating rate	HR: 10, 30, 50 C/min	Feedstock: safflower seed press cake; pyrolysis: 400, 450, 500, 550, and 600 °C	With increasing HR, the S and pore volume decreased from 3.41 to 2.47 m²/g at PT 600 °C and from 0.0064 to 0.0047 cm³/g. The yields of biochar changed from 34.18% to 29.70% at PT 400 °C. This effect was weaker for higher PT.	[58]
Pyrolysis atmosphere	at-CO_2 and N_2 atmosphere	Feedstock: red pepper stalk; pyrolysis: flow rate of agent gas: 500 mL min^{-1}	The S of biochar produced in CO_2 increased from 32.46 to 109.15 m²g^{-1}. Pore volume increased from 0.02 to 0.09 cm³/g.	[60]
Supporting process	Steam activation	Feedstock: burcucumber; pyrolysis: 300 and 700 °C, HR 7 °C min^{-1}, RT 2 h. Steam activation after pyrolysis: flow rate 5 mL min^{-1} for 45 min	Steam-activated biochars produced at PT 300 and 700 °C showed increased ash content from 25.40 to 28.67% and from 43.72 to 70.66%, increased S from 0.85 to 1.22 m²/g and from 2.31 to 7.10 m²/g, and decreased yield from 51.83 to 50.21% and from 27.52 to 18.90%.	[77]
Supporting process	Microwaves	Feedstock: willow wood chips and mixed straw pellets consisting of wheat and rape straw; pyrolysis: HR 5 °C min^{-1}, 200, 250, 300 and 350 °C, RT 10 min; microwave pyrolysis: MW power 1200 W, vacuum, 170 and 200 °C	Yield of char derived from willow wood and straw pellets was the lowest for microwave-assisted pyrolysis, reaching 27.3 and 33.7% as compared to 39.8% and 49.9% for conventional pyrolysis conducted at 350 °C. The S (BET) was higher for microwave-assisted biochars as compared to conventionally obtained biochars and reached 3.87 and 1.14 m²/g for biochars derived from willow wood and straw pellets as compared to 0.17 and 0.51 m²/g for biochars produced conventionally at 350 °C.	[78]
Post-pyrolysis Modification				
Chemical modification	KOH, HNO_3, H_2SO_4, H_2O_2, and $KMnO_4$	Feedstock: rice straw; pyrolysis: 550, 650, 750 °C, HR 50 °C/10 min, N flow 300 mL/min	KOH treatment increased S (179.7 m²/g at 650 °C), followed by H_2O_2 and $KMnO_4$, whereas H_2SO_4 treatment decreased S, followed by HNO_3. HNO_3 and H_2SO_4 treatment contributed to higher mesoporosity. H_2O_2 treatment contributed to the highest microporosity, while KOH increased both high micro- and mesoporosity.	[72]
Chemical modification	NaOH + methanol	Feedstock: rice husk; pyrolysis: fast, 723–773 K	Increase in the S from 51.86 m² g^{-1} to 65.97 m² g^{-1} for modified char, decrease in carbonyl groups, and increase in ester and hydroxyl groups after modification.	[79]
Chemical modification	H_2O_2 solution (1, 3, 10, 20, 30% *w/w*)	Feedstock: pinewood; pyrolysis: 400 °C	Biochar treated with H_2O_2 showed higher CEC (increase from 17.95 to 31.37 cmol/kg) and lower pH (decrease from 7.16 to 5.66).	[73]
Chemical modification	CO_2 and NH_3 at 600 °C	Feedstock: cotton stalk; pyrolysis: 600 °C	As compared to untreated biochar, modifications with NH_3 and CO_2 resulted in increase of the S of micropore from 224 m²/g to 252 and to 352 m²/g and N content change from 1.09 to 3.48 and to 1.02 wt (%).	[74]

Physical modi-fication	Particle size reduction in ball milling process	Feedstock: pine wood, spruce and fir; pyrolysis: 525 °C	Milled biochar particles showed higher S (47.25 m²/g) compared to raw biochar (3.12 m²/g). Raw biochar adsorbed less than 14% of carbamazepine, milled biochar—more than 98% in 3 h.	[67]
Modification with nanoparti-cles	Loading MnO_2 nano-particles on biochar	Feedstock: water hyacinth; pyrolysis: 450 °C, HR 5 °C min⁻¹, RT 3 h	Increasing loading of MnO_2 from 0.6 to 18.4 wt% increased the S from 3.5 to 120.2 m²/g and decreased it at higher MnO_2 loading. A 26.6% loading can show high capacity for heavy metals removal from electroplating wastewater.	[80]

PT—pyrolysis temperature; HR—heating rate; RT—residence time; S—surface area.

Biochars are becoming more popular as a carrier for various beneficial microorganisms, which are added to soils for remediation or naturalization purposes. Despite the fact that the properties of the biochar may be, in principle, modified by microorganisms, there are far fewer reports on this subject than for those on the pre-pyrolysis, pyrolysis, and post-pyrolysis modifications described above. The experimental works indicate that several chemical and physicochemical characteristics of biochars may be more beneficial for the habitat conditions of microorganisms and, thus, for innovative biopreparations production. The literature shows that properties of biochar such as pH, porosity, content of minerals, organic matter, free radicals, volatile matter, or nutrients may affect microbial activity, promoting or suppressing their community [76]. The initial period of bioaugmentation seems to be critical for creating a proper micro-habitat, allowing for microorganisms' survival and further expansion. Therefore, to promote better habitat conditions for microorganisms, the selection of advantageous physicochemical properties of biochar seems to be of the utmost importance, which can be achieved by targeted biochar modification. Again, the physicochemical properties of the biochar matrix are of primary importance.

3. Optimal Properties of Biochar as a Carrier and Habitat for Microorganisms

3.1. Valuable Features of Microbiological Carriers

Interest in agriculture sustainability and environmental quality attracts attention to microbial inoculants. These are products containing living microorganisms capable of having beneficial effects on the development of various plant species [81]. Microbial inoculants could be made of a singular strain of microbe, two or more microbe strains, or different types of organisms [82]. They are a viable alternative to harmful chemicals in increasing food production by improving plant growth and productivity and preventing pest and disease attacks without endangering human health and the environment [83–85]. Most of the microorganisms used as microbial inoculants can be broadly classified as plant growth-promoting bacteria (PGPB) or root-colonizing rhizobacteria (PGPR) and their fungal counterparts [86].

The key obstacle in microbial inoculation is the heterogeneity of soil colonized by other microorganisms, making it difficult to find an empty niche in the soil. The introduced microorganisms are confronted with factors detrimental to their viability, such as UV radiation; variable soil properties such as temperature, pH, and texture; and repeated drying–wetting cycles depending on the frequency of precipitation. For these reasons, the effective use of inoculants requires appropriate carriers that support the growth and delivery of microorganisms to the rhizosphere [87–91]. A summary of the valuable features of microbial carriers is presented in Figure 2.
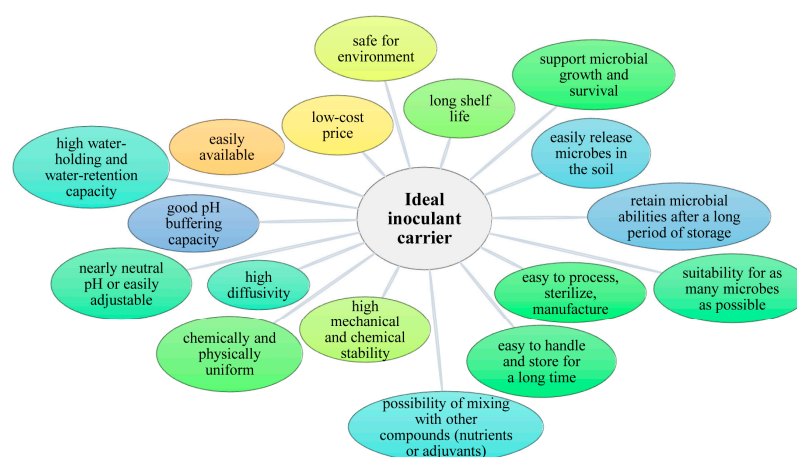

Figure 2. Characteristics of an ideal inoculant carrier.

Immobilization of microorganisms takes place by attaching or trapping cells in organic or inorganic water-insoluble materials [92,93], which is expected to preserve metabolic activity and reduce the pressure from heterogenic environments and metabolic products [89,94,95]. An appropriate carrier should not negatively interfere with the activity of the introduced microorganisms, e.g., by adsorbing signaling compounds, antibiotics, and plant growth hormones excreted by cells [96]. The viability of inoculants may be affected at various stages before and during the application; thus, the product should have a sufficiently long shelf life that determines its stability at the time of the production process, packaging, storage, and transport [82,97]. Additionally, immobilization should provide protection from predators [98,99], greater resistance to toxic compounds, higher cell density, and better reusability of biopreparations [18]. Ideal inoculants should be easy to use, cost-effective in production, and safe for people, animals, and plants. Moreover, they should work effectively under different field conditions and types of soil and be compatible with routine field practices [87,88]. It is also important to prepare carriers with acceptable physical and chemical properties, i.e., high water-holding capacity, buffering capacity, nearly neutral or easily adjustable pH, high diffusivity, high mechanical and chemical stability, and chemical and physical homogeneity [87,100,101].

One of the most attractive materials that can act as an inoculum carrier is biochar [87]. Broad availability and low cost of feedstock for biochar production and its high sterility make this carbonaceous material more economically profitable than other carriers [102]. Several studies certify that biochar can be a carrier for microbial inoculants under various climatic and environmental conditions, improving soil productivity and promoting plant growth and nutrient uptake [103–105]. These beneficial microorganisms are involved in carbon and nutrient cycling and soil biochemical processes [32]. Moreover, biochar-based carriers enriched with microorganisms have shown great potential in various environmental pollutant removals [55,106,107]. The effectiveness of soil bioremediation increases through the sorption of organic pollutants onto biochar modified with bacteria selected for their ability to degrade organic pollutants [108]. Biochar also enhances the ability of bacteria to survive and grow in contaminated soils while promoting the effectiveness of bioremediation [55,107]. Due to its unique features, biochar can affect microbial habitats and soil physicochemical properties by regulating soil pH, improving water retention and nutrient transport, and modifying soil porosity and pore structure. It was shown that biochar-based formulations increased cell viability and the functioning of the bacterial community [104,106], supported plant growth and nutrient uptake in unfavorable conditions [105], promoted seed germination, and improved the physical and chemical properties of soils, acting as a sustainable substitute for chemical fertilizers [103,109,110]. Biochar as a microorganism carrier creates a safer and more valuable way of agricultural practice, which contributes to improving soil health and ecological sustainability. Table 2 summarizes the use of biochar as a microbial carrier.

Table 2. Feedstock type, pyrolysis conditions, and the effects of biochar used as a carrier of beneficial microorganisms. NA in the table means no available data.

Feedstock/Pyrolysis Conditions	Microorganism(s)	Most Important Effects of Using Biochar as a Carrier	Ref.
Maize silage, batch-wise hydrothermal carbonization at 210 °C Maize, pyrolysis at 600 °C Wood, pyrolysis at 800 °C	*Bradyrhizobium* sp.	The highest survival of bacteria was observed in hydrochar from maize silage. An effective carrier for inoculum, improving growth and nutrient uptake of lupins under drought conditions.	[105]
Agricultural waste (leaves of cabbage, spinach, cauliflower, and green gram leaves) Pyrolysis at 250 °C	*Burkholderia* sp. *Bacillus* sp.	Improving the physical, chemical, and biological properties of the soil Higher microbial viability was observed up to 270 days of storage. Increasing the parameters of the tested plant.	[109]
Agricultural wastes (discarded cabbage, cauliflower, leguminous plants, leafy vegetables) Pyrolysis at 600 °C	*Burkholderia* sp. *Bacillus* sp.	Increased the shelf life of bacteria. Increased seed germination and promoted tomato (*Lycopersicon esculentum*) growth and yield. Improved soil physical and chemical properties and enhanced dehydrogenase activity.	[103]
Cassava stem Pyrolysis at 300 °C	*Arthrobacter* sp. *Micrococcus* sp.	Enabling the survival and growth of bacteria in cadmium-contaminated soil. Promoting the efficiency of cadmium phytoextraction by *Chlorophytum laxum* R.Br.	[107]
Rice straw, rice husks, soybean straw, peanut shells, corn cobs, wood	*Arthrobacter defluvii* *Burkholderia cepacia* *Bacillus megaterium* *Pseudomonas frederiksbergensis* *Rhodanobacter* sp. *Streptomyces prasinopilosus* *Variovorax paradoxus*	Biochar affected survival and functioning of the bacterial community. Improved rape growth and phosphate uptake. The available P content of the biochar was recognized as dominant factor affecting bacterial community structure.	[104]
Tea leaves Pyrolysis at 350 °C and 600 °C	*Bacillus cereus*	Biochar produced at a higher temperature was a suitable carrier and revealed longer shelf life. Biochar enhanced community of *Bacillus cereus* as compared to peat alone. Improved soil properties, growth, and yield of mung bean.	[110]
Lantana camara biomass Pyrolysis at 300 °C	*Azotobacter chroococcum*	The biochar-based carrier revealed no contamination during storage. The highest moisture content and maintained microbial viability at the end of the storage.	[111]

Crop straws from cotton, peanut, maize, soybean, wheat Pyrolysis at 300 and 600 °C	*Bacillus megaterium*	Improved survival of bacteria by cotton straw biochar that acts as a carrier. Improved soil available phosphorus.	[112]
Wheat straw Pyrolysis at 700 °C	*Acinetobacter calcoaceticus*	Effective immobilization of bacteria was observed on biochar-based carriers due to the developed specific surface area and porous structure that supported the nutrients. Increased bioremediation of lead-contaminated soil.	[55]
Waste of *Eichhornia crassipes* Pyrolysis at 400, 500, and 600 °C	*Pseudomonas plecoglossicida*	Biochar alleviated the cytotoxicity towards microorganisms and promoted cell proliferation. Biochar stimulated extracellular substances' secretion and sheltered microorganisms under unfavorable conditions. Biochar promoted effectiveness in reducing the availability of Cu and Pb and the degradation of 2,2′,4,4′-tetrabrominated diphenyl ether (BDE-47).	[106]
Pinewood Modified with Luria–Bertani broth, a worm-casting extract, or mixed with earthworm castings Pyrolysis at 600°C	*Pseudomonas putida*	Biochar of neutral pH was near optimal for *Pseudomonas putida*. A high population abundance was sustained after five months of storage. Carrier supplementation does not promote increased shelf life or inoculum efficacy.	[98]
Four biochars types consisting: rice husk, coconut shell, oil palm empty bunch, corncob Pyrolysis at 500–600 °C	*Paenibacillus alvei* *Burkholderia cepacian* *Acinetobacter baumannii* *Penicillium variabil*	Optimal pH and higher nutrient content of biochar influenced higher cell density. Effective adhesion of microbes within pore space (2–4 μm) and at the surface was observed only for coconut shell biochar. Biochars were found to increase the survival of microbial inoculants up to six months. Due to limited moisture content, biochar does not present any storage problem.	[102]
Three biochar types prepared from *Eucalyptus Marginata*, *Eucalyptus marginata* and *Eucalyptus wandoo, Acacia saligna* Pyrolysis at 550–650 °C and 380 °C	NA	All biochars contributed potential habitats for soil microorganisms due to the high porosities and surface areas. Transient effects of particle size within each biochar source were observed at earlier stages of the incubation.	[113]
Cotton stalks Pyrolysis at 400 °C and 600 °C	*Bacillus subtilis*	Biochars stimulate the growth rate of *B. subtilis* in a liquid nutrient broth medium. Bacteria immobilization increased with biochar particle size decrease and with larger specific surface area. The contact area between the biochar particles and microorganisms was one of the most important factors in cell immobilization. Gradual release of bacteria from the biochar surface and tubular structure was observed.	[114]

Biochar source	Microorganism	Effect	Ref.
Wood pellets biochar, a mixture of spruce and pine wood without bark (poor in ash and nutrients) Biochar from chicken manure (rich in ash and nutrients) Pyrolysis at 550 °C	*Rhizophagus irregularis*	Arbuscular mycorrhizal fungi (AMF) used biochar as a growth matrix and nutrient source. AMF were shown to grow on two contrasting types of biochar particles, strongly attaching to their inner and outer surfaces. Contact of hyphae with the biochar surface stimulated phosphorus uptake and its translocation to associated host roots.	[115]
Waste products (palm fronds, pine wood, coconut shells, pistachio nut shells, stone fruit pits) Pyrolysis at 300 °C and 600 °C	*Enterobacter cloacae*	Chemical properties of biochar, particularly nitrogen content and pH, were among the most important characteristics affecting initial inoculum survival and shelf life. Physical features, including surface area, pore diameters, and water-filled pore spaces, were more closely associated with inoculum survival after incorporation into the soil.	[96]
Acacia wood and coconut shell	*Azospriliium lipoferum*	Coconut shell-based biochar with the highest specific surface area, water holding capacity, and nutrient availability increased the survival of *Azospirillum lipoferum* up to 6 months.	[116]
Corn stalk Pyrolysis at 250 °C, 550 °C, and 850 °C	*Acinetobacter lwoffii* *Bacillus megaterium* *Bacillus subtilis* *Pseudomonas aeruginosa* *Enterobacter* sp.	Biochar produced at a temperature of 550 had the greatest effect on the growth, phosphorus solubilization, and acid phosphatase activity of phosphate-solubilizing bacteria. A reduction of residual atrazine and an increase in the content of total and available phosphorus were observed.	[117]

As shown previously, the physical and chemical properties of biochar vary with the source material, pyrolysis conditions, and production process. All these properties can affect the interactions between biochar and microbial colonization [105,107]. The next sub-sections discuss the key physical and chemical parameters of biochar that determine its effectiveness as a carrier and habitat for microorganisms.

3.2. Physical Properties of Biochar

Figure 3 summarizes the physicochemical properties of biochar affecting the immobilization of microorganisms in the biochar matrix.
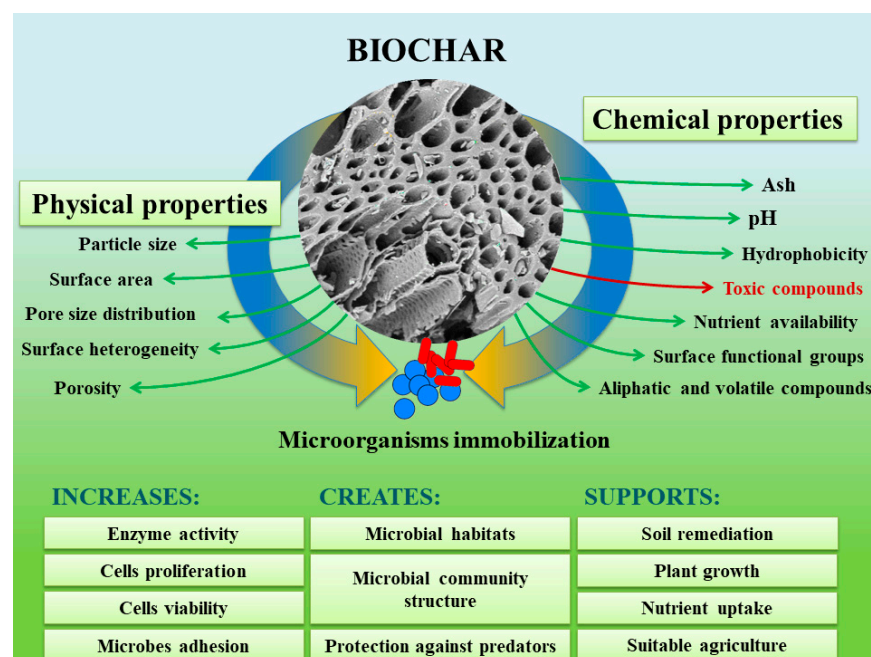

Figure 3. Selected physicochemical properties of biochar affecting immobilization and vitality of beneficial microorganisms within biochar matrix and after soil application.

The immobilization process of microorganisms on the surface of biochar can involve several mechanisms such as adsorption, cell entrapment, and surface binding (electrostatic or covalent), which are governed by properties of biochar, such as ash content, particle size and shape, and surface area of pores [118–121]. These properties vary with the type of raw biomass and the pyrolysis conditions [121–125]. Depending on the size, shape, and tortuosity of the pores, the surface area may be accessible to various groups of microorganisms and, therefore, it is expected that different biochars provide various degrees of protection of bacteria and fungi against predators or competitors, as well as unfavorable environmental conditions such as drought [109,113,126]. The specific surface area of different types of biochar can vary significantly from several to several hundred square meters per gram [127] and is often associated with smaller biochar particles and higher surface heterogeneity [18,113]. The adsorption of microorganisms onto biochar usually increases with particle size decrease due to higher contact area between biochar particles and microorganism cells [114]. Jaffar et al. [113] assessed the potential interactions between biochar from different sources and of various particle sizes with soil microbial properties in a short-term incubation study. Colonization of hyphae was observed on the biochar surface and in larger pores after 56 days of incubation. The authors concluded that all biochars created suitable habitats for soil microorganisms due to preferable porosity and specific surface area. It was also found that smaller biochar particles often have more micropores and retain water more strongly than larger particles [116,128], thus creating a better habitat for microbial populations [90,108]. It is believed that a moisture content

higher than 40% is optimal for microbial growth [118]. The osmotic potential and matrix potential below this limit diminish the amount of water available to microorganisms [102,120]. Numerous authors emphasize that biochar can affect soil water characteristics and improve infiltration, permeability, and water-holding capacity [18,129–132]. Therefore, while microorganisms would be stressed in the soil by periodic droughts exposing them to dormancy or even death [133], applying biochar could improve microbial inoculants' survival and distribution [110]. However, as Feng et al. [134] pointed out, the impact of biochar will vary depending on soil type and the amount of used biochar, which should be considered before its application.

Accessible surface area and a well-developed pore structure of biochar may determine the immobilization of various groups of microorganisms, including bacteria [96,114,120] and fungi [115,135]. Tao et al. [114] revealed that cells of bacteria entered the tubular structure of biochar prepared from cotton stalks, and only some population was attached to the biochar surface. Lou et al. [136] reported that an enhanced number of bacterial cells was immobilized on bamboo biochar than on wood biochar. The authors concluded that bamboo biochar had more open and rough surfaces, larger internal pores, and denser and more irregular pore arrangements that constituted a better matrix for microbes' adhesion. It is noteworthy that a pore diameter that is too large may reduce microorganism's adhesion to biochar due to pore curvature [137]. An average pore diameter smaller than 0.3 μm limits the occurrence of colonies [138] and is effectively uninhabitable for most microbes due to cell exclusion [139].

The pore size range from 2 to 80 μm was found to promote fungal activity [113]. Hammer et al. [115] reported that the morphology of wood biochar and the size of macropores ranging from 1 to 15 μm in diameter enables hyphal colonization from 1 to 10 μm. Similarly, Ascough et al. [135] showed that the colonization of the biochar surface was faster when the biochar contained many fractures in which the fungi could grow. The optimal pore size of the carrier should be at least two to five times larger than the largest dimension of the immobilized cell [91,102,140]. Simultaneously, materials with large volumes of nano- to micropores that are not accessible to microbes do not reflect the functional capacity of the material as an inoculum carrier [96].

3.3. Chemical Properties of Biochar

The combination of favorable physical characteristics with the chemical properties of biochar, such as the relatively high content of C, N, H, O, and other nutritional elements, e.g., Na, K, Ca, and Mg, makes it a potential carrier material [109]. The sorption capacity of biochar determines the retention of water and nutrients that provide favorable conditions for colonization by microorganisms, especially in poor soils [141,142]. Biochar contains both inorganic and organic forms of N [143], but the content of available N decreases with the increase of pyrolysis temperatures due to volatilization and formation of nitrogen heterocyclic compounds [122,144,145]. Biochars also have great potential to increase the availability of potassium, which is both a macroelement for plants and an essential nutrient for the growth and development of microorganisms [59,146,147]. High-temperature biochars with high mineral ash content have higher total K content, whereas lower temperatures increase the water-soluble and exchangeable potassium fractions [146,148]. Phosphorus content increases with increasing pyrolysis temperature, as well [149]. Biomass phosphorus is converted via carbonization from organic to inorganic forms and can act as a soil nutrient or as a sorption site for heavy metals [149–151].

Numerous studies confirm that the concentration and availability of alimentary elements in biochar may have a significant impact on the viability and abundance of bacteria [90,104,144,152] and fungi [115,153]. Hammer et al. [115] found that hyphae grow in biochar pores, especially when the surrounding medium conditions were poor in nutrients. They demonstrated that mycelium in direct contact with the biochar surface captured adsorbed phosphates, which constitutes a competitive advantage for the fungus and associated host plant under P deficiency conditions. Vanek et al. [91] found that plant-derived

ash nutrients in biochar, such as K, Mg, and P, supported the survival of *Rhizobium tropici*. They also observed higher growth on hardwood biochar produced at 450 °C compared to biochar treated with acetone, which suggests the presence of easily mineralized carbon substrates for bacteria on unmodified biochar [91]. The physicochemical properties of biochar and metabolically available labile-C compounds may also shift the soil microbial community structure and biogeochemical function [154]. Gomez et al. [155] reported that the addition of wood-derived biochar in a higher dose determined the microbial community change towards a more Gram-negative bacteria compared to fungi and Gram-positive bacteria. They stated that readily available carbon could only partially explain the increase in microbial biomass, suggesting the involvement of additional abiotic factors.

The lability of biochar is strongly controlled by the relative amount of more aliphatic and volatile components [156–158], including thermally untransformed cellulosic and hemicellulose fractions [159]. While biochars generated at elevated temperatures are dominated by aromatic compounds and longer carbon chain hydrocarbons, biochars produced at temperatures below 350 °C usually sorb volatile substances consisting of short carbon chain aldehydes, furans, and ketones [160]. The above findings were supported by Hass et al. [161], who stated that some pyrolysis products were trapped within the biochar after anoxic, pyrolytic heat treatment of poplar wood samples. They concluded that these products may increase the biochar value if applied as a fertilizer and soil conditioner because of the presence of less recalcitrance carbon that can be used by microbes [161]. It should be noted that the differences in nutrient availability and the composition of organic compounds in biochar may play a role in a wide variety of microbial responses. This argues for the need to identify chemical classes of substances as substrates or toxins for microorganisms' growth [91,160]. There are reports of reduced root colonization by arbuscular mycorrhizal fungi [162,163], reduced biological N_2 fixation above a certain threshold of biochar doses [164], as well as lower microbial carbon use efficiency within the biochar space as compared to the surrounding soil [139]. Some authors also revealed that products formed during biomass pyrolysis may be toxic to plants and microorganisms and limit their abundance and activity [165–167]. These include high levels of salts and heavy metals, acidic and phenolic compounds derived from lignin and hemicellulose materials, and polycyclic aromatic hydrocarbons [165,168,169].

Numerous functional groups present in biochar matrices, such as carboxyl, amino, hydroxyl, carbonyl, and sulfhydryl groups, are effective for the immobilization and proliferation of microbial cells through weak physical interactions or chemical covalent bonds [94,170]. The biochar carrier having a small number of such groups may require further chemical or physical modification, as described in previous sections. Moreover, the quantity and quality of surface functional groups may be crucial for the effectiveness of biopreparations in agricultural and environmental conditions because these structures determine the chemical properties of biochar, including acidity and alkalinity, wettability, surface charge, or cation exchange capacity [127,149]. Functional groups located on biochar surfaces can retain and provide nutrients for the growth of microorganisms in the soil [146,171]. Biochar is dominated by negatively charged functional groups; therefore, it mostly affects cation retention [172]. However, due to the pH-dependent nature of the surface, it can also contribute to a smaller extent to an anion adsorption [173,174].

Higher pyrolysis temperature decreases polar functional groups on the biochar surface, increasing its hydrophobicity [76,175]. The hydrophobicity may affect the adhesion of microbial cells, particularly in external pores and macropore residues [176]. Typically, microorganisms prefer to interact with hydrophobic versus hydrophilic biochar surfaces [141] and penetrate the pores via the formation of biochar-associated biofilms [177] and through the production of extracellular polymeric substances [178,179].

The development, community structure, and functions of microorganisms are also very sensitive to pH [180,181]; thus, the pH and buffering capacity of biochar can alter living conditions [182]. Generally, pH values favoring the availability of macroelements as well as the growth and activity of most microorganisms range from ~6.0 to ~8.0 [94];

however, fungi exhibit wider tolerance for pH variation than bacteria [183]. Husna et al. [102] showed that biochar from coconut shells was characterized by an optimal pH (7.74) for bacterial isolates, which resulted in higher cell density and a final population size. Similarly, Hale et al. [96] indicated that among the physical and chemical properties of biochars, pH and nitrogen content were the key factors influencing the initial survival of the *Enterobacter cloacae* and, therefore, the shelf life of the biopreparation. The authors also emphasized that after the application of preparations to the soil, physical features such as surface area, pore opening diameters, and water-filled pore spaces determined the survival of the inoculum. However, more studies on the influence of various production conditions and physicochemical properties of biochar on microbial community structure are still needed [184–186].

4. Microbiological Indicators for Assessing the Impact of Biochar in Soil

Soil microorganisms are key indicators of soil quality since they participate in many biochemical processes essential for soil functioning [187]. Therefore, it is important to elucidate changes in soil microbial communities that are caused by soil management practices such as biochar applications [188]. Changes in soil conditions accompanying different biochar application rates have a significant impact on microbial activity in the soil and on changes in the community structure [118,189]. The response to a biochar addition in soil can be revealed by activity, diversity, and soil microbial community abundance, which, in turn, can cause positive outcomes such as the facilitation of biochemical cycling, enhancement of the activity of soil enzymes, improvement of soil structure through microaggregates, contaminant degradation (transformation into less toxic forms by oxidation, reduction, and hydrolysis) or immobilization (sorption into cells, formation of complexes/precipitates), and promotion of plant growth [190]. However, according to Pathy et al. [191] and Gomez et al. [155], the exact mechanism by which biochar affects the microbial population is not precisely known yet. They suggested that changes in microbial functions and community structure are only possible when biochar application rates are high enough to significantly alter soil moisture retention capacity, pH conditions, and nutrient concentrations. Nevertheless, the accepted opinion is that there are several microbiological properties that are sensitive to biochar addition. They are usually used as indicators for assessing the impact of biochar in soil, and they are presented in Figure 4.
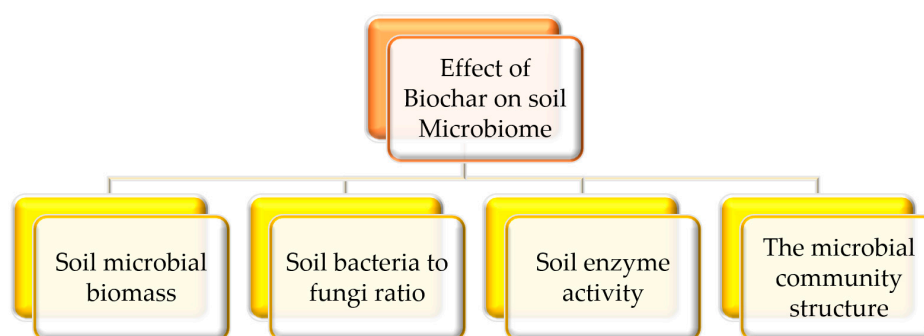

Figure 4. Microbiological properties affected by biochar.

4.1. Microbial Biomass

The term "soil microbial biomass" defines all soil organisms with a volume of less than $5 \times 10^3 \, \mu m^3$, other than living plant tissue. That is the so-called living part of soil organic matter [192]. Significant changes in the structural and functional diversity of microbial populations can be caused by the addition of organic materials to the soil, including biochars. It is associated with changes in the intensity of microbial degradation of organic connections [193,194]. According to Ajema [195], the type of soil into which biochar

has been introduced determines the effect of biochar on the number and biomass of microorganisms, their activity, and their effectiveness in colonizing plant roots. Domene et al. [196] reported that biochar application increased microbial biomass by nearly 100% after three years due to the increase in soil moisture. Similar results were presented by Jones et al. [197], where an enhancement of microbial growth rates was observed two years after biochar application. However, as reported by Murphy et al. [198], the addition of biochar to soil reduced the carbon content of microbial biomass, simultaneously contributing to the reduction of the soil organic matter pool. These different results draw attention to the fact that special care should be taken when applying biochar to the soil. Particularly, some negative effects, such as reduction of the mass, activity, and structural diversity of microorganisms, are possible due to the excessive use of biochar. Such negative changes in microbial communities may hinder plant production [191].

4.2. Microbial Abundance

In biochar-amended soil, microbial abundance can be determined by various methods, including total genomic DNA extraction, culturing and plate counting, fumigation extraction, phospholipid fatty acid (PLFA) extraction, staining and direct observation of individual biochar, or with techniques including qPCR, DGGE, TGGE, and DNA and RNA analyses [118,190]. The assessment of the impact of biochar on microbial communities in bulk soils is the subject of an increasing number of studies. According to Domene et al. [196], the addition of biochar in a dose of 30 t/ha to a sandy loam soil increased microbial abundance from 366.1 μgCg^{-1} (control) to 730.5 μgCg^{-1}. Domene et al. [199] proved that the rates of corn stover biochar from 0 to 14% in temperate loamy soil at different pre-incubation times of 2–61 days caused microbial abundance to increase by 5–56%. Liao et al. [200] found that total PLFA concentrations increased with increasing biochar rates in the alluvial, gray desert soil; however, only the rate of 4.5 t/ha showed significantly different results from the control. A rate of 4.5 t/ha caused an increase in PLFAs of Gram-negative bacteria, Gram + bacteria, and actinomycetes, and a rate of 2.25 t/ha of biochar caused an increase in PLFAs of Gram-negative bacteria and actinomycetes. Several possible reasons that may be responsible for enhancing the number of microorganisms after biochar application include an increase in the availability of nutrients or the presence of labile organic matter on the biochar surface [195]. However, research presented by Plaza et al. [201] on three distinct arable soils with contrasting textural classes (loamy sand, sandy loam, and clay) showed that 12.5 and 50 g/kg^{-1} doses of biochar had negligible effects on apparent PLFA. A strong decrease in PLFA extraction efficiency (−77%) in four temperate soils, Luvic Phaozem, Haplic Luvisol, Haplic Gleysol, and Gleyic Phaeozem, with biochar addition rates of 1, 5, 10, and 20% was observed by Gomez et al. [155], which can be related to the adsorption of PLFA by the biochar. However, other studies [202–206] showed that there is no or only a slight impact of biochar on microbial abundance. Mitchell et al. [202] showed that Gram-positive-bacteria-specific PLFA concentrations and Gram-negative bacteria and actinomycetes counts decreased during the first 16 weeks of biochar (made from sugar maple wood) addition to the forest soil (Cambisol).

Soil microbial communities are influenced by biochar applications [195,202]. Biochar can cause the rapid growth of certain microorganisms, resulting in increased microbial biomass. However, its diversity can be decreased [207]. It was found that in soils enriched with biochar, the bacteria were generally dominated by *Bacteroidetes*, *Firmicutes,* and *Actinobacteria*, while the fungi were dominated by *Basidiomycota*, *Trichoderma*, and *Paecilomyces* [208–211]. Soil enrichment with biochar generally resulted in a reduction in the abundance of *Acidobacteria, Proteobacteria*, and *Planctomycetes* [209,210,212,213]. According to Abujabbah et al. [208], the abundance of *Nitrospirae* and *Verrucomicrobia* increased by the application of wood-derived biochar on black clay and brown sandy soils but decreased on red clay soils. The relative abundance of *Chloroflexi* increased in field studies after the application of rice straw biochar to an Acrisol and decreased after the application of bam-

boo biochar to Ferrisol derived from granite. In the laboratory incubation, rice straw biochar application caused a decrease in those microbes [210,213,214]. According to Anderson et al. [154], the application of biochar manufactured from *Pinus radiata* at rates of 15 and 30 t/ha to silt loam soil increases the relative abundance of *Bradyrhizobiaceae* and *Hyphomicrobiaceae* bacteria. In other studies, the relative abundance of *Adhaeribacter*, *Rhodoplanes*, and *Pseudoxanthomonas* bacteria increased in the calcareous soil after the addition of tobacco stalk biochar, while the abundance of *Kaistobacter*, *Pirellula*, and *Lacibacter* decreased [215].

In most studies, the impact of biochar on microbial communities is examined over 1–2 years [154,207,216]. Yao et al. [217] conducted a long-term study to assess the impact of biochar on soil microbial communities over a period of 3–3.5 years. They concluded that changes in bacterial community compositions were closely related to soil characteristics, such as pH, total C, total N, and total K, which were highly correlated with biochar concentration. These results suggest that the long-term impact of biochar on the soil bacterial community was indirectly driven by changes in soil properties. Assessment of the impact of biochar application on root-associated bacterial communities, despite their being important components of soil ecosystems, has been the subject of only a few research studies [154,217,218]. According to Kolton et al. [212], biochar may influence the role of root-associated microbial populations and, as a consequence, plant growth and resistance to pests and diseases. Cheng et al. [219] proved that biochar application significantly influenced the diversity and composition of bacterial communities in the calcareous rhizosphere and the bulk soil. They observed a significant increase in the relative abundance of *Gemmatimonadetes* and a decrease in the relative abundance of *Bacteroidetes*, *Firmicutes*, and *Cyanobacteria* in the rhizosphere. The relative abundance of *Proteobacteria* and *Chloroflexi* increased in bulk soils with increasing biochar addition, contrary to *Bacteroidetes* and *Verrucomicrobia*. The biochar application influenced the relative abundance of some important C and N cycles of bacterial taxa from the *Firmicutes*, *Pedomicrobium*, and *Bradyrhizobium* groups. These results suggest that the addition of biochar may lead to important long-term effects on C and N cycling in soil.

Ascough et al. [135] showed that biochar and its surfaces can be colonized by fungal hyphae. Fungi can colonize the surface and interior of biochars prepared at 300 °C and 400 °C for over three months [220,221]. Warnock et al. [126] reported that biochar has a positive effect on the two most commonly occurring fungi types: arbuscular mycorrhizal and ectomycorrhizal. According to Ascough et al. [135,221] and Jaafar et al. [222], soil microorganisms colonize habitable biochar pore spaces, and this may depend on microbial community composition as well as on changes that occur to biochar particles in soil over time. During the interaction of biochar with the soil, the physical and chemical properties of the biochar are modified, and soil particles attach to the biochar surface, which may lead to changes in the suitability of the habitat and the activity of microorganisms. There is a need for further investigation of the fate of biochar particles once deposited in the soil. Such research will clarify which biochar pores, structural cracks, pore connectivity, and surfaces affect biochar as a habitat for soil microorganisms [108,118].

4.3. Bacteria to Fungi Ratio

Usually, the literature reports indicate significant changes in the structure of soil microbial communities and an increase in the dominance of bacterial communities after biochar addition [197,223]. Liao et al. [200] observed that the bacteria-to-fungi ratio in the 4.5 t/ha biochar treatment was significantly greater than in the 2.25 t/ha treatment or in the control. Mitchell et al. [202] found that the bacteria-to-fungi ratio increased during 16–24 weeks of sugar maple wood biochar application. Similar results were also reported by Chen et al. [223] and Ippolito et al. [147], who observed an increase in bacterial abundance and a decrease in fungal abundance after biochar addition. Bamminger et al. [224] reported, however, higher fungal-to-bacterial ratios in Stagnic luvisol after the addition of biochar. Different responses of bacteria and fungi to biochar may result from differences

in their abilities to cope with biochar in the soil environment, differences between bacteria and fungi mobility and colonization of biochar pores, and decomposition of the biochar by some fungal species [225].

4.4. Enzyme Activity

Microorganisms are among the main sources generating enzymes in the soil [226]. Several studies report that biochar increases the activity of various extracellular and intracellular enzymes related to the C, N, P, and S cycles [227–229]. Biochar application can improve the action of dehydrogenase, catalase, urease, alkaline phosphatase, β-glucosidase, arylsulfatase, oxidase, and fluorescein diacetate hydrolase, which are involved in microorganisms activity [227,230]. According to Raiesi et al. [231] and Sandhu et al. [232], sorption of enzyme molecules to functional groups present on the biochar surface affects the course of enzymatic reactions. Biochar influences the activity of soil extracellular enzymes responsible for C degradation, N mineralization, or P solubilization. This effect depends on biochar properties, soil, and enzyme type [233–235]. However, a reduction of C-degrading enzyme (α-glucosidase, β-cellobiosidase, and β-glucosidase) activity was also observed after biochar amendment [233,235]. According to Guo et al. [236], an increase in biochar pyrolysis temperature resulted in a reduction of C-degrading enzyme activity and a moderate increase in the ratio of C-acquiring enzymes to N-acquiring enzymes. Different results regarding the effect of biochar on N-mineralizing or P-solubilizing enzyme activity have been observed in numerous studies [235,237–239]. Wang et al. [240] reported that biochar addition to a fluvo-aquatic soil increased the activity of some extracellular enzymes involved in soil C and sulfur (S) cycling: ß-glucosidase, ß-D-cellobiosidase, ß-xylosidase, and a-glucosidase and sulfatase. This effect depended on the rate of biochar addition. According to Pokharel et al. [234], biochar increased the activity of urease by 23.1% and alkaline phosphatase by 25.4%. Keiluweit et al. [124] and Gujre et al. [241] reported that the abiotic aging of biochar caused lactase and peroxidase activities increase that was due to the changes in aromatic groups and the introduction of aliphatic groups in the biochar structure. According to Yang et al. [242], the interactions of biochar with the oxidoreductase enzymes might be influenced by the presence of free radicals on the biochar surface and their participation in electron transport. According to Gorovtsov et al. [141], oxidoreductases and hydrolases have been studied much more extensively than the other enzyme classes.

Among all soil biological indicators, dehydrogenase activity is considered the most important parameter that is useful for the general assessment of soil conditions. The amount of these intracellular enzymes is directly related to the number of active cells/living microorganisms, so the dehydrogenase activity in soils amended with biochar is used to evaluate its impact [243,244]. The addition of different types of biochar increases dehydrogenase activities under various soil conditions [243,245,246]. According to Gascó et al. [229], the most important in this respect is the labile and volatile organic matter content of the used biochars. Demisie et al. [247] show that dehydrogenase activity in degraded red soil was elevated by oak wood and bamboo biochar at 0.5% (*w/w*) dose. Irfan et al. [246] observed that 1% biochar stimulated the activity of dehydrogenase.

Biochar can impact enzyme activities in two ways: directly through surface adsorption and indirectly through microbial synthesis. The active sites of enzyme molecules may be directed to the biochar surface, which reveals a reduction of the enzyme activity. Indirectly, biochar can impact enzyme activity by changing the microbial population and activity due to changes in the physicochemical properties of the surrounding environment, especially pH and EC [132,141,248–251]. Furthermore, PAHs, benzofurans, and heterocyclic compounds can be released by biochar, which can lead to the inhibition of several enzymes [248].

Table 3 provides a review of publications reporting the impact of various biochars on soil microbiome properties, such as the number and diversity of microorganisms and enzymatic activity in various types of soils and biochar application conditions.

Table 3. The impact of various biochars on the properties of the soil microbiome.

Type of Soil	Type of Biochar	Dose of Biochar	The Number of Microorganisms/Microbial Biomass and Enzymatic Activity	Type of Study	Ref.
Typical brown soil, Eutric Cambisol	Wheat straw biochar prepared at 300 °C	0, 0.2, 0.5, 1, and 2%	The addition of biochar with nutrients increased the number and intensified the activity of soil microorganisms.	Laboratory experiment	[187]
Forest rhizospheric soil	Tea leaf biochar prepared at 350 °C (B1) and 600 °C (B2)	1% (*w/w*)	B2 + BC (*Bacillus cereus*) significantly ($p \leq 0.05$) enhanced the *Proteobacteria* (11%), *Firmicutes* (46%), *Actinobacteria* (20%), and *Cyanobacteria* (33%) community as compared to control and improved soil properties, i.e., enzyme activity urease (12%), dehydrogenase (40%), and phosphatase (49%).	Vase experiment	[110]
Drip-irrigated desert soil	Cotton straw biochar	0, 2.25, or 4.5 t/ha	Rate: 4.5 t/ha: increased microbial biomass C (by 32%), microbial biomass N (by 58%), and basal respiration (by 13%); increased total PLFA (by 27%) compared with the control; shifted the microbial community toward bacteria and actinomycetes; and increased enzyme activities related to N cycling. Both rates of biochar increased the activities of three key enzymes related to C cycling.	Field study	[200]
Salt-induced soil (Salinity gradients (S0: control, S1: 2, S2: 4, S3: 6 EC iw))	Peanut shell biochar	B0—control; B1—2.5%; B2—5%; B3—10% *w/w*	Soil enzyme activities depended on biochar rate, day of incubation, and type of enzyme. B1 showed highest dehydrogenase (20.5 mg TPF g^{-1} soil h^{-1}), acid phosphatase (29.1 mg pnpg^{-1} soil h^{-1}), and alkaline phosphatase (16.1 mg PNP g^{-1} soil h^{-1}), and B2 increased the urease (5.51 mg urea-N g^{-1} soil h^{-1}) and fluorescein diacetate hydrolyzing activities (3.95 mg fluorescein g^{-1} OD soil h^{-1}) in soil.	Laboratory experiment	[245]
Three acidic soils: black clay loam (BCL) Vertosol, loamy red Dermosol (RL), and brown sandy loam (BSL)	Eucalypt green waste biochar prepared at 650–750 °C	0, 2.5, 5, and 10% (*w/w*)	Biochar addition has a greater impact on the bacterial diversity in RL and BSL soils than in BCL soil. The abundance of nitrifying bacteria increased with increasing biochar rate, especially AOB (ammonia-oxidizing bacteria) in BCL soil. The abundance of *Nitrospira* and NOB (nitrite-oxidising bacteria) was greater than AOB in all biochar-amended soils.	Pot experiment	[208]
Silt loam soil	Biochar manufactured from *Pinus radiata*	0, 15, and 30 t ha^{-1}	Comparing biochar-amended soils with controls, temporal changes in bacterial family abundances that were >5% included *Bradyrhizobiaceae* (~8%), *Hyphomicrobiaceae* (~14%), *Streptosporangineae* (~6%), and *Thermomonosporaceae* (~8%), where the biochar had a positive influence, either promoting an increase in abundance or reducing the magnitude of the loss, and *Streptomycetaceae*	Pot experiment	[154]

			(~−11%) and *Micromonosporaceae* (~−7%), where biochar had a negative effect on bacterial family abundance.		
Calcaric cambisol	BC made from tobacco stalks	0, 1, 2.5, and 5.0% (*w/w*)	Compared to the control, the BC addition increased the diversity and richness of bacteria; increased the relative abundance of *Adhaeribacter*, *Rhodoplanes*, *Pseudoxanthomonas*, and *Candidatus Xiphinematobacter;* and lowered the relative abundance of *Lacibacter*, *Pirellula*, and *Kaistobacter*.	Pot experiment	[215]
Calcareous soils in a karstic region of southwestern China	BC produced from crop straws of maize and rapeseed at 550 °C	0, 1, 5, and 10% (i.e., 0, 12.8, 64, and 128 t ha⁻¹)	In rhizosphere soils with increasing BC amendment, the relative abundances of *Gemmatimonadetes* increased while those of the *Bacteroidetes*, *Firmicutes*, and *Cyanobacteria* decreased. In bulk soils with increasing BC application levels, the relative abundances of *Proteobacteria* and *Chloroflexi* increased while those of the *Bacteroidetes* and *Verrucomicrobia* decreased.	Field study	[219]
Haplic Podzol (pzha) originating from glaciofluvial fine-grained loamy sand	Biochar from wheat straw produced at temperature of 650 °C	Biochar at rates of 10 (BC10), 20 (BC20), and 30 (BC30) t ha⁻¹	Enzymatic activity of dehydrogenase, phosphatase, and urease were lower in successive years of the study, regardless of the biochar rate.	Field study	[252]
Sandy and clay soils	Three corn stalk biochars prepared at 200, 400, and 600 °C	0.5 and 1% (*w/w*)	Compared with the control, the biochar addition stimulated the activities of catalase, dehydrogenase, cellulase, invertase, and protease, which varied with pyrolysis temperature biochar dose as well as soil texture. The positive effects of biochar addition on soil enzymes were greater for 1% than 0.5% biochar doses and greater for sandy soils than clayey soils for catalase, dehydrogenase, and invertase.	Laboratory experiment	[253]
An arable Typic Haplocalci	Biochar from maize residue produced at 600 °C	Two addition rates (low, 0.5%, and high, 1.0% *w/w*)	BG (β-glucosidase) showed an increase in potential enzymatic activity (81%) in the biochar-amended sandy loam soil but only at a higher biochar dose. In the clayey soil, biochar decreased potential BG activity (by 10–29%).	Laboratory experiment	[231]
BS—black soil; FS—fuvo-aquic soil; and RS—red soil	Biochar produced from wheat straw at 500 °C	0, 1, and 2% of biochar addition	Biochar did not change the bacterial richness and diversity in BS, FS, and RS but shifted all the soil bacterial community structures. Biochar mainly increased the growth of low-abundance bacteria in FS and high-abundance bacteria in RS. The most abundant bacterial phylum in BS and FS, *Proteobacteria*, increased after biochar addition, while *Chlorofexi*, the most abundant phylum in RS, decreased.	Laboratory experiment	[211]

Soil from field in Bregentved Estate, Zealand, Denmark	Wheat straw biochar (type not specified) (GBC)	6–8 and 0.8–1.4 t/ha of high and low doses, respectively	No significant effect of biochar was observed on microbial biomass content. In a higher dose of BGC, only a minor effect on the soil community composition was observed. An increase in phenol oxidase activity and decrease in cellulase activity were reported. In a lower dose of GBC, the relative abundance of the rare members, and, thus, diversity of soil microorganisms, increased. Filed study [205]
Silty loam texture soil from Pistoia	Wood-derived biochar	30 and 60 t/ha	No significant effect of biochar was observed on microbial biomass-C, microbial quotient, or genetic diversity. Biochar stimulated soil microbial activity with no disruptions, but this positive effect was very short. Filed study [204]

5. Implications of Using Biochar and Biochar-Based Products for The Environment and Agriculture

In recent years, biochar has been increasingly used in agricultural production [254,255], soil improvement [256], water pollution treatment [257], greenhouse gas mitigation [258], waste management [259,260], and many others. Various applications of this material have been widely researched. Bibliometric network analysis conducted by Kumar et al. [261] showed that most studies are focused on the use of biochar for soil improvement (more than 1000 papers). However, the application of biochar may have disadvantages and limitations, as well.

5.1. Limitations in the Use of Biochar and Biochar-Based Products

Biochar composition depends on the kind of raw material [262] and its moisture [263]. To facilitate pyrolysis, the size of various feedstocks is frequently reduced by cutting or crushing, which, along with reducing water content, requires additional costs. The above factors should be considered before selecting appropriate input materials for biochar production.

Another limitation may arise from processing logistics. According to Zilberman et al. [264], feedstock transport is a linear cost function of the distance between the point of the feedstock origin and the pyrolysis location. As reported by Kung et al. [265], the feedstock transport distance of 9.22 miles to the pyrolysis facility adds 11% to the overall production costs. The pyrolysis process has to consider raw materials located close to the site of their implementation [264] or the application of mobile pyrolyzers. Also, complementary industries (e.g., wood or paper) may place a pyrolysis unit directly in their plant [266].

Biochar can permanently alter soil and water ecosystems due to its very long half-life (>100 years) [267]; however, it does not always improve the soil's agricultural productivity. Potential disadvantages of biochar application include the binding and deactivation of agrochemicals such as herbicides and nutrients in the soil, oversupply of nutrients, increase in soil EC and pH, adverse impact on germination and soil biological processes, and release of possibly toxic substances such as heavy metals and PAHs [268]. Agricultural applications of biochar of low mechanical strength and dusty nature may be dangerous in wind- or water-eroded soils. The main barrier to the agricultural use of biochar is that once introduced into the soil, the biochar cannot be removed [269].

Biochar is a resistant material; however, it can undergo biotic degradation (incorporation of microorganisms or oxidative action), C respiration, and abiotic processes such as chemical oxidation, photo-oxidation, or solubilization [270]. As reported by Cheng et al. [271], the biochar incubated for a year spontaneously underwent significant surface oxidation with an increase in the number of carboxyl and phenolic functional groups, oxygen, and the disappearance of positive surface charges. Drivers of biochar mineralization can be microbial networks or erosion streams [270,272]. There is no clear information on what controls the durability vs. degradation of various forms of biochar. The durability of biochar cannot be characterized by a single factor because its aging and decomposition differ at different biochar doses and soil–atmosphere conditions.

The colonization of biochar by microorganisms depends on the composition of the biochar, which depends mainly on the biomass feedstock, residence time, pyrolysis temperature, and type of reactor [273]. Other important factors are soil properties, the abundance and composition of consortia of pre-existing microorganisms in the soil, and the contact time of biochar with the soil [203,274,275]. According to Mukherjee et al. [276], the aging process of biochar can significantly change its properties and lead to changes in the dynamics of colonization by microorganisms. Wang et al. [277,278] claim that the addition of an aged biochar increases microbial activity, while the opposite phenomenon was observed in the case of a fresh biochar. The most likely reason is the toxic effect of freshly prepared biochar caused by a high content of pyrolysis products [141].

Although biochar's application as a carrier of beneficial microorganisms is constantly growing, only biochar with optimal properties will ensure the proper development and survival of the immobilized microorganisms. An inappropriate carrier for microbial immobilization usually results in a dramatic decline in the population of most of the inoculated microorganisms [279], which is related to the need to compete with better-adapted, more aggressive native microflora and predators of soil microfauna. Moreover, cells of immobilized microorganisms may show metabolic differences compared to their free-living counterparts [280]. The clogging of biochar pores by small soil particles, as well as the formation of a thin biofilm on the surface of the carrier, may limit the diffusion of oxygen and nutrients, reducing the growth and activity of immobilized microorganisms. This promotes the production of toxic metabolites and the degradation of the preparation introduced into the soil, including progressive cell lysis.

Biochar can be a matrix for the immobilization of microorganisms that supports the reduction and removal of various types of pollutants below critical values [281,282]. A limitation of this bioremediation method may be the generation of a toxic environment for the immobilized microorganisms due to the strong binding of pollutants by biochar and, as a consequence, the reduction of their degradation rate [283]. Further separation of contaminated microbial biomass is hardly possible, which also limits the microorganism-enriched biochars' usage. The solution may be a pre-selection of substrates and the creation of reusable biochar-based carriers that could allow simple and safe separation of contaminated media. It is worth noting that the effectiveness of immobilization relating to practical applications, including pollutant removal and overall improvement of soil productivity and quality, will also be limited due to variable environmental conditions. More attention should be paid to external factors responsible for the immobilization process and protection of valuable microorganisms, which are of key importance in the microbiologically enriched biochar efficiency. Solutions are also needed regarding techniques for the immobilization of microorganisms on biochar on a large scale, as well as methods of storing and transporting the produced microbiological preparations to the place of their final use [284].

5.2. Environmental Risk Resulting from the Use of Biochar

Biochar may have unfavorable properties such as harmful ingredients, e.g., heavy metals, polycyclic aromatic hydrocarbon (PAH) content, environmentally persistent free radicals, dioxins, and perfluorochemicals. Their occurrence may arise from, among others, improper selection of raw biomass, preparation conditions, and production methods [258]. Indeed, the latest research draws attention to the negative environmental effects of biochar due to its potentially harmful ingredients or various negative interactions with the environment [284,285].

The type of biomass determines the content and bioavailability of heavy metals in biochar. Biochar produced from biomass rich in heavy metals can increase heavy metal content in the environment by leaching, exchange, or decomposition. An example is the energy plant Miscanthus. It often grows in sludge or sewage farms and shows a high accumulation of trace metals [286]. According to Oleszczuk et al. [287], Miscanthus-derived biochar showed higher hazardous metal content than other biochars and may release heavy metals into the environment. Another extremely important aspect of controlling the content of heavy metals in biochar is the proper selection of the pyrolysis temperature. According to Devi and Saroha [288], the content of Cu, Pb, and Zn in biochar increased significantly with temperature, governing the organic matter decomposition. When the pyrolysis temperature increased from 200 °C to 700 °C, the content of the above three metals increased by 61%, 73%, and 65%, respectively. However, they observed that the bioavailability of heavy metals in biochar may decrease as the pyrolysis temperature increases. They also observed that the maximum ability to leach heavy metals from biochars

occurred at pH 3 (as low pH generally increases the dissolution of metals). After increasing the solution pH from 3 to 7, the leached amount decreased, and the increase in solution pH from 7 to 13 led again to an increase in leaching.

During the pyrolysis process, polycyclic aromatic hydrocarbons may be formed, which are harmful to human health and the environment. A diminishing pyrolysis rate and increasing residence time lead to lower PAH yield. At low temperatures (<500 °C), PAHs with low molecular weight are usually formed, while at high temperatures (>500 °C), PAHs with high molecular weight dominate. PAH content depends on the original composition, mineral and moisture content in the raw material, and the availability of O_2 during pyrolysis [289].

Biochar applied to soil or water can adsorb anthropogenic chemicals such as steroids, hormones, and heavy metals, which is promoted by large specific surface area, microporous structure, active functional groups, and high pH [290]. Soil microbial diversity can dramatically change over time with the introduction of increased durability carbon pool because microorganisms, especially heterotrophs, derive energy from decomposition of organic matter. If microorganisms are dependent on a non-degrading carbon source (biochar), negative effects on soil ecological conditions can occur [268].

5.3. Environmental Benefits from the Use of Biochar

Biochar is relatively stable in most environments. With residence times exceeding 1000–1500 years, it has been found in various environments, for example, in Amazonian dark earth [291], moist tropical forest soils [292], soils of Costa Rica [293], and ocean sediments [294]. The aromatic structure, surface area, and sorption properties against the other organic compounds and mineral phases increase the resistance of biochar to degradation, leaching, or chemical oxidation [295]. Thanks to its high potential to increase soil quality, fertility, and carbon sequestration in the soil, biochar is a suitable prospect for sustainable agriculture and climate management [296]. Lai et al. [297] found that the application of biochar at the rate of 2–5% increased carbon sequestration by 46–58% in rice and beet fields.

Biochar contains nutrients derived from the raw material, so when applied to the soil, biochar can directly serve as a source of several nutrients. Lehmann et al. [298] reported that the immediate beneficial effects of a secondary forest charcoal addition on crop productivity in tropical soils were associated with an increase in availabilities of Ca, Cu, K, P, and Zn. When biochar is modified with exogenous nutrients, it can be used as a slow-release fertilizer [299]. The slow desorption of nutrients is governed mainly by a biochar porous network that creates structural obstacles for physical wrapping, chemical sorption, and retarded diffusion. Biochar can be used as a carrier of plant protection products such as pesticides, herbicides, and antifungal agents [87,300].

Biochar addition modifies the physical and mechanical properties of soils. Busscher et al. [301] observed that the addition of ground pecan shells pyrolyzed at 700 °C to Norfolk sandy loam reduced soil strength and improved soil water content during free drainage, but this improved neither aggregation nor infiltration rate. According to Glaser et al. [291], improving soil moisture retention may be one of the key factors explaining the positive effect of biochar/charcoal on crop yields. According to Manyà [302], the improved agronomic productivity of biochar-amended soils can be attributed to an increase in soil surface area and porosity, which in turn improves water-holding capacity. Glaser et al. [291] noted an increase in water-holding capacity after charcoal addition, which was probably due to better soil aggregation. As reported by Atkinson et al. [303], the biochar application increases water availability, improves soil water storage capacity, and protects soil moisture. However, Mukherjee and Lal [304] stated that soil moisture retention could only be improved by applying biochar on coarse-textured soils. Biochar reduces soil bulk density and promotes aggregation [128,305–307]. Reduced bulk density and increased porosity, water-holding capacity, pH, and nutrient resources have a positive impact on the number and activity of soil microorganisms [118,193]. The long-term biochar interactions

with soil components may rebuild the soil structure and potentially improve the overall resistance of the soil to compaction forces.

Biochar is usually alkaline, and its alkalinity depends on the raw material and processing conditions. It can reduce soil acidity due to high buffering potential. Soil deacidifying effect is mainly due to the dissolution of carbonates and (hydro)oxides present in the biochar ash [283].

5.4. Future Research

Biochar is considered a potential tool for carbon sequestration, but there are conflicting reports on the benefits of its use. Therefore, there is a need for the development of standard guidelines for the production and quality assurance of biochar. Developing such guidelines will ensure that the end product is nutrient-rich and non-toxic and has the appropriate pH, EC, and metal content for its widespread and sustainable use [268]. According to Carvalho et al. [308], to assess the environmental impact of biochar production and its use in different contexts, a comprehensive life cycle assessment (the assessment of greenhouse gas emissions, water consumption, and potential leaching of pollutants) should be carried out. It would serve for the identification of areas where improvements can be made to minimize environmental risk and optimize biochar production.

Biochar quality standards and certification programs should be elaborated to enable proper decisions regarding the acquisition, production, and use of biochar. The next step is to develop an integrated framework and decision support tools that will assess and combine the potential impact of biochar use in various sustainable development goals. This aspect involves assessing the economic viability, social acceptability, and environmental impacts of biochar projects in specific contexts [309]. To increase the overall sustainability and impact of biochar-based environmental solutions, it is most attractive and important to explore the synergies between biochar production for bioremediation and other sustainable practices such as organic farming or renewable energy production [310].

To further increase the attractiveness of biochar for advanced remediation technologies, replacing traditional pyrolysis and other thermal conversion methods should be developed. To fully exploit the potential of biochar as a highly effective environmental bioremediation tool, despite its already existing potential in this context, further research is needed to remove commercial obstacles related to resource optimization, scale-up, and life cycle sustainability assessment [311].

Regarding the use of biochar-based microbial carriers, there are still many research gaps and uncertainties that need to be resolved based on long-term experiments conducted in natural conditions. Attention should be placed on optimizing the properties of biochar-based carriers through the appropriate selection of substrates and pyrolytic and post-pyrolytic modifications based on the assumed management goals and soil properties. One of the research goals should be to reduce the decline in the population of immobilized cells during the preparation and use of biochar-based products, especially those that initially revealed positive effects on a laboratory scale. The classification of biochar in terms of physicochemical properties, including potentially toxic compounds, should be adapted to environmental requirements to improve soil properties, reclamation, or plant yield. Research relating to the mechanisms of interaction of immobilized microorganisms with soil microorganisms is also needed to gain better insight into the direct effects of the use of modified biochar on soil microorganisms that have a significant impact on soil biogeochemical cycles.

6. Conclusions

The growing interest in biochar's potential to improve soil quality contributes to its further development. The prospective research direction is the modification of biochars to obtain a product with the desired properties. In agriculture, one interesting application of such modifications may be to optimize the properties of biochar, which could serve as an

optimal environment for the life of beneficial soil microorganisms and thus act as its ecological carrier.

The properties of biochar can be shaped at every stage of the production process, including the selection of starting materials, through adjusting the conditions of the pyrolysis process and ending on post-pyrolysis modifications.

- The selection of appropriate biomass should primarily consider the relationship between the content of lignin, cellulose, and hemicellulose and the temperature at which the pyrolysis will be carried out. Biomass with high lignin content favors the formation of biochar with higher carbon content, larger specific surface area, and lower nitrogen and ash contents. However, lignin substrates may require higher temperatures to be decomposed. Pyrolysis of biomass with excessive moisture content may negatively affect the economic balance of the process.
- The achievement of desired biochar properties at the stage of pyrolysis may depend on several different factors regulating this process simultaneously, among which the temperature generates clear, strong, and predictable effects. An increase in pyrolysis temperature increases porosity, specific surface area, pH, and ash content and decreases the negative surface charge, the contents of volatile matter, and the oxygen-containing functional groups.
- Post-pyrolysis modifications can lead to remarkable improvements in porosity, specific surface area, or reactivity (e.g., treatment with acids, bases, or hydrogen peroxide) but also to the generation of completely new properties (e.g., treatment with nanoparticles of metal salts and oxides). Detailed research is still needed in this area because the effectiveness of modification does not always increase with the concentration of the modifying agent. Moreover, modification with aggressive chemicals generates the problem of waste, while the production of nanocomposite or magnetic biochars meets issues of the appropriate durability of the product.

Modified biochars can constitute a beneficial environment for the life and development of bacteria and fungi. Microbiological indicators showed that various biochars could affect the development, community structure, and functions of various microorganisms differently.

- Biochar's physical properties, such as high surface area and porosity, are primary factors providing a suitable habitat for various microorganisms. Given that the porosity and surface area of biochar increase with the temperature of the pyrolysis, biochar can be designed specifically for particular microbes and various soil conditions. The highly porous nature of biochars increases their capacity for cell adhesion as well as for water and nutrient retention and prefers the survival of microorganisms under storage conditions and after application to the soil, improving the protection and proliferation of microorganisms in the soil and plant rhizosphere.
- The chemical properties of biochar depend on the selection of raw materials, pyrolysis temperature, residence time, and pyrolytic gaseous environment, and it is obvious that they are not the same for different biochars. Depending on the CEC, buffering properties, kind of surface functional groups, and mineral content, biochar can be a source of nutrients with different accessibilities for the microbial community, affecting the metabolism and colonization of functional microorganisms. Some biochars can have an inhibitory effect on microorganisms by releasing residual toxic chemicals, hindering nutrient availability, and inhibiting the biofilm formation process. The quality and quantity of surface functional groups influence the immobilization process of microorganisms on the biochar surface through electrostatic, ionic, and hydrophobic interactions.

Finally, it should be emphasized that the development of technology for biochar production and its use as a soil conditioner and a matrix for beneficial microorganisms has the potential to support the productivity of agroecosystems and maintain plant produc-

tion at an appropriately high level. However, this prospect still requires extensive research, specifically, a detailed investigation of factors that determine biochar production with respect to a specific strain of inoculated microorganisms, soil, and kinds of cultivated plants.

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