

Research Note

CHARACTERIZATION OF COFFEE SHRUB BIOCHAR CHARGED WITH COW MANURE AND EFFECTIVE MICROORGANISMS^{1,2}

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Soil erosion is one of the primary concerns of this century. The loss of the fertile top layer of soil decreases its capacity to store carbon and retain water, impoverishing the soil's chemical, physical and biological quality. Loss of soil quality has detrimental effects on food security. There is a need to improve soil's carbon storage capacity and soil quality to assure food security. Reliable agricultural and agroecological technologies need to incorporate practices such as the pyrolysis of biomass to make an impact on soil carbon storage and usage. The solid material obtained from the pyrolysis of biomass is termed biochar when used in agriculture (IBI, 2012). The use of biochar to enhance the quality of soil requires a more extensive study to understand why, under specific conditions, applying biochar improves soil quality, but in other situations has none or detrimental effects on agricultural soil health (Cheng et al., 2018; Yuan et al., 2017; Wang et al., 2019; Han et al., 2020). There is a need for proper treatment and processing of charged biochar to increase its benefits to agricultural soils. Charged biochar is the biochar surface coated with labile organic carbon and having functional groups ready to absorb nutrients. The use of biochar has the potential to promote long-term soil carbon sequestration and at the same time to improve and recover soil quality.

Biochar is carbon skeleton material obtained from the pyrolysis of biomass and has negatively and positively activated surfaces and high electric conductance properties, which depend on pyrolysis (Yuan et al., 2017). These biochar properties could promote critical soil chemical and biological electron transfer processes (Yuan et al., 2017), which can have either positive or adverse agrarian effects. In agriculture, tons of biomass produced and nutrients applied are lost when the biomass is removed from fields and inappropriately processed. In the coffee industry, where coffee trees need pruning and stumping to maintain productivity, ideally the removed biomass should be transformed, charred, and kept on the production fields where its carbon can have a positive effect on soil health and production.

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Charred coffee shrubs (CSB) were anaerobically fermented with a mixture of cow manure and effective microorganisms (EM) to activate them with active biological organics. The physicochemical properties of the CSB associated with agronomical benefits were assessed. The CSB properties include labile organic carbon, electrical conductivity, cation exchange capacity, and acidity. The high-temperature biochar was obtained using a bottomless open kiln (Chong et al., unpublished results). The pyrolysis process of coffee shrub biomass in the kiln reached maximum temperatures of 815° C and the burn lasted about six hours. The CSB was then milled and sieved to a <1 mm size before treatment and analysis.

In this study, we evaluated the chemical properties of untreated and treated biochar (charged) combined with different proportions of cow manure slurry and mixed with activated effective microorganisms (AEM). The AEM solution, prior to distilled water dilution, was made with equal parts of EM-1 (Teraganix, Alto, TX)⁶ and 5% molasses in distilled water and incubated for two weeks, after which it reached a pH of 3.5 and was then mixed with the distilled water. The different concentration slurries were prepared to attain a percentage of 0, 3.13, 6.25, 12.5 and 25% (w/v) of cow manure in a mixture of distilled water with equal parts of AEM solution (Table 1). The equal parts of distilled water and AEM solution were used to stabilize the cow-manure (CM) and AEM slurry. A total of 40 mL of each dilution treatment, CM with EM (CMEM), was then used to activate 5 g of CSB. The suspension was incubated at 37° C in sealed one-liter, plastic zip-lock bags for seven days. After incubation, the biochar was placed over a glass fiber filter and vacuum-washed with deionized water until the electrical conductivity dropped to 300 µS/cm. This process removed non absorbed salts, tars, and organic matter from the CSB. The washed biochar samples were characterized for pH, electrical conductivity (EC), oxidation-reduction potential (ORP), labile organic carbon, cation exchange capacity (CEC), functional groups identification using Fourier-transform infrared spectroscopy (FTIR) and stereo microscopy imaging techniques described below.

The biochar pH, EC and ORP were determined from the slurry after seven days of incubation. The CSB-CMEM suspensions were then measured for both pH and EC using calibrated meters. The oxidation-reduction potential was measured by the CSB-CMEM supernatant using an Orion ORP electrode. The ORP electrode combines a platinum electrode with an internal Ag/AgCl reference electrode calibrated against a Zobell solution.

The CEC of the CSB samples were determined according to the unbuffered salt extraction method described by Sumner and Miller (1996). Ten grams of each untreated

TABLE 1.—Cow manure (CM) proportions for CMEM slurry preparation.

Treatment (%)	CM weight (g)/400 mL distilled water + EM solution
0.0	0
3.1	12.5
6.3	25
12.5	50
25.0	100

⁶Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

and treated biochar was washed with deionized water until the electrical conductivity was lower than 300 $\mu\text{S}/\text{cm}$. Then, the samples were dried at 70° C for two days. One half gram sample was mixed with five consecutive portions of 30 mL of 0.2M ammonium chloride solution for 1 h, and then washed with an 80% ethyl alcohol aqueous solution until ammonium was not detected. Thirty milliliters of a 0.2M potassium nitrate aqueous solution was added to each sample and then agitated for one hour. The samples were then filtered, and the amount of ammonium was analyzed using a calibrated ammonia-selective electrode. The CEC was then calculated using the amount of ammonia adsorbed on CSB active sites. Total nitrogen was measured by the Kjeldahl method. Briefly, a 0.5 g CSB sample was mixed with 4 mL of concentrated sulfuric acid in a 100 mL Kjeldahl digestion tube. The sample was pre-digested overnight at room temperature and heated to 440° C using a digestion block for 2 h. The sample was allowed to reach room temperature and diluted to 100 mL with deionized water. The nitrogen content was measured by a colorimetric method using an Autoanalyzer.

Labile organic carbon (LOC) was measured by mixing 3 g CSB with 30 mL of deionized water in a 50 mL polypropylene centrifuge tube. The tube was capped, shaken, and partially uncapped to avoid water vapor overpressure. The sample was autoclaved at 80° C for 16 h, shaken for 10 s and the suspension filtered through a 0.45 μm nylon membrane into a 40 mL TOC vial. The labile organic carbon in the leachate was measured by the catalytic oxidation method using a Torch TOC (Total Organic Carbon analyzer) with NDIR detector (Teledyne Tekmar, Ohio, USA).

For the FTIR analyses, approximately 1 g of charged and uncharged CSB was ground into powder using an agate mortar and passed through a 500 μm sieve prior to spectral acquisition. All the spectra were collected using an IRTracer-100 spectrophotometer (Shimadzu Corp.) equipped with a high-luminance ceramic source and a DLATGS detector with temperature control system. The spectra were in absorbance units, and baseline and ATR corrections were applied. The attenuated total reflection (ATR) sampling device was QATR-10 single reflection with diamond-coated accessory. The sample was placed on the diamond optical path and consistent contact pressure was applied by way of a stainless steel rod, monitoring the applied pressure using the spectral signal displayed. Forty-five scans were collected in the range of 4,000 to 400 cm^{-1} at a resolution of 4 cm^{-1} .

Stereomicroscopy images of untreated and charged CSB were evaluated after oven drying the washed biochar particles to observe the surface optical microstructure under halogen light. An optical stereomicroscope (Nikon model SMZ745T stereomicroscope equipped with G-AL-2X objective) with reflected light was used to identify morphological changes after CMEM treatments.

Statistical analyses for pH, EC, CEC, LOC, ORP and total nitrogen were conducted using R Statistical Software Version 3.6.1. We used an analysis of variance (ANOVA) model with Tukey tests to evaluate the difference induced by CMEM on biochar properties.

The average of EC of biochar in distilled water after incubation for seven days was 2.85 mS/cm and it increased without significant difference with the CMEM solutions of 0, 3.1 and 6.3%. Thereafter, at CMEM levels between 12.5 and 25%, the EC seemed to have plateaued (Figure 1). The pH of the slurry increased for all the CM concentrations after seven days of incubation. We suggest that this increase may be explained by the production of ammonium and hydrolysis of proteins from the cow manure.

The Fourier-transform infrared spectroscopy (FTIR) is a method to obtain an infrared spectrum of the biochar surface in order to access its functional groups. The band regions of the FTIR provide an insight into the surface functional groups which strongly influence the chemical reactivity and acidic properties of the biochar. The high electrical conductivity associated with biochar (Figure 1) is indicative of a highly delocalized

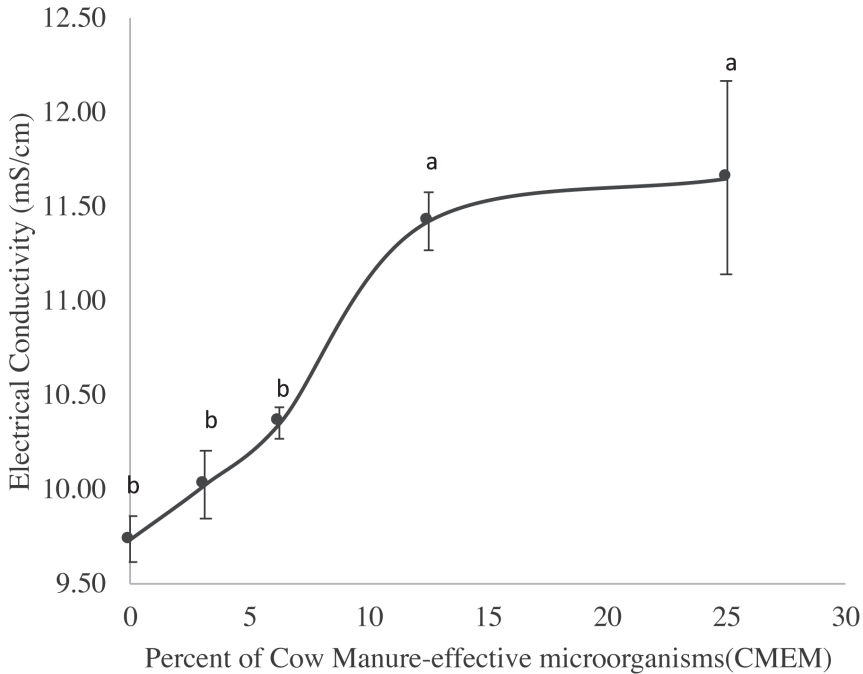


FIGURE 1. Electrical conductivity vs. percentage of Cow Manure-EM (CMEM) ratio of the slurry after an incubation period of seven days. Values are averages of triplicates. The error bars represent standard errors, and bars with different letters indicate statistically significant ($P < 0.05$) differences.

electron-rich material proper of its aromatic nature, denoted by a stretch band found in the CSB FTIR spectrum at $1,570\text{ cm}^{-1}$ (Figure 2, Line A). Figure 2 shows the FTIR spectrum of untreated CSB (Line A), whereas the absence of carbonyl and carboxyl bands in the region of $3,000$ to $3,700\text{ cm}^{-1}$; $1,700$ to $1,800\text{ cm}^{-1}$; and $1,200$ to $1,300\text{ cm}^{-1}$ on the FTIR spectrum is denoted. Carbonyl and carboxyl groups contribute to the negative surface charges and to the cation exchange capacity (CEC) of this material, the data of which denote that the untreated CSB is a low oxidized biochar. Low functionalized untreated CSB had a high pH (alkaline) indicative of its low content of carboxylic groups. The low CEC of the CSB can be attributed to a high pyrolysis temperature (815° C) and extended time (6 h) for its preparation, which were not the best conditions for CSB synthesis for agricultural purposes. A high pyrolysis temperature for char preparation is associated with high biochar surface area and ash content, and low CEC (Cheng et al., 2018). The data is in accord with the results of Cheng et al. (2018), which showed that increasing the pyrolytic temperature from 250 to 550° C decreased the CEC from 69 to 22 cmolc/kg . Cheng et al. (2018) data suggest that when the pyrolytic temperature is above 690° C , the CEC could have values as low as 1 cmolc/kg . The data indicates that the decrease of CEC with an increase of the pyrolytic temperature is due to the loss of oxygen-containing functional groups. The absence of oxygen-containing functional groups is shown in the FTIR spectra of the CSB (Figure 2, Line A). Also, Figure 2 shows the FTIR spectra of charged CSB (Line B) where the appearance of signals in the carbonyl and carboxyl

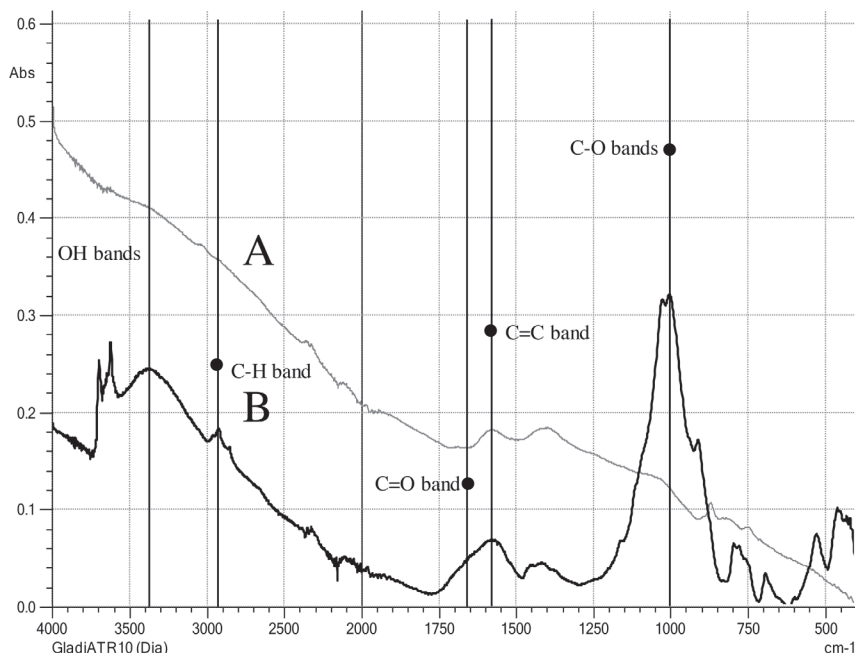


FIGURE 2. FTIR spectra of untreated (A) and charged (B) CSB showing principal labile organic matter signals.

regions and a significant improvement of the C-O bands in the region between 1,000 to 1,050 cm^{-1} in the charged sample are indicative of the formation of an organic layer over CSB surfaces. Additional data that confirms these findings is the low ORP observed in biochar mixed with AEM and cow manure under anaerobic incubation conditions in comparison with water-biochar samples at the same liquid-solid ratio which had positive values, which suggests lower negative charges on the CSB surface (Table 2 and Figure 3).

The activation of CSB with CMEM had the effect of increasing slightly the CEC in the 3.13% and 6.25% CMEM treatments. However, at a higher CMEM ratio, there were no significant differences relative to the control samples. This finding suggests that the CSB surface coverage with an organic carbon layer from CM and EM treatments reached a critical level, after which active sites were blocked by an organic matter with low electrostatic surfaces impeding cation attraction. Therefore, CSB has impeding cation surfaces, an indicator of little buffering and capacity to hold exchangeable cations (CEC), which means a low oxygen content surface representing a substantial limitation for agronomic purposes from the standpoint of available nutrients. Surface modification of low oxygen functionalized biochar is an alternative to improve its CEC. For example, pinewood biochar prepared by slow pyrolysis at 400° C has been shown to increase the biochar CEC value (Huff et al., 2018) significantly by ozonization. Thus, an alternative to enhance the agronomic properties of CSB is to improve its CEC through surface oxygenation.

Labile organic carbon from the aqueous CSB extracts shows a significant increase after EM and CMEM treatments (Table 3). These findings are indicative of an LOC layer formed over the CSB surface. The reduction in LOC concentration at 25% of CMEM

TABLE 2.—Chemical-physical properties of the slurry of Coffee Shrub Biochar (CSB), activated effective microorganisms (EM) and distilled water with different concentrations of cow manure (CM) (0, 3.13, 6.25, 12.50, 25%) before and after an incubation period of seven days.

Concentration of CM mixed with CSB-EM (%)	pH		EC ¹ (mS/cm)		ORP ² (mV)
	Initial	Final	Initial	Final	Final
0	5.02 ³	6.75 b ⁴	9.16	9.73 b	-163 b
3.1	5.12	7.19 b	7.93	10.02 b	-290 c
6.3	5.30	6.88 b	10.09	10.35 b	-292 c
12.5	5.54	7.31 b	11.05	11.42 a	-303 c
25.0	6.35	7.36 b	12.80	11.65 a	-428 d
Distilled water	9.69	9.50 a	4.22	2.85 c	30 a

¹EC denotes electrical conductivity. ²ORP denotes oxi-redox potential; ORP measured only after incubating the samples. ³All values represent means (n = 3). ⁴Means having different letters in each column indicate statistically significant (P < 0.05) differences.

treatment in the aqueous solution from the charged CSB could be the result of an increase of carbon humification process on biochar when the cow manure rate reached 25% (Table 3). The drastic ORP drop when the CM increased from 12.5 to 25% supports

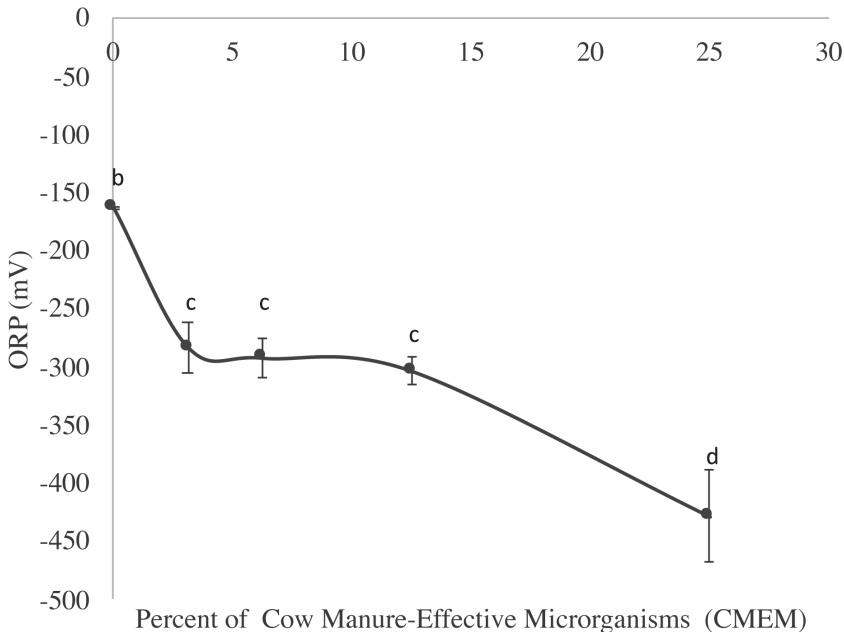


FIGURE 3. Oxidation-reduction potential for solution vs. Cow Manure-EM ratio. Values are averages of triplicate. The error bars represent standard errors, and bars with different letters indicate statistically significant (P < 0.05) differences.

TABLE 3.—*Chemical properties of the previously incubated Coffee Shrubs Biochar (CSB) with activated effective microorganisms (EM), distilled water and cow manure (CM) (0, 3.13, 6.25, 12.50, 25%) after washed with water.**

CSB treated with different CM Concentration (%)	CEC ¹ (cmolc/kg)	N total ² (%)	LOC ³ (ug/g)
0	0.61 bc ⁴	1.1 a	9,739 ab
3.1	1.02 ab	1.3 a	9,170 ab
6.3	1.51 a	1.6 a	10,499 a
12.5	0.47 bc	1.3 a	10,833 a
25.0	0.62 bc	1.3 a	8,282 b
water	0.25 c	1.2 a	1,644 c

¹CEC denotes cation exchange capacity. ²N total denotes total nitrogen. ³LOC denotes labile organic carbon. All values represent means (n = 3). ⁴Means having different letters in each column indicate statistically significant (P < 0.05) differences. *Incubated CSB washed with deionized water until the electrical conductivity of the filtrate dropped to 300 µS/cm.

that an increase in the humification process was happening, because the ORP drop is indicative of a higher anaerobic condition, favoring anaerobic microorganisms which promote humic acid synthesis and accumulation. Thus, the coat of organic carbon from the CMEM slurry over the CSB could have lower water solubility because of higher adsorptive forces between the organic layer and CSB and/or a higher polymerization degree, decreasing the hot water-soluble LOC amount.

Figure 4 shows the stereo microscopic images of untreated CSB and CSB charged with CMEM. The charged CSB (right) has a smoother and less bright surface than the untreated sample, indicating a labile organic carbon layer over the CSB. This image supports LOC data previously mentioned because the smooth organic coating on the CSB surfaces is denoted by a lower reflectance of the CSB surface. The smooth surface in the charged CSB suggests that surface porosity may have been blocked after CMEM treatments. Table 2 shows the values of pH and EC of the aqueous slurry in contact with the CSB. The pH of the biochar in contact with water was 9.50, and biochar incubated with CM+EM ranged from 6.88 to 7.36 with non-significant differences among treatments. The increase of organic carbon soluble in hot water indicates the LOC layer formation over CSB after the seven days of incubation. The covering of biochar with a LOC layer has positive effects on the agrochemical properties of biochar. Biochar charged in this way is not a nutrient sink but a source of nutrients and energy for the soil microflora. Biochar is applied at field levels at a broad range, between 0.5 and 5 kg/m² (Major, 2010). The extensive range of biochar application in the soil is a result of problems associated with material availability, cost, and decreasing crop yield observed when a high amount of biochar application occurs with some crops. The biochar application usually has little effect on available nutrients supplied with the material. The main benefits are related to physical and biological parameters, both associated with the labile and recalcitrant organic carbon fraction, and the avoidance of nutrient losses due to the increasing CEC available surfaces. Biochar with high EC is associated with nutrient sources for plant nourishment, and the recalcitrant component is associated with electron shuttle mechanisms, which benefit soil microorganisms. This study shows that CMEM charged CSB has better properties from an agronomical standpoint than untreated CSB due to the increase in LOC and enhanced hydroxyl, carbonyl, alkenyl, and alkoxy bands observed in the IR spectra. These findings denote the importance of the treatment of biochar before

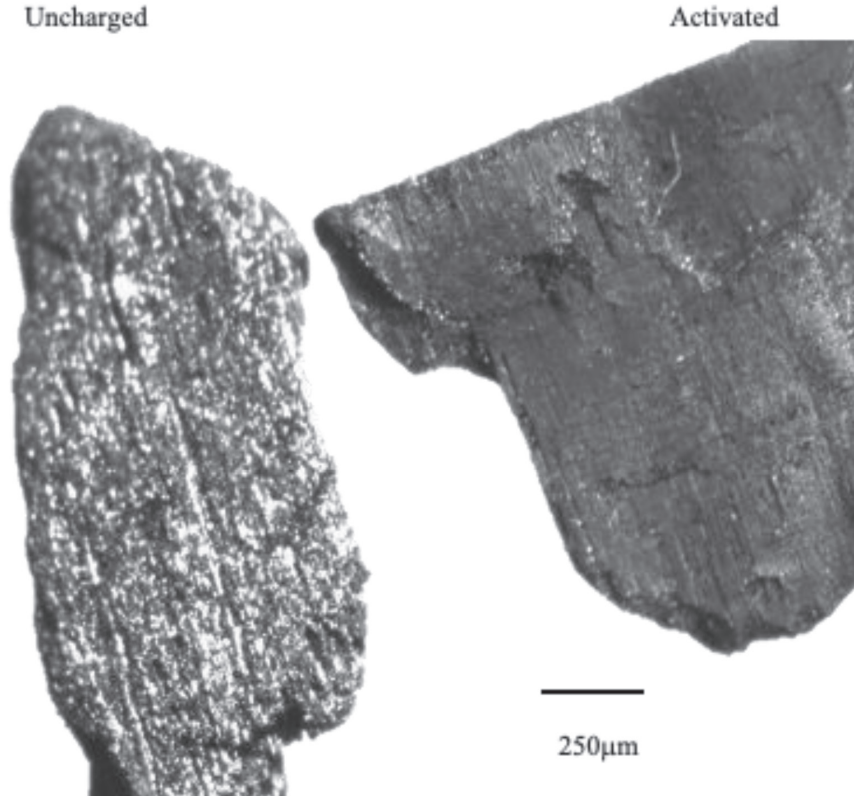


FIGURE 4. Stereo microscopy image of the uncharged (left) and charged (right) CSB showing the labile organic carbon layer formation. Scale bar 250 μm .

agronomical application to normalize and increase the benefits associated with its use. The low CEC capacity and lack of functionality on the uncharged CSB denote the importance of improving the manufacturing process and reducing the pyrolysis temperature, condition, and production time. These results pinpoint the importance of performing more studies to optimize biochar pyrolysis conditions and activation processes.

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