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Effects of Alkali-Activated Algae Biochar on Soil Improvement after Phosphorus Absorption: Efficiency and Mechanism

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Abstract: Biochar is often used for the removal of phosphorus in wastewater. However, the improper treatment of adsorbed biochar might cause secondary pollution. In order to promote the recycling and harmless utilization of biochar with adsorbed phosphorus, a new modified biochar (ABC) was prepared from cyanobacteria in this study. The maximum adsorption capacity of ABC calculated from the Langmuir isotherm model was $38.17 \text{ mg}\cdot\text{g}^{-1}$. ABC was used to absorb phosphorus in wastewater, whose product (ABC/P) was used for soil improvement and soybean cultivation. The results showed that adding the proper amount of ABC/P could significantly increase the pH of the soil (from 6.52 ± 0.04 to 7.49 ± 0.08), organic matter content (from 34.02 ± 0.41 to $47.05 \pm 0.14 \text{ g}\cdot\text{kg}^{-1}$), cation exchange capacity (from 3.01 ± 0.18 to $3.76 \pm 0.07 \text{ cmol}\cdot\text{kg}^{-1}$), water-holding capacity (from 28.78 ± 0.34 to $35.03 \pm 0.31\%$), effective phosphorus content, and total phosphorus content. Meanwhile, the soil alkaline phosphatase activity was improved. The plant height, root length, and fresh quality were promoted by planting soybeans in ABC/P-improved soil and were better than those of the control group. Therefore, ABC/P, as a new type of phosphorus fertilizer, has the potential for soil amendment for legume crops.



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Keywords: cyanobacteria; alkali-activated algae biochar; soil amendment; phosphorus; soybean cultivation

1. Introduction

It is well known that excessive phosphorus in natural water bodies leads to eutrophication [1–3]. At present, the main methods for removing phosphorus in water include chemical precipitation, bioremediation, and adsorption [4,5]. Among them, the adsorption method is considered to be a simple and efficient removal technique [6]. However, the adsorbent after adsorption contains a large amount of phosphorus and organic matter. If it is treated as solid waste, it will increase the processing cost. Therefore, it is crucial to find a resource utilization method to deal with the waste material.

Coastal areas and freshwater lakes in many countries are now experiencing severe eutrophication, accompanied by an overgrowth of algae [7–9]. In order to solve these problems and uphold the concept of turning global, social, and environmental problems into opportunities, many researchers have carried out research on the utilization of algae resources [10,11]. Among them, the conversion of algae biomass into biochar is currently a popular research direction. Algae biochar has the following advantages: (i) algae grow fast; (ii) it is a new and promising renewable raw material; (iii) it can be used to fix carbon, improve soil fertility, and for the bioremediation of wastewater [12]. Among many algae, cyanobacteria are one of the most widely distributed and common algae in fresh water [13]. The efficient utilization of filamentous green algae and preparing it into biochar provide a new direction for the utilization of algae resources [14].

The ability of biochar to absorb phosphate anions is limited because it is negatively charged and lacks metal cations. In recent years, various metal-modified biochar have been used to remove phosphorus from sewage, and all have shown a good removal performance [15]. However, there are few reports on the utilization of modified algae

biochar for soil amendment for resource utilization after adsorbing phosphorus. Therefore, this research is focused on the preparation of magnesium-modified algae biochar (ABC) and the phosphorus adsorbed product (ABC/P). ABC/P was used to conduct soil improvement experiments, including evaluating the effects on soil physical and chemical properties, the content of different forms of phosphorus, enzyme activity, and soybean planting. This technology is expected to provide a scientific basis for the harmless utilization of algae biochar after phosphorus absorption.

2. Materials and Methods

2.1. Experiment Material

2.1.1. Experimental Materials and Instruments

The filamentous green algae used in this experiment were obtained from a lake in Nanjing, Jiangsu. The water used in the experiment was distilled water, and all reagents were of analytical grade, purchased from China Aladdin Company. Experimental instruments include: electric heating constant temperature blast drying oven (DHG-9240, Zhejiang Xinfeng Medical Equipment Co., Ltd., Shaoxing, China), desktop high-speed centrifuge (H/T18MM, Hunan Hexi Instrument Equipment Co., Ltd., Changsha, China), scanning electron microscope (JSM-6360LV, Japan Electronics Corporation, Tokyo, Japan), and BET specific surface area tester (TriStar II Plus, McMuritik, Shanghai, China).

2.1.2. Tested Soil and Plants

The test soil was collected from the topsoil (0–20 cm) of farmland in the suburbs of Nanjing City, Jiangsu Province. After the soil samples were air-dried and crushed through a 100-mesh sieve, their basic physical and chemical properties were determined according to the method in [15]. Potted test plants are selected soybeans that are more sensitive to phosphorus.

2.2. Experiment Method

2.2.1. Preparation and Characterization of Modified Algae Biochar (ABC)

The cyanobacteria were washed, freeze-dried for 24 h, and then ground and crushed, and 10.00 g cyanobacteria powder was placed in a shaker at a temperature of 25 °C and a rotation speed of 200 r·min⁻¹, followed by 100 mL of 2.5% KOH solution, and 100 mL of 0.0125 mol·L⁻¹ MgCl₂ solution was impregnated and modified for 2 h and then filtered out and placed in an oven at 60 °C for 12 h. Finally, it was transferred to a crucible, placed in a muffle furnace, heated to 600 °C at a rate of 10 °C·min⁻¹, and heated at a constant temperature for 2 h. After cooling, it was ground through an 80-mesh sieve to obtain ABC. A quantity of 2.0 g ABC was added into a serum bottle (150 mL), and then 100 mL of simulated phosphorus-containing wastewater (500 mg·L⁻¹) was added and placed in a constant temperature water bath shaker at a temperature of 25 °C and a rotation speed of 200 r·min⁻¹ for 1 h. After the reaction, the filtered material was placed in an oven and dried at 60 °C for 12 h to obtain the final phosphorus-containing algae biochar soil amendment (ABC/P). The basic properties of ABC and ABC/P are shown in Table 1.

Table 1. The basic properties of the experimental materials.

Test Indicators	OM/(g·kg ⁻¹)	Total N/(g·kg ⁻¹)	Total P/(g·kg ⁻¹)	Total K/(g·kg ⁻¹)
ABC	82.35	13.79	7.28	25.38
ABC/P	63.47	14.02	27.76	23.77

2.2.2. ABC Adsorption Experiment on P

The phosphorus sorption isotherms of ABC were carried out in batch experiments. For the sorption isotherms, 0.2 g ABC was added to 50 ml polyethylene centrifuge tubes with 25 ml of various phosphorus solutions (10, 20, 40, 50, 100, 150, and 150 mg P/L) at a pH of 7.0. The tubes were placed on a constant temperature shaker (25 °C) for 24 h

at 220 rpm to ensure complete mixing. The solutions were then centrifuged and filtered through a 0.45 µm membrane. The filtered solutions were kept at 4 °C for further analysis.

2.2.3. Soil Improvement Test

Plastic pots with diameters of 20 cm and heights of 20 cm were used. Each pot was filled with 500 g of soil. Then, ABC and ABC/P were added at a ratio of 10 g·kg⁻¹ and mixed with the soil thoroughly to keep the soil moisture content at about 20%. Then, the cultivated soybeans were planted into the soil according to the sowing rate of 5 soybeans per pot. In order to facilitate the simultaneous study of the effects of ABC/P on soil and plants after improving soil, a total of 3 test groups were set up, namely the soil group (control group), soil + ABC group, soil + ABC/P group, with 4 pots in each group. One sample pot corresponds to one culture time. All potted plants were cultivated in a sunny greenhouse. The cultivation period was set to 40 days. The soil and plant data of the sample pots were collected for the corresponding cultivation time every 10 days. All experiments were set to be repeated 3 times.

2.3. Analytical Method

Phosphorus concentrations in the solution and extract were determined spectrophotometrically at 700 nm (UV-2550), according to the molybdenum blue method. The determination of basic physical and chemical properties of soil refers to padronized methods [15]. P fractions were determined in this study according to the method of Hieltjes and Lijklema [16].

2.4. Data Processing and Analysis

Microsoft Excel 2010 was used for data processing, Origin 9.0 was used for drawing, and SPSS 11.5 was used to test the significance of differences ($p > 0.05$).

3. Results and Discussion

3.1. Morphological Characterization of ABC

ABC was prepared by the hydrothermal carbonization of cyanobacteria biomass. As shown in Figure 1a,b, there are many folds and sintered holes on the surface of ABC, which indicates that ABC has a large surface. The BET specific surface area (Figure 1c,d) showed that the specific surface area of ABC was as high as 25.95 m²·g⁻¹, which was 3.41 times higher than cyanobacteria. This can be explained by the change in the number and diameters of holes after hydrothermal carbonization at a high temperature. The higher specific surface area indicated that ABC has a potentially strong phosphorus adsorption capacity [17,18].

3.2. Adsorption Isotherm of ABC for P

The P adsorption of ABC was evaluated at various phosphorus solutions (10, 20, 40, 50, 100, 150, and 200 mg P/L) at 25 °C (298 K) with an initial pH of 7.0. The results show that ABC has an excellent adsorption capacity for phosphorus. The Langmuir and Freundlich isotherm equations are commonly used to describe the process in sorption experiments. The linear form of the isotherm equations can be given as follows (Equation (1) for Langmuir isotherm and Equation (2) for Freundlich isotherm):

$$\frac{C_e}{q_e} = \frac{C_e}{q_{\max}} + \frac{1}{K_L \cdot q_{\max}} \quad (1)$$

$$\ln q_e = \ln K_f + \ln C_e/n \quad (2)$$

where C_e is the equilibrium phosphate concentration in the solution (mg·L⁻¹); q_e is the P concentration on the sorbent (mg·L⁻¹); q_m represents the maximum sorption of the sorbent at given concentrations (mg·g⁻¹); K_L , K_F and n are constant parameters of the above models. The isotherm parameters are shown in Figure 2 and Table 2. The appli-

cability of the isotherm equation was compared by the correlation coefficient R^2 . The results showed that the Langmuir isotherm equation showed a more satisfactory result ($R^2 = 0.9698$). The maximum adsorption capacity calculated from the Langmuir isotherm model was $38.17 \text{ mg}\cdot\text{g}^{-1}$.

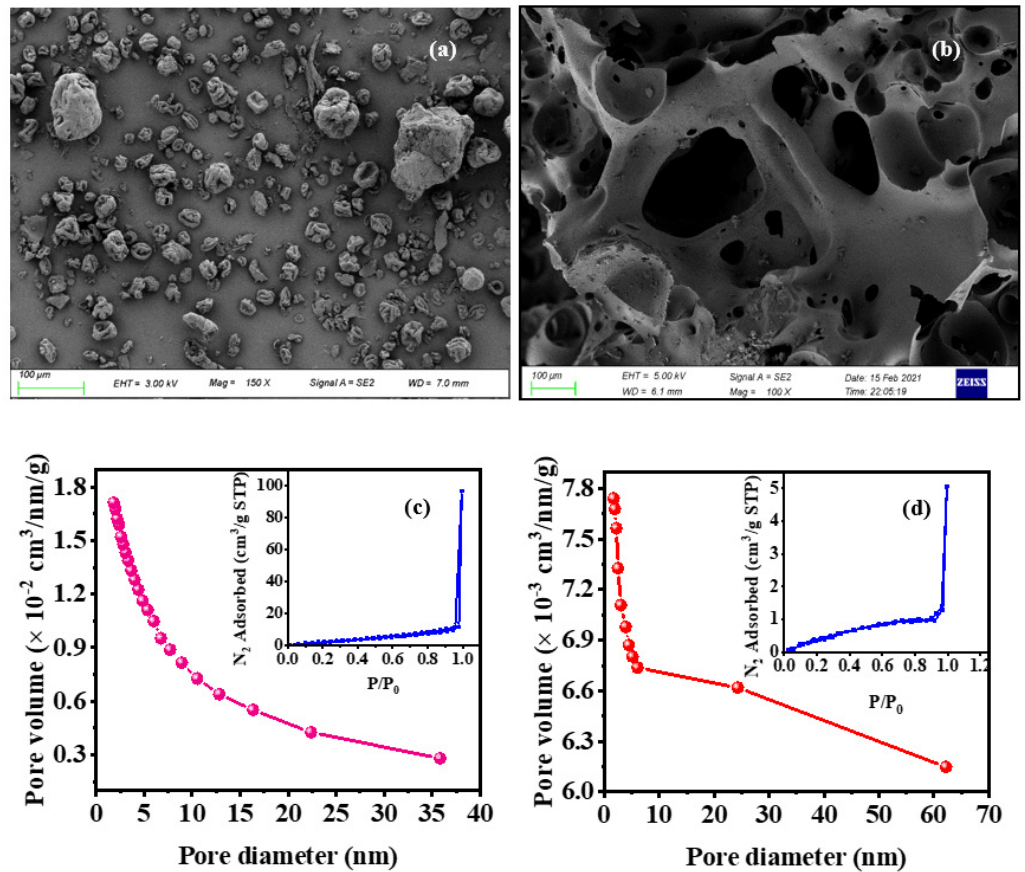


Figure 1. SEM of cyanobacteria (a) and ABC (b). Pore size distributions of cyanobacteria (c) and ABC (d).

Table 2. Parameters of Langmuir isotherm and Freundlich isotherm for the phosphorus adsorption by using ABC.

T (K)	Langmuir Isotherms			Freundlich Isotherms		
	q_{\max} ($\text{mg}\cdot\text{g}^{-1}$)	K_L ($\text{L}\cdot\text{mg}^{-1}$)	R^2	n	K_f ($\text{mg}\cdot\text{g}^{-1}$)	R^2
298	38.17	0.0721	0.9698	0.33	7.1446	0.8560

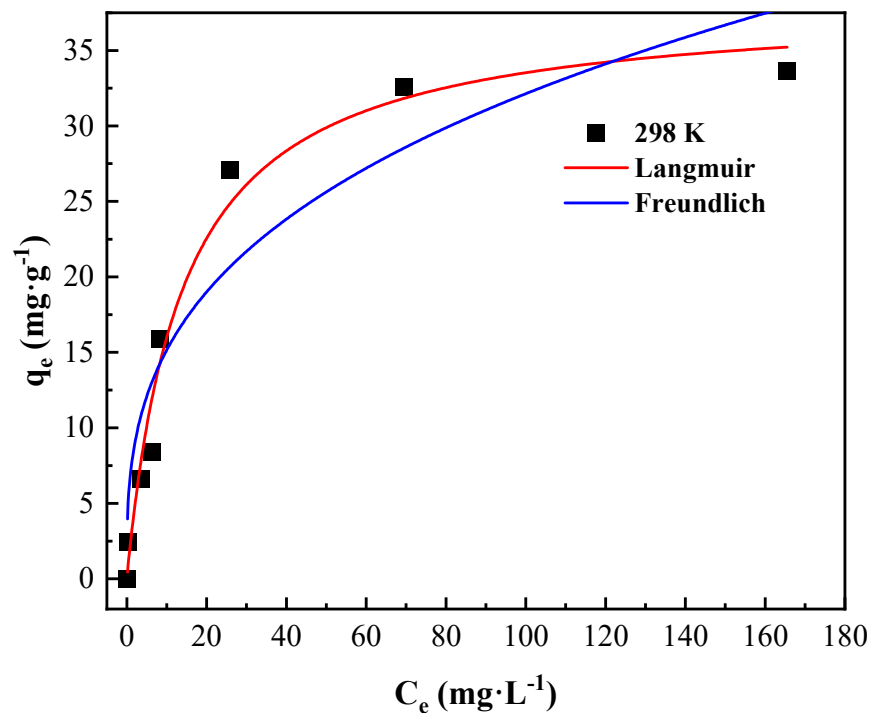


Figure 2. Adsorption isotherms for ABC on P at 298 K fitted with Langmuir and Freundlich model.

3.3. Effects of ABC/P on Soil Physical and Chemical Properties

The addition of ABC and ABC/P significantly increased soil pH, organic matter content, cation exchange capacity, water-holding capacity, available phosphorus content, and total phosphorus content (Table 3). The cation exchange capacity, available phosphorus, and total phosphorus in the experiment group showed a decreasing trend with the increase in incubation time, which might be due to the continuous consumption of soil nutrients by the soybean plants without the supplementation of external ions. The pH value is generally considered to be the main variable of soil [19]. As shown in Table 3, the addition of ABC and ABC/P could improve the soil pH and regulate the soil pH from weakly acidic to neutral and weakly alkaline, which was similar to the results reported by Z. Yuan et al [20]. ABC and ABC/P are relatively alkaline and can also increase soil pH by increasing soil base saturation, reducing exchangeable aluminum levels, and consuming soil protons. On the other hand, the soil cation exchange capacity (CEC) is an important basis for evaluating soil fertility retention, soil improvement and rational fertilization [21]. The addition of ABC and ABC/P improved the soil variable charge status and increased the soil cation exchange capacity, mainly due to the biochar itself having a negatively charged surface and a certain anionic functional group [22]. Moreover, ABC/P contains a large amount of phosphate, which releases a large quantity of phosphorus-containing anions in the soil, making the soil colloid adsorb various cations. Phosphorus in the soil is an indispensable nutrient for plant growth. It can be seen from Table 3 that the amount of effective phosphorus and total phosphorus of the soil increased slightly, by 19.59% and 14.08%, respectively, after adding ABC compared to the soil group. The main reason was that ABC has a large amount of soluble phosphate residues and stable physical and chemical properties and the ability of strong anti-decomposition and antioxidation, which ensured a long-lasting supply of phosphorus. Therefore, the addition of ABC to soil will inevitably lead to the accumulation of phosphorus and improve the release capacity of soil phosphorus. After adding ABC/P, the contents of total phosphorus and available phosphorus in the soil increased by 242.18% and 729.99%, respectively, compared to the soil without added substances. This is mainly because ABC/P not only has the characteristics of ABC, but also absorbs a large amount of phosphate in wastewater. The accumulation and promotion of soil phosphorus were more

significant than ABC when ABC/P applied to soil. The addition of ABC and ABC/P to the soil effectively improved the soil organic matter content and water-holding capacity since they contained a certain amount of organic matter and have abundant pores, which was consistent with the results of Shin H et al. [23].

Table 3. Effects of different treatments on soil physical and chemical properties.

Experimental Groups	Culture Time/d	pH	OM (/g·kg ⁻¹)	CEC (/cmol·kg ⁻¹)	Water-Holding Capacity/%	Effective P/(mg·kg ⁻¹)	Total P/(mg·kg ⁻¹)
Soil	0	6.23 ± 0.03	29.76 ± 0.25	4.26 ± 0.17	28.78 ± 0.34	38.25 ± 1.23	736.78 ± 15.73
	10	6.37 ± 0.05	—	4.13 ± 0.09	—	42.28 ± 1.05	684.38 ± 23.75
	20	6.41 ± 0.07	—	3.72 ± 0.15	—	45.12 ± 0.49	635.71 ± 16.93
	30	6.42 ± 0.09	—	3.32 ± 0.21	—	46.81 ± 0.56	584.78 ± 9.35
	40	6.52 ± 0.04	34.02 ± 0.41	3.01 ± 0.18	—	42.01 ± 0.34	544.85 ± 22.61
Soil + ABC	0	6.79 ± 0.04	34.84 ± 0.21	4.52 ± 0.08	36.35 ± 0.24	45.12 ± 0.33	784.93 ± 24.34
	10	7.05 ± 0.11	—	4.25 ± 0.21	—	42.23 ± 1.05	728.45 ± 12.46
	20	7.12 ± 0.08	—	3.94 ± 0.23	—	47.92 ± 1.31	696.36 ± 16.88
	30	7.21 ± 0.04	—	3.72 ± 0.15	—	43.81 ± 0.69	648.21 ± 23.45
	40	7.18 ± 0.07	43.04 ± 0.13	3.25 ± 0.08	—	50.24 ± 0.39	621.55 ± 19.04
Soil + ABC/P	0	6.92 ± 0.03	35.02 ± 0.08	4.82 ± 0.13	35.03 ± 0.31	843.92 ± 14.82	2845.28 ± 23.45
	10	7.43 ± 0.09	—	4.54 ± 0.11	—	736.23 ± 18.75	2473.23 ± 28.93
	20	7.62 ± 0.01	—	4.34 ± 0.16	—	543.98 ± 21.35	2234.48 ± 27.35
	30	7.54 ± 0.12	—	4.06 ± 0.04	—	431.37 ± 33.45	2082 ± 18.94
	40	7.49 ± 0.08	47.05 ± 0.14	3.76 ± 0.07	—	348.65 ± 27.34	1864.38 ± 22.54

3.4. Effects on Soil Phosphorus Forms

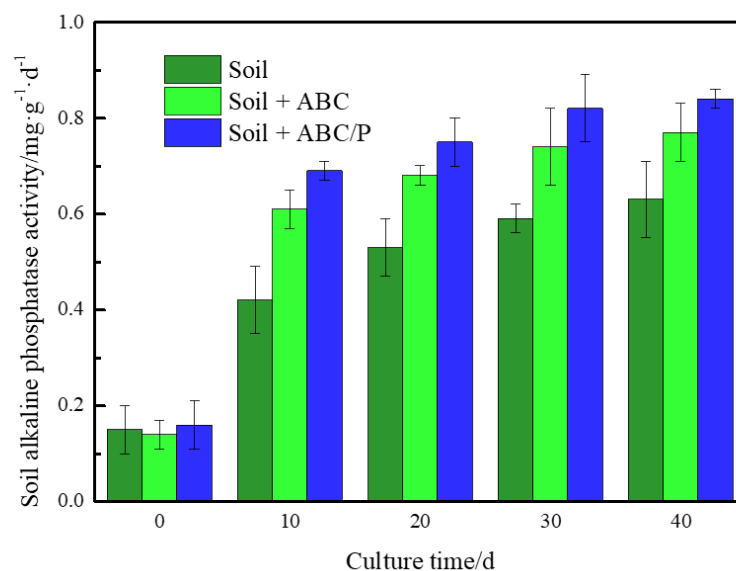
It can be seen from Table 4 that the addition of ABC did not significantly increase the contents of various forms of inorganic phosphorus in the soil during the 40-day cultivation period. The conversion of different forms of inorganic phosphorus had no obvious promotion effect, which might be due to the small amount of biochar added and short culture time. After adding ABC/P to the soil and culturing for 40 days, the contents of Al-P, Fe-P, Ca₂-P, and Ca₈-P in the soil were significantly increased. Compared to the natural soil, it increased by 4.45, 3.51, 7.51, and 9.03 times, respectively, and was 1.08, 1.42, 1.18, and 1.16 times that of the soil added with ABC. This was mainly due to the adsorption of a large amount of phosphorus in ABC/P, and phosphorus is released into the soil and quickly converted into Ca₂-P, Ca₈-P, Al-P, and Fe-P in a short time after mixing with the soil, thus significantly increasing the high-efficiency phosphorus source (Ca₂-P) and slow-acting phosphorus source (Ca₈-P, Al-P, and Fe-P) in the soil. After 40 days of culture with ABC/P, there was no significant change in soil Ca₁₀-P compared with to the other two groups, which might be due to the rapid absorption of various active phosphorus by plants and the shorter culture time [24]. The contents of highly active Ca₂-P in the soil with added ABC/P tended to decrease gradually during the 0–40-day cultivation process, while the contents of Ca₈-P, Fe-P, and Al-P all tended to increase gradually. This is because the highly active Ca₂-P was gradually being transformed into the relatively stable Ca₈-P, Fe-P, and Al-P during the culture process [25].

3.5. Effects on the Soil Alkaline Phosphatase Activity

The activity of soil alkaline phosphatase reflects the status of soil phosphorus to a certain extent. As shown in Figure 3, the addition of ABC/P could effectively increase soil alkaline phosphatase activity.

Table 4. Variation of inorganic phosphorus content in different treatments ($\text{mg}\cdot\text{kg}^{-1}$).

Experimental Groups	Culture Time/d	Al-P	Fe-P	Ca ₂ -P	Ca ₈ -P	Ca ₁₀ -P
Soil	0	57.37 ± 4.25	97.63 ± 3.89	42.34 ± 1.87	38.78 ± 1.34	76.25 ± 1.23
	10	44.47 ± 3.12	86.73 ± 3.71	45.28 ± 2.45	33.45 ± 2.01	80.24 ± 1.32
	20	36.41 ± 1.91	82.01 ± 2.45	40.06 ± 1.86	40.38 ± 1.04	86.12 ± 2.49
	30	38.21 ± 2.17	74.83 ± 1.96	43.32 ± 1.21	43.73 ± 2.05	86.81 ± 0.96
	40	39.45 ± 1.83	79.02 ± 4.92	43.05 ± 0.98	36.23 ± 1.06	94.05 ± 2.16
Soil + ABC	0	59.79 ± 1.04	114.89 ± 2.89	44.42 ± 1.28	36.35 ± 1.25	83.23 ± 5.21
	10	52.05 ± 2.11	103.81 ± 6.27	44.25 ± 1.41	34.94 ± 2.03	85.38 ± 1.05
	20	47.62 ± 3.08	100.25 ± 4.13	49.94 ± 1.23	41.38 ± 1.38	87.34 ± 1.38
	30	40.25 ± 1.04	118.45 ± 6.37	53.72 ± 1.45	45.07 ± 2.41	93.81 ± 1.69
	40	42.72 ± 1.27	112.95 ± 3.29	45.34 ± 2.08	39.47 ± 3.05	104.25 ± 3.39
Soil + ABC/P	0	186.32 ± 10.03	221.34 ± 6.53	634.98 ± 11.14	335.53 ± 6.31	114.53 ± 2.38
	10	162.43 ± 9.34	242.27 ± 4.43	484.54 ± 0.71	312.39 ± 3.94	124.25 ± 1.75
	20	183.62 ± 12.01	263.26 ± 11.22	396.37 ± 3.16	331.02 ± 5.21	137.98 ± 2.38
	30	167.33 ± 6.12	272.47 ± 7.04	340.56 ± 6.43	320.35 ± 10.25	139.33 ± 3.02
	40	175.45 ± 12.02	277.05 ± 10.14	323.56 ± 5.07	327.48 ± 9.27	148.65 ± 2.31

**Figure 3.** Effects of different treatments on the soil alkaline phosphatase activity ($p > 0.05$).

The alkaline phosphatase activity in the three test groups increased continuously with the culture time, which was primarily due to the continuous growth of soil microorganisms and soybean roots and the production of a large amount of alkaline phosphatase. The application of ABC and ABC/P in soil could further improve the living environment of soil microorganisms and increase the number of soil microorganisms, thus increasing the alkaline activity of phosphatase [26]. Therefore, the alkaline phosphatase activity in the ABC/P group and ABC group was significantly higher than that in soil group (Figure 3).

3.6. Effects on Soybean Growth

It can be seen from Table 5 that the plant height, root length, and fresh weight of soybeans in the three test groups increased with the increase in cultivation time. The growth status of soybeans in the ABC/P treatment group was the best after 40 days of cultivation. Compared to the soil group, plant height, root length, and fresh weight were increased by 35.56%, 48.92%, and 119.56%, respectively. The addition of ABC/P could effectively promote the growth of soybeans, which is mainly due to the adsorption of a large amount of phosphorus in wastewater on ABC/P, and the application of ABC/P to

soil could significantly increase Ca₂-P and Ca₈-P in the soil. Inorganic phosphorus with strong activity, such as Fe-P and Al-P, increased the content of nutrient elements that could be directly used by crops in the soil. In addition, ABC/P also has a large internal surface area, which provides a large space for nutrient adsorption, water retention, and microbial community survival, thereby improving environmental conditions, such as soil physical and chemical properties and soil enzyme activity, and promoting crop growth [23].

Table 5. Effects of different treatments on soybean growth.

Experimental Groups	Culture Time/d	Plant Height/cm	Root Length/cm	Fresh Weight/g
Soil	10	14.15 ± 5.65	7.15 ± 4.16	—
	20	25.83 ± 6.23	7.48 ± 3.92	—
	30	31.48 ± 10.06	8.73 ± 5.01	—
	40	45.05 ± 7.82	9.57 ± 4.16	21.57 ± 5.38
Soil + ABC	10	20.72 ± 4.78	9.25 ± 1.38	—
	20	31.08 ± 7.36	10.28 ± 4.02	—
	30	38.03 ± 8.03	10.83 ± 3.42	—
	40	54.27 ± 4.27	11.05 ± 2.74	30.61 ± 2.49
Soil + ABC/P	10	26.83 ± 6.66	10.03 ± 3.58	—
	20	37.46 ± 5.48	11.88 ± 2.59	—
	30	43.12 ± 9.66	12.98 ± 3.12	—
	40	61.64 ± 8.25	14.25 ± 3.36	47.36 ± 3.42

4. Conclusions

(1) The modified ABC prepared from cyanobacteria has a porous surface and a large specific surface area of 25.92 m²·g⁻¹, whose maximum adsorption capacity calculated from the Langmuir isotherm model was 38.17 mg·g⁻¹.

(2) Compared to the control group, adding the proper amount of ABC/P could significantly increase the soil pH, organic matter content, cation exchange capacity, water-holding capacity, effective phosphorus, and total phosphorus content. Meanwhile, the soil alkaline phosphatase activity was improved.

(3) In the pot experiment, planting soybeans with ABC/P-improved soil could significantly promote the growth of soybeans. Therefore, ABC/P is a promising soil amendment for legume crops.

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