International Journal of Design & Nature and Ecodynamics
Vol. 20, No. 1, January, 2025, pp. 137-146
Journal homepage: http://iieta.org/journals/ijdne

Pilot Study: Impact of Biochar Derived from Activated Sludge with *Pseudomonas putida* on Cherry Tomato Cultivation

Thana Majid Zayer Noor[1*], Noor S. Naji[2], Salah M. Abdullah[3], Aseel F. Alwan[3], Nibras Mohammed A. Alsaffar[4], Ibtisam Fareed Ali Karm[4]

[1] Department of Biology Science, Mustansiriyah University, Baghdad 10052, Iraq
[2] Department of Biology, University of Babylon, Babil 51015, Iraq
[3] Chemistry Department, Mustansiriyah University, Baghdad 46079, Iraq
[4] Market Research and Consumer Protection Center, University of Baghdad, Baghdad 10071, Iraq

Corresponding Author Email: thananoor20@uomustansiriyah.edu.iq

https://doi.org/10.18280/ijdne.200115

Received: 11 November 2024
Revised: 13 December 2024
Accepted: 20 December 2024
Available online: 31 January 2025

Keywords:
biochar, pseudomonas putida, VITEK 2, WWTP, soil productivity, greenhouse gas mitigation

ABSTRACT

Biochar produced from activated sludge boosts soil fertility by supplying nutrients, improving water retention, and optimizing nutrient accessibility. Its durable carbon composition sequesters carbon in the soil for long-term storage. Reduces greenhouse gases such as CH_4 and N_2O by modifying microbial dynamics and enhancing soil aeration. Furthermore, biochar captures pollutants, minimizing environmental hazards and promoting sustainable farming practices. This study investigates the impact of biochar derived from activated sludge and biochar loaded with *Pseudomonas putida* on cherry tomato growth. Biochar was produced from activated sludge at the Babel wastewater treatment plant, with *Pseudomonas putida* isolated from the same source. In a 90-day pot experiment, four biochar treatments were tested: two concentrations (1% and 5%) and the same concentrations loaded with *Pseudomonas putida*. Results showed that 5% biochar loaded with *Pseudomonas putida* significantly enhanced cherry tomato growth, with the highest fresh shoot weight (179.9 g) and chlorophyll content (62.22 SPAD), All biochar treatments significantly enhanced soil chemical properties, such pH, electrical conductivity, and level of phosphorus, carbon, and nitrogen, leading to enhance plant growth and productivity ($P < 0.0034$) compared to the control ($P < 0.0045$). Scanning Electron Microscopy (SEM) analysis revealed a reduction in biochar particle diameter from 33.95 nm before pyrolysis to 16.17 nm after pyrolysis. These findings suggest that 5% biochar loaded with *Pseudomonas putida* is effective for small-scale agricultural applications.

1. INTRODUCTION

Biochar consists of carbon, oxygen, sulfur, hydrogen, nitrogen, and minerals found in ash. It's created through pyrolysis, a process where biomass undergoes thermal decomposition in a low-oxygen setting. Black, porous, and finally grained, biochar has a lightweight nature, significant surface area, and an impact on soil pH, all beneficial for soil applications. To combat agriculture soil degradation, biochar is applied to enhance soil quality by stabilizing biomass [1]. This process modifies soil to improve crop productivity and reduce pollution. Biochar, a carbon-rich by-product from biomass pyrolysis, depends on biomass type and processing conditions. Its production in low-oxygen, high-heat environment creates co-products like oil and gas, with quantities varying by process setting [2].

Biochar from organic waste can enhance soil properties and generate farm-based renewable energy. Biochar quality varies based on soil type, metal content, biomass source, and application rate. When added to soil, biochar raises pH, carbon content, nitrogen, electrical conductivity, cation-exchange capacity and phosphorus levels [3]. Research shows that biochar's porous, nutrient-rich structure improves heavy metal absorption in soil, aids nutrient retention, and enhances microbial support. When combined with *Pseudomonas putida*, biochar promotes plant growth by improving microbial activity and nutrient availability while increasing disease resistance. This synergy also enhances soil water retention and reduces compaction risks [4].

Biochar and *Pseudomonas putida* improve soil properties and ecosystem sustainability through complementary effects. Biochar increases soil organic carbon by 20-30%, phosphorus by up to 40%, and aggregate stability by 30-60%, while sequestering 50-80% of its carbon. *Pseudomonas putida* enhances nitrogen availability by 15-25%, phosphorus solubilization by 10-15%, and aggregate stability by 25-50%, while promoting carbon and nitrogen cycling by 10-30%. Together, they optimize nutrient content, stability, and cycling processes in soil [5]. The combined application of biochar and *Pseudomonas putida* improves soil quality and ecological sustainability through synergistic mechanisms through soil fertility. Biochar supplies stable carbon and retains nutrients

(N, P), while *Pseudomonas putida* enhances nutrient cycling through nitrogen fixation and phosphorus solubilization. Porous structure inside biochar particles improves water retention and aggregation, complemented by *Pseudomonas putida* produced exopolysaccharides. Since biochar sequester 50-80% of carbon, reducing greenhouse gases, while *Pseudomonas putida* accelerates organic matter decomposition and nutrient release. Most heavy metals adsorbed by biochar who works as pollutant management and *Pseudomonas putida* degrade organic pollutants, minimizing toxicity [6].

The aim of this study is to describe the composition, production, and benefits of biochar, particularly its role in enhancing soil quality and supporting sustainable agriculture. This study describes how biochar improves soil properties, aids nutrient retention, especially when combined with *Pseudomonas putida,* in addition to that promotes plant growth and resilience, ultimately contributing to sustainable farming practices and environmental stability.

2. MATERIALS AND METHODS

2.1 Experimental site description

The experimental location is located in the School of Science, Biology Department backyards at Mustansiriyah University in Baghdad. The experiment occurred at the end of winter season in Iraq, spanning from February 2023 to April 2023, during which the average annual temperature was 20℃. The soil in the area is a combination of clay soils.

2.2 Soil preparation

Soil samples were gathered from the backyards of the School of Science, Biology Department at Mustansiriyah University in Baghdad. After air-drying for 14 days, soil samples were sieved with 2.5 mm and then autoclaved and utilized for cultivation experiments. The resulting biochar was transformed into powder formulations. Figure 1 and Figure 2 display the characteristics of soil and biochar at day 0 of the experiment.

2.3 Experimental design

Production of biochar from activated sludge via pyrolysis

The wastewater treatment facility in Babel province, Iraq, uses an activated sludge system for secondary municipal wastewater treatment. The sludge is processed through anaerobic digestion and then dewatered with a belt filter press. The dewatered sludge was dried in an oven at 105℃ for 24 hours, then crushed, sieved, and stored in an airtight plastic bag.

The pyrolysis process was conducted in a muffle furnace (Linn High Therm) at 500℃, with a nitrogen flowing at 200 ml/min to create an oxygen free environment. The temperature increased at 17℃/min until reaching the target temperature, where the sample was held for 90 minutes (residence time). The resulting biochar was then cooled in a desiccator, weighed, and stored in airtight plastic containers as shown in Figure 3(A & B) [7]. The fundamental characteristics of soil and biochar, as obtained from the literature by Liu et al. [8], are summarized below:

The soil was classified as sandy loam in texture, whereas no specific texture was noted for biochar. In terms of organic matter, the soil contained 8.04 g kg^{-1}, while the corresponding value for biochar was not provided. For total carbon content, no data were available for the soil, but biochar had a significantly high carbon concentration at 597.7 g kg^{-1}. Regarding total nitrogen, the soil had 0.56g kg^{-1}, while biochar showed a much higher level at 13.4 g kg^{-1}. Similarly, the total phosphorus content was 1.09 g kg^{-1} for soil and 2.47g kg^{-1} for biochar. The total potassium as 15.36 g kg^{-1} for soil and 29.8 g kg^{-1} for biochar. Lastly, the cation exchange capacity (CEC) of the soil was measured at 5.40 cmol kg^{-1} which was considerably lower compared to biochars CEC of 17.0 cmol kg^{-1}. These findings highlight the enhanced nutrient and cation exchange properties of biochar compared to soil.

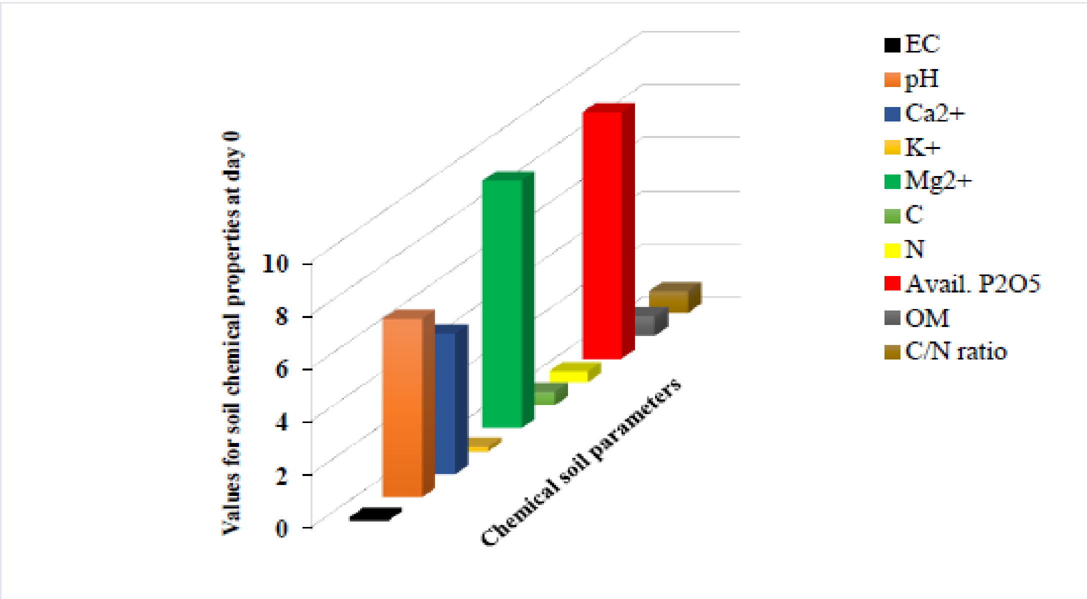

Figure 1. Chemical characteristics for soil samples at day 0

EC, electrical conductivity (ds m^{-1}); Ex. cations: exchangeable cations (cmol kg^{-1}); (Ca^{2+}, K^{+} and Mg^{2+}); C, carbon (g kg^{-1}); N, nitrogen (g kg^{-1}); P_2O_5, available phosphorus (mg kg^{-1}) and OM, organic matter (g kg^{-1}). Values represent by means ± SD.

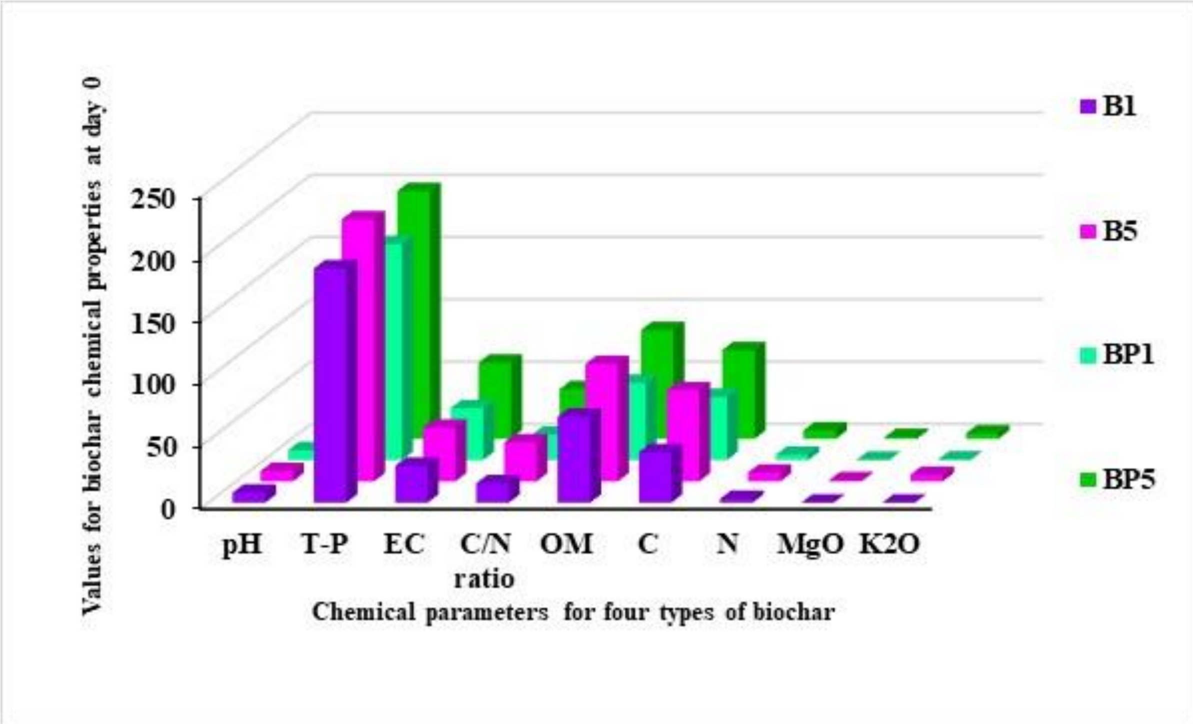

Figure 2. Chemical characteristics for biochar samples at day 0

T-P, total phosphorus (mg kg^{-1}); EC, electrical conductivity (ds m^{-1}); C/N ratio; OM, organic matter (%), C, carbon (g kg^{-1}) and N, nitrogen (g kg^{-1}); B1: 1% of biochar; B5: 5% of biochar; BP1: 1% of biochar loaded with *Pseudomonas putida* and BP5: 5% of biochar loaded with *Pseudomonas putida.* Values represent by means ± SD.

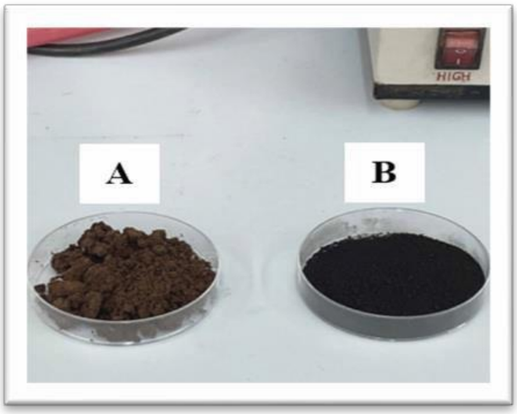

Figure 3. (A) Activated sludge before pyrolysis application; (B) Biochar produced through pyrolysis of activated sludge

Samples collections and identification of Pseudomonas isolates

Activated sludge samples were collected in triplicate from the wastewater treatment plant in Babel province, Iraq, using sterile 100 ml bottles under aseptic conditions. These samples were promptly transferred to the laboratory, stored in a dry and clean environment, and subjected to serial dilution and culture procedures using King A and King B medium. Each sewage samples of 100 microliters were diluted up to 10^{-6} and cultured later on the same media. following the manufacturer's instructions. The cultured medium was poured into plates for further procedures. Different bacterial colonies from activated sludge samples were then grown on King A and King B media plates to verify that they belonged to the *Pseudomonas* genus. Identification of the isolates was based on morphological characteristic like colony appearance, pigmentation, and motility. Furthermore, a biochemical test like oxidase activity and substrate utilization were performed on suspected isolates, lastly a phenotypic traits like VITEK test that include resistance to specific antibiotics or environmental conditions was applied. These combined methods ensure accurate differentiation within microbial communities [9].

VITEK test conditions

Utilizing the VITEK 2 Compact machine, *Pseudomonas putida* was isolated from activated sludge samples. Various microbial colonies were obtained through serial dilution techniques applied to all collected samples. Specifically, 100 microliters from the sixth dilution were transferred to prepared agar (King A and King B medium), spreaders, and incubated at 30°C for 24 hours. Following incubation, individual colonies were examined, sub-cultured to obtain pure colonies, and used for the suspension preparation for ID Cards. Additionally, pure colonies were stored in 20% glycerol in the freezer for further tests [10].

Produced biochar loaded with isolated *Pseudomonas putida*

The *Pseudomonas putida* bacterial strain, initially isolated from activated sludge, was identified with a 98% probability using the VITEK 2 Compact machine. Prior to utilizing the pre-prepared biochar from activated sludge, all samples underwent pulverization and passed through a 0.25 mm sieve. To prepare the *Pseudomonas putida* loaded biochar inoculant, the bacterial strain was initially inoculated in beef extract peptone medium at a 5% concentration and cultivated in a thermostatic shaker at 200 rpm, until the OD 600 value reached 1.0. The biochar was then combined bacterial culture at 1:3 (w/v) ratio and incubated for 8 hours before use [11]. The use of a 1:3 (w/v) ratio of biochar to bacterial isolates like *Pseudomonas putida* is often chosen to balance microbial inoculum with biochars surface area and nutrient retention properties. This proportion supports effective microbial colonization, boosts nutrient cycling, and enhances plant growth. This ratio tested in our study to assess its impact on soil quality and plant productivity under specific conditions.

Soil incubation experiment applications

In this study, soil samples were collected from the topsoil (0 – 25 cm) layer of the soil in the backyards of the Biology Department at Mustansiriyah University, Baghdad. The soil was air-dried and sieved to remove stones and organic debris. A 90 days incubation experiment was conducted at 20℃ in a thermostatic chamber. Soil samples were treated with either biochar or *Pseudomonas putida*-loaded biochar at 1% and 5% ratios (based on biochar dry weight in soil), labeled as B1, B5, BP1, and BP5, respectively. Soil moisture was kept at 40% of water-holding capacity during incubation. All treatments and controls (S, without amendment) were preformed out in triplicate, with subsequent analyses conducted on air-dried, sieved soil samples.

Cherry tomato cultivation experiments

This experiment took place in a glass greenhouse at Mustansiriyah University, School of Science/Biology department. Cherry tomatoes were chosen, and 90 days after planting, seedlings were placed in pots (1/5,00 Wagner pots) and grown. Biochar was added based on soil weight. The study involved a control group with no treatment (Control) and treatment groups with 1% biochar, 5% biochar, 1% biochar loaded with *Pseudomonas putida* and 5% biochar loaded with *Pseudomonas putida.* Three repeated experiments were conducted for each treatment group using the egg mass method, Cultivation followed the agricultural technology method of the Rural Development Administration, and inorganic fertilizer adhered to the Administration's standards.

Soil and biochar analysis

Soil parameter and properties encompass electrical conductivity (EC), pH, exchangeable cations (Ca^{2+}, K^{+} and Mg^{2+}), total carbon and nitrogen, available phosphoric acid, organic matter and carbon-to-nitrogen ratio. The pH and EC were assessed with a pH and EC meter (ORION versastar pro, Thermo, UK) using a 1:5 method of soil and distilled water method. Available phosphoric acid was determined through the Lancaster method, while total carbon and nitrogen were measured with an Elemental analyzer (Flash 1102 series EA, Thermo, UK).

Exchangeable cations were leached with 1N-ammonium acetate solution adjusted to pH 7.0 and then an inductively coupled plasma spectrometer (ICAP 7000 series ICP spectrometer, Thermo, UK). For biochar samples the MgO and K_2O were assessed using an inductively coupled plasma spectrometer (ICAP 7000 series ICP spectrometer, Thermo, UK). The total phosphorus (T-P) in biochar samples were assessed using acid digestion followed by colorimetric analysis, measuring the product color with spectrophotometer (TX-TP800 TESTRONIX-INDIA) [12]. Values were represented as means ± SD. All results were explained in Figures 2 and 3.

Data analysis

All experiments were performed in triplicate, and the results were reported as mean values with standard deviations. Statistical analyses were conducted using Graph Pad Prism 8 software. A one-way analysis of variance (ANOVA) was used to evaluate the effects of different amendment treatments on the measured parameters, with Bartlett's test applied to identify differences between treatment means at a significance level of $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Characterization of Pseudomonas putida loaded biochar

Various images of biochar products were captured at different scales using Scanning Electron Microscopy (SEM). The highest quality images, with a resolution of 100 nm, provided detailed insights into the biochar surface morphology, pore, structure, and other microstructural features. Additional images were captured at resolutions of 200 and 500 nm. These high-resolution images are crucial for understanding the properties and quality of the biochar, which directly impacts its effectiveness in applications like soil amendments, the focus of this study [13]. After pyrolysis, the size of the biochar particles decreased due to the breakdown of organic matter and the loss of volatile components [14].

SEM images of the biochar (Figure 4(A1)) reveal a coarse surface with porous structures, with particles shrinking from 33.95 nm before pyrolysis to 16.17 nm after pyrolysis (Figure 4(B1)). This reduction in size may enhance the attachment and growth of *Pseudomonas putida*. The fine structure of biochar allows *Pseudomonas putida* to adhered effectively, with cells either scattered or grouped on its surface; some cells may even penetrate the biochars pore structure due to their small size (< 2 μm). Microbial colonization of biochar is affected by both microbial physiological characteristics and the biochar properties. A study [11] found that Geobacter metallicreducens and Geobacter sulfurreducens could co-colonize the biochar surface within a short time frame (10 days). In our current research, we anticipate that *Pseudomonas putida* will efficiently colonize the biochar surface, showing rapid colonization within (10 h). SEM images are crucial for examining the microstructural changes in biochar that impact its effectiveness as a soil amendment. Figure 4 reveals essential features like porosity, surface area, and cracks or irregularities created during pyrolysis. These properties influence biochars capacity for water retention, nutrient adsorption, and providing microbial habitats. Monitoring how these structures change under various environmental conditions or treatments aids in understanding and optimizing biochars role in enhancing soil quality.

3.2 Variations in soil pH throughout the incubation period

The use of biochar and biochar loaded with *Pseudomonas putida* initially increased soil pH on day 6, especially with a 5% application rate, compared to untreated soil samples. Over time, all treatments showed a decreasing trend in soil pH. In previous researches [15, 16], they observed a similar pH fluctuation during incubation with biochar from crop residues, attributing the initial pH rise to the release of alkaline substances. The *Pseudomonas putida*-loaded biochar treatment, particularly BP1 and BP5, significantly lowered soil pH during the 90-day incubation period. In particular, the BP5 treatment significantly reduced the pH recording ($P < 0.0001$) as explained in Figure 5. This suggests that a high dose of *Pseudomonas putida*-loaded biochar effectively influences soil pH. While *Pseudomonas putida* may release organic acids causing pH reduction, the soil pH in this study remained within the natural-alkaline range after biochar amendments. Despite this, the pH-buffering ability of *Pseudomonas putida*-loaded biochar is an intriguing phenomenon, deserving further investigation regarding its impact on crop productivity and soil health. The addition of *Pseudomonas putida* to biochar enhances soil pH through synergistic biochemical processes. Biochar, with its alkaline nature, neutralize acidity and support microbial colonization. Meanwhile, *Pseudomonas putida* breaks down organic acids, solubilized phosphates, and generates alkaline compounds like ammonia during nitrogen cycling. these combined actions effectively reduce soil acidity and improve nutrient availability, promoting a balanced pH for optimal soil health [17].

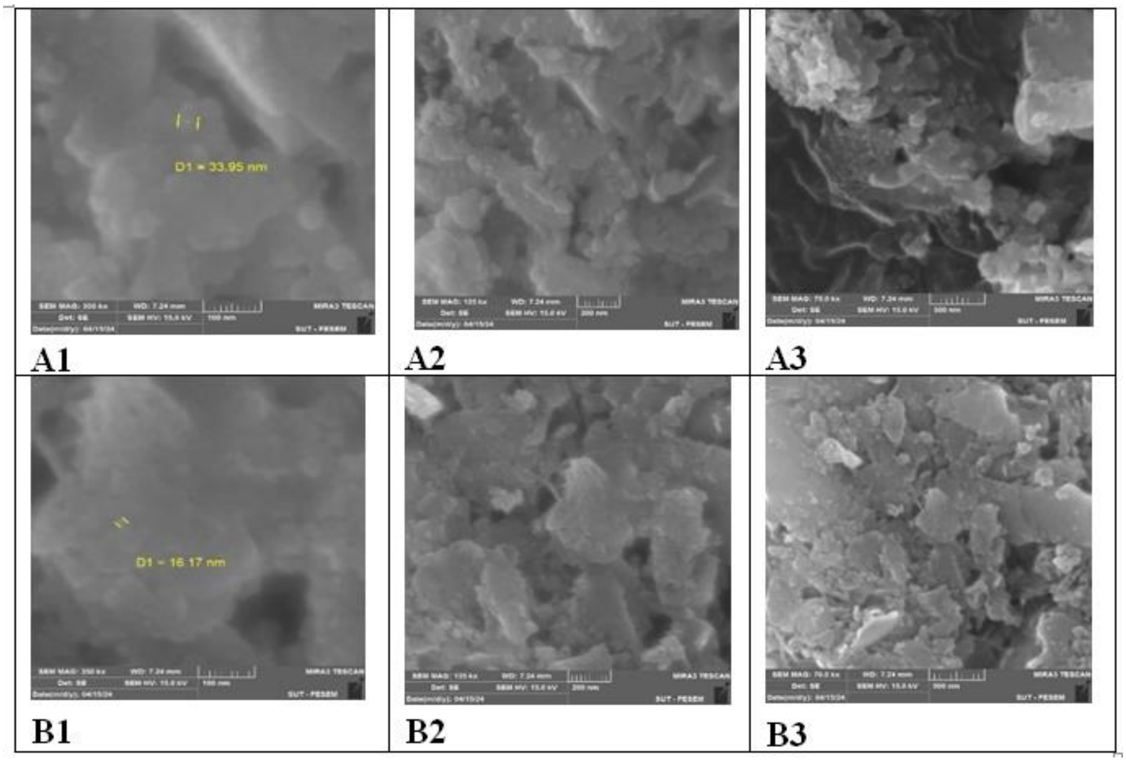

Figure 4. (A1) Biochar before pyrolysis at 100 nm, (A2) Biochar before pyrolysis at 200 nm, (A3) Biochar before pyrolysis at 500 nm, (B1) Biochar after pyrolysis at 100 nm, (B2) Biochar after pyrolysis at 200 nm, (B3) Biochar after pyrolysis at 500 nm

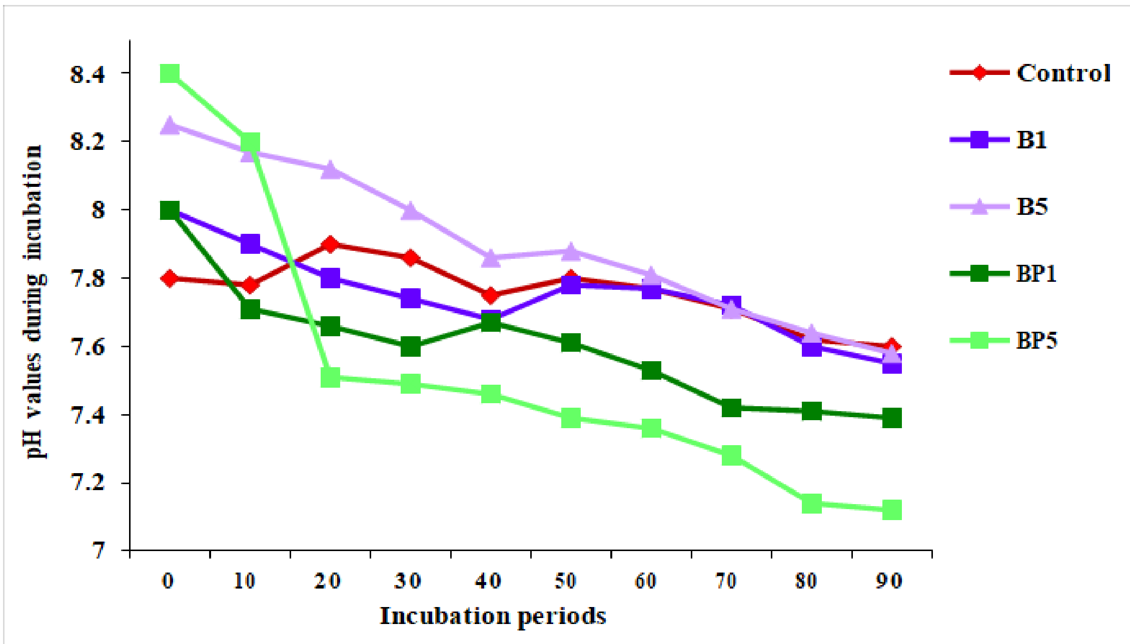

Figure 5. Measurements of pH for five soil samples (Control - without any amendments, B1-1% biochar, B5-5% biochar, BP1-1% biochar loaded with *Pseudomonas putida*, BP5-5% biochar loaded with *Pseudomonas putida*) during the 90-day incubation period

3.3 Cherry tomato growth and quality characteristics

The results of the cherry tomato growth are presented in Figure 6. Biochar treatment (B1, B5, BP1, and BP5) was found to positively impact cherry tomato crop growth compared to the control (soil without amendments). The tallest cherry tomato plants were observed in samples treated with 5% biochar loaded with *Pseudomonas putida* reaching a height of 147.4 cm, while the control samples had the lowest height at 58 cm. Regarding fresh weight and Chlorophyll, the highest values were recorded in samples treated with 5% biochar loaded with *Pseudomonas putida* measuring 179.9 g and 62.22 SPAD, respectively. Control samples (soil without amendments) had the lowest values for these measurements. In terms of fruit measurements, samples treated with 5% biochar loaded with *Pseudomonas putida* exhibited the highest number of cherry tomato fruits per plant (39.9) and total fruit weight (111.1g). However, fruit length and diameter (cm) were greatest in samples treated with 5% biochar alone, without *Pseudomonas putida*, suggesting a lesser effect of the loaded bacteria on these specific fruit dimensions. RM-One Way ANOVA showed $p < 0.0045$ for treated samples and control. The p value summary was significant recording **.

The increase of each of chlorophyll content, fruit number and weight stems from various factors. Initially, incorporating biochar improve soils capacity to retain water and nutrients, resulting in the improvement of soil quality and structure leads ultimately to fosters plant growth [18]. Moreover, Biochar loaded with beneficial bacteria like *Pseudomonas putida* aiding in plant growth and development. *Pseudomonas putida* assist in nitrogen and phosphorus metabolism cycle, improving nutrient absorption by plants [19]. In addition to that, these bacteria aids in the sequestration of heavy metals such as cadmium leading to decrease their harmful effects on plant and enhance soil quality [20].

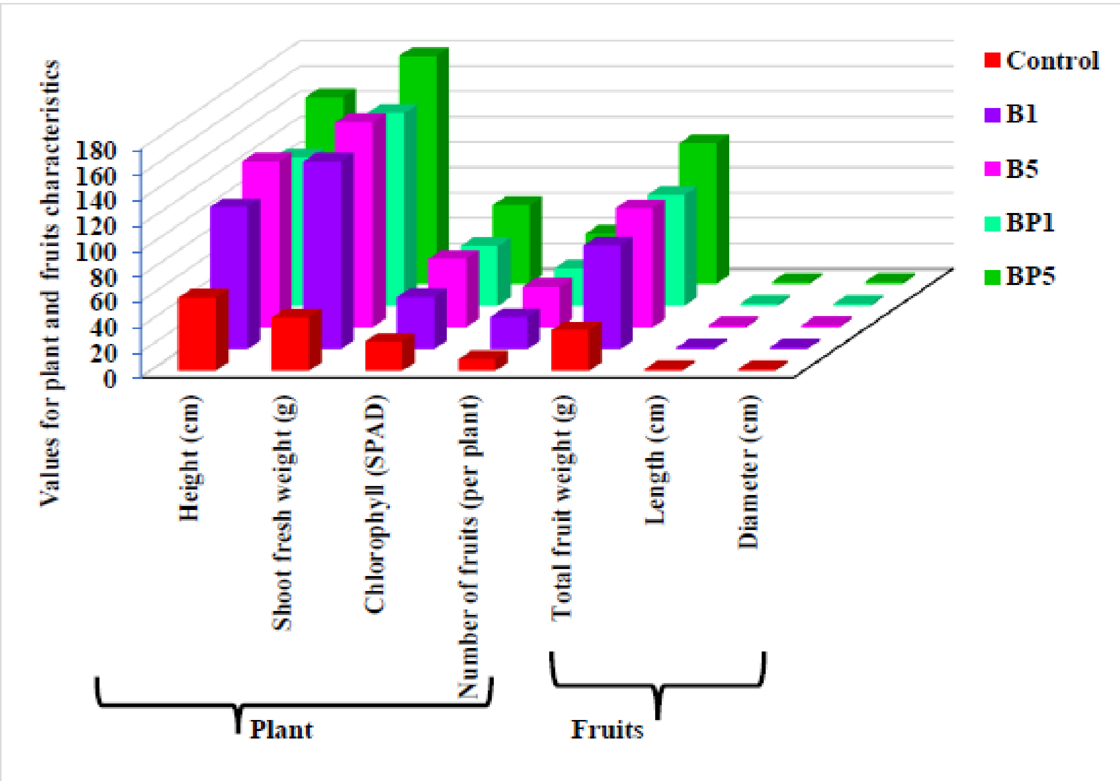

Figure 6. Growth characteristic of cherry tomatoes at end of experiment (Control – soil without any amendments, B1-1% biochar, B5-5% biochar, BP1-1% biochar loaded with *Pseudomonas putida*, BP5-5% biochar loaded with *Pseudomonas putida*)
One-way analysis of variance, ANOVA, Post Hoc Tests by GraphPad Prism 8.0.

3.4 Chemical properties of soil mixed with four types of biochar at end of 90-day

The characteristic of soil mixed biochar (B1, B5, BP1, BP5 and control) explained in Figure 7, including EC, pH, Ca, K, Mg, T-C, T-N, P_2O_5 and C/N ratios, can be affected by various factors and may differ based on specific conditions and experimental setups. In certain instance, adding biochar has been observed to raise pH levels and improve the availability of specific nutrients for example potassium and nitrogen. However, the biochar impact on the rest nutrients such as phosphorus may vary [21]. The carbon content of biochar is also subject to variation based on the source material. The influenced of biochar on these aspects is complicated and influenced by factors like biochar type and application rate, soil attributes, and the particular crop or plant being cultivated. Applying biochar at different rates, such as 1% and 5% to soil can produce diverse effects on soil chemical properties. It can alter the physical characteristics of soil, including particle-size distribution, electric conductivity, pH, and zeta potential [22]. The application of both biochar and modified biochar noted to significantly influence the availability of potassium, phosphorus, cation exchange capacity (CEC), electrical conductivity (EC), and nitrogen in soils contaminated with chromium (Cr) [23]. Furthermore, varying rates of biochar application can improve soil carbon stabilization over time and impact the activities of soil enzymes as well as the composition of bacterial communities [24].

The utilization of biochar enriched with *Pseudomonas putida* was demonstrated to enhance crop quality. Previous researches indicated that applying biochar can impact soil microbial health, resulting to increase soil organic matter and nutrient level as well, consequently influencing crop yield and quality. Moreover, the combined effects of biochar and the plant growth promoting bacteria *Pseudomonas putida* TSAU1 revealed to enhance wheat growth, nutrient absorption, and soil enzyme activities [25]. These findings suggest that utilizing biochar infused with *Pseudomonas putida* considered as advantageous for improving crop quality.

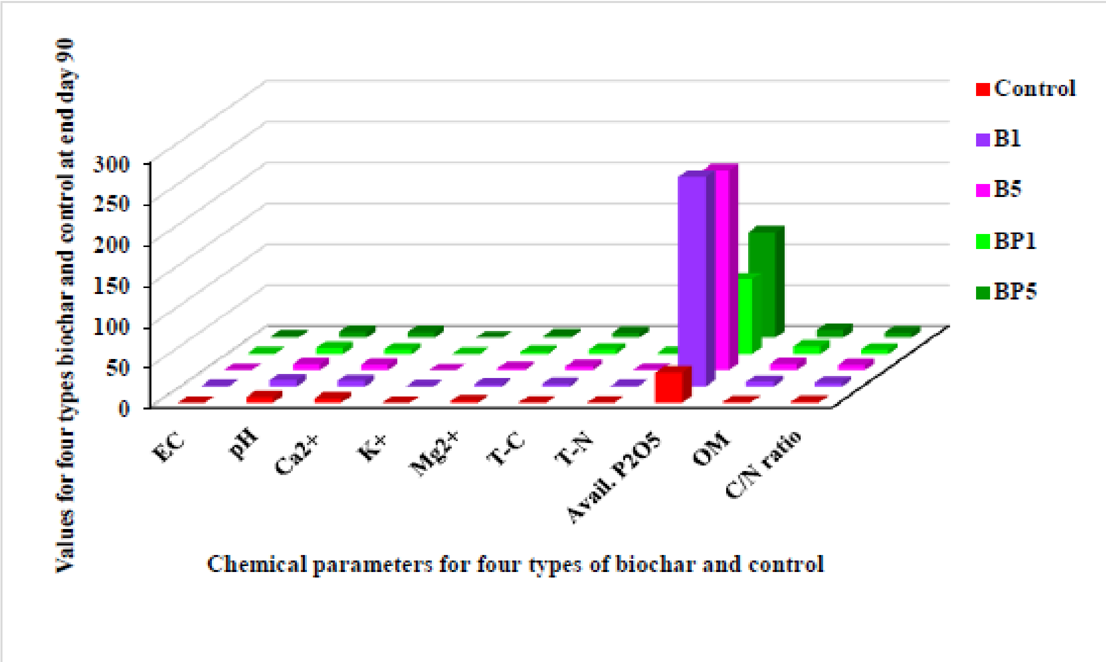

Figure 7. Chemical parameters of treated samples and control at the end of the 90-day

The introduction of biochar containing microorganisms can yield diverse effects on soil electrical conductivity (EC). In a study, the addition of biochar elevated EC in saline soils, particularly under high salinity conditions. Furthermore, biochar application resulted in increased soil pH and electrical conductivity across different moisture levels [26]. Hence, the influence of biochar loaded with microorganisms on soil EC may vary based on factors like soil salinity, presence of contaminants, and moisture content. Biochar was found to increase soil electrical conductivity (EC) when incorporated into soil, as indicated by study [27], approved that EC levels increased in soils treated with biochar or biochar combined with *Pseudomonas putida* compared to untreated soils. The highest EC, recorded at 1.51 dSm^{-1}, was observed in soil treated with 5% biochar loaded with *Pseudomonas putida*. This highlights the significant potential of incorporating 5% biochar with *Pseudomonas putida* to enhance soil EC.

Range varying depending on the crops salt tolerance. Effective soil management practices, such as appropriate irrigation and drainage, are crucial for regulating soil EC and mitigating the adverse effects of high salt levels on crop cultivation.

In general, cherry tomatoes thrive within a soil EC range of 1.5 to 2.5 dSm^{-1}. Notably, cherry tomatoes exhibit lower salt tolerance compared to specific types of crops, underscoring the importance of maintaining a balanced and suitable EC level to ensure their optimal growth and yield. The utilization of saline water can diminish crop quality and yield. Salinity adverted effects on the morphology and yield parameters of tomatoes, with yield decreasing as salinity levels rise [28]. Various salinity levels in irrigation water negatively impact chlorophyll content and biomass accumulation in cherry tomatoes [29]. Nevertheless, cherry tomatoes can be grown in nutrient solutions made with salt- containing groundwater as evidenced in Malta, resulting in reduced yield but improved fruit quality [30]. Overall, maintaining an EC range of 1.5 to 2.5 dSm^{-1} is crucial for the optimal growth and yield of cherry tomatoes, aligning with our findings.

Each of calcium (Ca^{+2}), potassium (K^{+}), and magnesium (Mg^{+2}) are vital for plant growth. The concentration of each pervious elements in 5% biochar loaded with *Pseudomonas putida* (BP5) was highest with values (6.9, 0.18 and 2.8) subsequently in compare to other biochar treatment group and control, suggesting that *Pseudomonas putida* might influence soil nutrient cycling, potentially by competing for nutrients. The elevated levels of these elements can be ascribed to the influence of biochar on retaining nutrients and enhancing soil fertility. Biochar has the capacity to immobilize calcium (Ca^{+2}), potassium (K^{+}), and magnesium (Mg^{+2}) in the soil, creating an optimal environment for soil organisms and promoting plant growth. Furthermore, adding biochar to the soil can enhance its ability to retain water and nutrients, thereby increasing nutrient availability for plants. The presence of *Pseudomonas putida* within the biochar can also aid in nutrient cycling and availability contributing to higher concentrations of calcium (Ca^{+2}), potassium (K^{+}), and magnesium (Mg^{+2}) in the soil which is matching with the current study results [31].

The EC, total carbon, total nitrogen, organic matter content and C/N ratios increased in treatments containing biochar loaded with 5% *Pseudomonas putida* (BP5) compared to other treatment biochar group and control recording values (1.51, 5.9, 1.1, 9.4 and 6.37) subsequently at end of day 90. This increase suggested because of synergistic effect of biochar loaded with bacteria. This synergistic effect found to contribute the increase concentration of EC, T-C, T-N, OM and C/N ratios in treated soils. Biochar loaded with bacteria enhance the abundance of soil microbial community involved in carbon and nitrogen metabolism. These findings highlight the potential of biochar and bacteria synergism in promoting soil health and nutrient availability in agricultural systems [32, 33].

The pH level was the lowest values at end of day 90 for BP5 with value 7.11 in compare with other biochar treatment group and this is due to various factors like the production of organic acids by loaded bacteria with the biochar resulting in soil acidification. Moreover, the degradation of organic matter within the biochar releases acidic compounds into the soil, further lowering the pH levels. Additionally, the inherent acidity of biochar has influenced by the source of biochar itself. In the current study, source of produced biochar was domestic activated sludge at the Babel wastewater treatment plant based in Iraq. Overall, the convergence of these factors contributes to the observed pH reduction in soil treated with bacteria loaded biochar. Biochar loaded with 5% *Pseudomonas putida* recorded a high P_2O_5 value of 128.6, which was higher than those of BP1 and the control, but lower than those of B5 and B1, which recorded values of 246.1 and 244, respectively.

The decreased level of phosphorus for biochar loaded with bacteria due to the ability of the loaded bacteria to immobilize phosphorus, making it less available for plant uptake in the same time the loaded bacteria enhance phosphorus cycling and transformation processes resulting to raise retention of phosphorus in forms that are not accessible to plants. Additionally, the incorporation of biochar into the soil can alter soil properties and microbial communities in ways that affect phosphorus availability and mobility. RM-One Way ANOVA showed $p < 0.0034$ for treated samples and control. The p value summary was significant recording *.

4. CONCLUSION

In this study, results revealed the impacts of various treatments involving biochar (1% and 5%) and biochar loaded with *Pseudomonas putida*, sourced from the same activated sludge used to produce the biochar, alongside untreated soil, on the growth of cherry tomatoes and soil characteristics. The experiments were primarily conducted in a glass greenhouse at the College of Science and Biology Department of Mustansiriyah University. Results from assessments of cherry tomato growth parameters, including plant height, above-ground byproduct weight, chlorophyll content, fruit number, and weight, indicated superior growth in the group treated with 5% biochar loaded with *Pseudomonas putida* compared to other biochar treatments and the control group. Analysis revealed an increase in chlorophyll content, fruit yield number and weight in the PB5 added treatment group compared to other biochar treatments and the control. Moreover, soil pH, electrical conductivity (EC), available phosphorus levels, total carbon and nitrogen content, and organic matter content tended to rise in biochar loaded with *Pseudomonas putida* PB5. Based on these findings, the 5% biochar loaded with *Pseudomonas putida* treatment demonstrated positive effects on cherry tomato growth and induced changes in soil chemistry, suggesting its potential application as a soil conditioner in small-scale agricultural settings. Additionally, the 5% biochar loaded with *Pseudomonas putida* appeared to enhance cherry tomato growth. The local strain *Pseudomonas putida* functions as a direct biocontrol agent. Its presence in the rhizosphere initiates a systemic response in the plant, protecting the host from the pathogen's infection and proliferation. The results indicated that, when compared to using biochar alone, applying biochar inoculated with *Pseudomonas putida* at a 5% rate notably increased the soil pH during the initial incubation period. Subsequently, there was a slight decrease, bringing the pH to a neutral to alkaline range throughout the reaction. The treatment resulted in increases of 14% in chlorophyll content, 6% in fruit yield, 19% in total fruit number, 25% in plant weight, 1% in fruit length and 34% in plant height, along with 1% increases in plant diameter compared to the control. At the same time both chemical parameters of treated samples and control resulted in increases of 4% in EC, 11% in pH, 9% in Ca, 3% in Mg, 1% for each T-C and T-N, 67% in available P_2O_5, 2% in OM, and 3% in C/N. The combination of *Pseudomonas putida* with biochar offers significant scalability for boosting plant growth across various agriculture systems. Scaling efforts can involve producing biochar from local biomass to minimize costs and environmental impacts. Efficient application methods, such as incorporating *Pseudomonas putida* into irrigation systems or as seed coating, can improve adaptability for divers' crops by addressing specific soil and nutrient needs. Economically, it reduces reliance on chemical fertilizers, while environmentally, it promotes carbon sequestration and lowers greenhouse gas emission, supporting sustainable agriculture. In summary, this study demonstrates that biochar loaded 5% with *Pseudomonas putida* significantlyenhances plant growth and vitality.

ACKNOWLEDGMENT

The authors would like to sincerely thank Mustansiriyah University, College of Science, for their invaluable support and resources that made this research possible. We extend our deepest appreciation to the technical laboratory staff, whose expertise, dedication, and huge assistance in the laboratory were instrumental in the successful completion of this study. Their technical support and guidance greatly contributed to the quality and rigor of this work.

REFERENCES

[1] Leng, L., Liu, R., Xu, S., Mohamed, B. A., Yang, Z., Hu, Y., Chen, J., Zhao, S., Wu, Z., Peng, H., Li, H. (2022). An overview of sulfur-functional groups in biochar from pyrolysis of biomass. Journal of Environmental Chemical Engineering, 10(2): 107185. https://doi.org/10.1016/j.jece.2022.107185

[2] Nadda, A. K. (2023). Biochar and Its Composites: Fundamentals and Applications. Springer Nature.

[3] Alkharabsheh, H.M., Seleiman, M.F., Battaglia, M.L., Shami, A., Jalal, R.S., Alhammad, B.A., Almutairi, K.F., Al-Saif, A.M. (2021). Biochar and its broad impacts in soil quality and fertility, nutrient leaching, and crop productivity: A review. Agronomy, 11(5): 993. https://doi.org/10.3390/agronomy11050993

[4] Nguyen, C.T., Nguyen, T.H.H., Tungtakanpoung, D.,

Tran, C.S., Vo, T.K.Q., Kaewlom, P. (2023). Paraquat removal by free and immobilized cells of Pseudomonas putida on corn cob biochar. Case Studies in Chemical and Environmental Engineering, 8: 100376.https://doi.org/10.1016/j.cscee.2023.100376

[5] Pan, S.Y., Dong, C.D., Su, J.F., Wang, P.Y., Chen, C.W., Chang, J.S., Kim, H., Huang, C.P., Hung, C.M. (2021). The role of biochar in regulating the carbon, phosphorus, and nitrogen cycles exemplified by soil systems. Sustainability, 13(10): 5612. https://doi.org/10.3390/su13105612

[6] Lu, J., Liu, Y., Zhang, R., Hu, Z., Xue, K., Dong, B. (2024). Biochar inoculated with *Pseudomonas putida*alleviates its inhibitory effect on biodegradation pathways in phenanthrene-contaminated soil. Journal of Hazardous Materials, 461: 132550. https://doi.org/10.1016/j.jhazmat.2023.132550

[7] Agrafioti, E., Bouras, G., Kalderis, D., Diamadopoulos, E. (2013). Biochar production by sewage sludge pyrolysis. Journal of Analytical and Applied Pyrolysis, 101: 72-78. https://doi.org/10.1016/j.jaap.2013.02.010

[8] Liu, Q., Liu, B., Zhang, Y., Lin, Z., Zhu, T., Sun, R., Wang, X., Ma, J., Bei, Q., Liu, G., Lin, X. (2017). Can biochar alleviate soil compaction stress on wheat growth and mitigate soil N_2O emissions? Soil Biology and Biochemistry, 104: 8-17. https://doi.org/10.1016/j.soilbio.2016.10.006

[9] Nathan, P., Rathinam, X., Kasi, M., Rahman, Z.A., Subramaniam, S. (2011). A pilot study on the isolation and biochemical characterization of Pseudomonas from a chemical-intensive rice ecosystem. African Journal of Biotechnology, 10(59): 12653-12656. https://doi.org/10.5897/AJB11.1638

[10] Aumeran, C., Paillard, C., Robin, F., Kanold, J., Baud, O., Bonnet, R., Souweine, B., Traore, O. (2007). *Pseudomonas aeruginosa* and *Pseudomonas putida* outbreak associated with contaminated water outlets in an oncohaematologypaediatric unit. Journal of Hospital Infection, 65(1): 47-53. https://doi.org/10.1016/j.jhin.2006.09.008

[11] Tu, C., Wei, J., Guan, F., Liu, Y., Sun, Y., Luo, Y. (2020). Biochar and bacteria-inoculated biochar enhanced Cd and Cu immobilization and enzymatic activity in a polluted soil. Environment International, 137: 105576. https://doi.org/10.1016/j.envint.2020.105576

[12] Wei, M., Liu, X., He, Y., Xu, X., Wu, Z., Yu, K., Zheng, X. (2020). Biochar inoculated with *Pseudomonas putida* improves grape (*Vitis vinifera* L.) fruit quality and alters bacterial diversity. Rhizosphere, 16: 100261. https://doi.org/10.1016/j.rhisph.2020.100261

[13] Adhikari, S., Mahmud, M.A.P., Nguyen, M.D., Timms, W. (2023). Evaluating fundamental biochar properties in relation to water holding capacity. Chemosphere, 328: 138620. https://doi.org/10.1016/j.chemosphere.2023.138620

[14] Nguyen, M.K., Lin, C., Hoang, H.G., Sanderson, P., Dang, B.T., Bui, X.T., Nguyen, N.S.H., Vo, D.V.N., Tran, H.T. (2022). Evaluate the role of biochar during the organic waste composting process: A critical review. Chemosphere, 299: 134488. https://doi.org/10.1016/j.chemosphere.2022.134488

[15] Tomczyk, A., Sokołowska, Z., Boguta, P. (2020). Biochar physicochemical properties: Pyrolysis temperature and feedstock kind effects. Reviews in Environmental Science and Bio/Technology, 19(1): 191-215. https://doi.org/10.1007/s11157-020-09523-3

[16] Grzyb, A., Wolna-Maruwka, A., Niewiadomska, A. (2020). Environmental factors affecting the mineralization of crop residues. Agronomy, 10(12): 1951. https://doi.org/10.3390/agronomy10121951

[17] Zhang, S., Zhu, Q., de Vries, W., Ros, G.H., Chen, X., Muneer, M.A., Zhang, F., Wu, L. (2023). Effects of soil amendments on soil acidity and crop yields in acidic soils: A worldwide meta-analysis. Journal of Environmental Management, 345: 118531. https://doi.org/10.1016/j.jenvman.2023.118531

[18] Saleem, I., Riaz, M., Mahmood, R., Rasul, F., Arif, M., Batool, A., Akmal, M.H., Azeem, F., Sajjad, S. (2022). Biochar and microbes for sustainable soil quality management. In: Microbiome Under Changing Climate, pp. 289-311. https://doi.org/10.1016/B978-0-323-90571-8.00013-4

[19] Rawat, P., Das, S., Shankhdhar, D., Shankhdhar, S.C. (2021). Phosphate-solubilizing microorganisms: Mechanism and their role in phosphate solubilization and uptake. Journal of Soil Science and Plant Nutrition, 21(1): 49-68. https://doi.org/10.1007/s42729-020-00377-3

[20] Sharma, P. (2021). Efficiency of bacteria and bacterial-assisted phytoremediation of heavy metals: An update. Bioresource Technology, 328: 124835. https://doi.org/10.1016/j.biortech.2021.124835

[21] Li, H., Li, Y., Xu, Y., Lu, X. (2020). Biochar phosphorus fertilizer effects on soil phosphorus availability. Chemosphere, 244: 125471.https://doi.org/10.1016/j.chemosphere.2019.125471

[22] Ginebra, M., Muñoz, C., Calvelo-Pereira, R., Doussoulin, M., Zagal, E. (2022). Biochar impacts on soil chemical properties, greenhouse gas emissions, and forage productivity: A field experiment. Science of the Total Environment, 806: 150465. https://doi.org/10.1016/j.scitotenv.2021.150465

[23] Siddika, A., Islam, M.M., Parveen, Z., Hossain, F.M. (2023). Remediation of chromium (VI) from contaminated agricultural soil using modified biochars. Environmental Management, 71(4): 809-820. https://doi.org/10.1007/s00267-022-01731-7

[24] Moreno, J.L., Bastida, F., Díaz-López, M., Li, Y., Zhou, Y., López-Mondéjar, R., Benavente-Ferraces, I., Rojas, R., Rey, A., García-Gil, J.C., Plaza, C. (2022). Response of soil chemical properties, enzyme activities and microbial communities to biochar application and climate change in a Mediterranean agroecosystem. Geoderma, 407: 115536. https://doi.org/10.1016/j.geoderma.2021.115536

[25] Egamberdieva, D., Alaylar, B., Alimov, J., Jabbarov, Z., Kimura, S.B. (2023). Combined effects of biochar and plant growth-promoting bacteria Pseudomonas putida TSAU1 on plant growth, nutrient uptake of wheat, and soil enzyme activities. Turkish Journal of Agriculture and Forestry, 47(3): 357-363. https://doi.org/10.55730/1300-011X.3092

[26] Huang, J., Zhu, C., Kong, Y., Cao, X., Zhu, L., Zhang, Y., Ning, Y., Tian, W., Zhang, H., Yu, Y., Zhang, J. (2022). Biochar application alleviated rice salt stress via modifying soil properties and regulating soil bacterial abundance and community structure. Agronomy, 12(2): 409. https://doi.org/10.3390/agronomy12020409

[27] Jabborova, D., Wirth, S., Kannepalli, A., Narimanov, A., Desouky, S., Davranov, K., Sayyed, R.Z., El Enshasy, H., Malek, R.A., Syed, A., Bahkali, A.H. (2020). Co-inoculation of rhizobacteria and biochar application improves growth and nutrients in soybean and enriches soil nutrients and enzymes. Agronomy, 10(8): 1142. https://doi.org/10.3390/agronomy10081142
[28] Murugappan, V., Latha, M.R., Jagadeeswaran, R., Bhaskaran, A. (2007). Balanced fertiliseruse for sustaining soil fertility and maximizing crop yield–A review. Agricultural Reviews, 28(4): 254-261.
[29] El-Mogy, M.M., Garchery, C., Stevens, R. (2018). Irrigation with salt water affects growth, yield, fruit quality, storability, and marker-gene expression in cherry tomato. Acta Agriculturae Scandinavica, Section B—Soil & Plant Science, 68(8): 727-737. https://doi.org/10.1080/09064710.2018.1473482
[30] Agius, C., von Tucher, S., Rozhon, W. (2022). The effect of salinity on fruit quality and yield of cherry tomatoes. Horticulturae, 8(1): 59. https://doi.org/10.3390/horticulturae8010059
[31] Ghassemi-Golezani, K., Abdoli, S. (2023). Alleviation of salt stress in rapeseed (*Brassica napus* L.) plants by biochar-based rhizobacteria: New insights into the mechanisms regulating nutrient uptake, antioxidant activity, root growth, and productivity. Archives of Agronomy and Soil Science, 69(9): 1548-1565. https://doi.org/10.1080/03650340.2023.2201577
[32] Tsai, C.C., Chang, Y.F. (2020). Effects of biochar on excessive compost-fertilized soils on the nutrient status. Agronomy, 10(5): 683. https://doi.org/10.3390/agronomy10050683
[33] Yin, X., Peñuelas, J., Xu, X., Sardans, J., Fang, Y., Wiesmeier, M., Chen, Y., Chen, X., Wang, W. (2021). Effects of addition of nitrogen-enriched biochar on bacteria and fungi community structure and C, N, P, and Fe stoichiometry in subtropical paddy soils. European Journal of Soil Biology, 106: 103351. https://doi.org/10.1016/j.ejsobi.2021.103351