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The Impact of HTL-Derived Biochar on Seedling Growth of Lettuce (*Lactuca sativa*)

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Abstract

Biochar, also known as hydrochar, is a solid residue resulting from organic matter being combusted in low or no-oxygen environments, such as during the process of pyrolysis, gasification, or hydrothermal liquefaction (HTL). Biochar resulting from the HTL conversion process is considered a waste product and often an environmental liability, though there have been some studies on the potential uses for HTL biochar, such as a potential soil amendment. For this study, the biochar generated via the HTL conversion of various food waste feedstocks (*i.e.*, food waste produced from beer-making and coffee brewing) was utilized to evaluate the effects of biochar as a soil amendment on plant growth. A series of seedling plant growth studies utilizing controlled environmental conditions were conducted with the addition of 0.0%, 2.5%, 5.0%, and 7.5% (m/m) biochar added to the soil medium. After three weeks, seedling plants were measured to have an overall wet weight of 0.65 to 0.73 g/seedling, a dry weight of 0.05 to 0.06 g/seedling, and a total leaf area of 10.6 to 12.4 cm/seedling. The seedlings grown in the soil mixture without the addition of biochar (*i.e.*, the control) yielded the highest total leaf area, and the seedlings grown in the soil mixture with the addition of 2.5% (m/m) biochar yielded the second highest total leaf area. Overall, no statistically significant impact on seedling structure in soil containing between 2.5% and 7.5% (m/m) biochar was found as compared to seedling growth in soil without the addition of biochar. There is some evidence for increased biochar content reducing leaf area; however, further studies are required. Additionally, it is possible that biochar had some impact on seedling growth or structure, but the nutrients contained in the watering solution used in this study may have obscured its effects. Nevertheless, the results of this study are instrumental in evaluating a potential use of an HTL by-product (*i.e.*, biochar) that is often considered an environmental liability.

Keywords

Hydrothermal Liquefaction, Biochar, Seedling Study, *Lactuca sativa*

1. Introduction

Biochar is the solid residue resulting from organic matter being combusted in low or no-oxygen environments, such as pyrolysis, gasification, hydrothermal carbonization (HTC), hydrothermal gasification (HTG), or hydrothermal liquefaction (HTL). Biochar resulting from HTC, HTG, and HTL is also called hydrochar, as it is mixed with the aqueous co-product (ACP), also resulting from the reaction, and needs to be separated and dried before being used or discarded [1]. Though exact properties (e.g., porosity, chemical composition, etc.) can vary due to feedstock, reaction type, and reaction conditions, biochar can usually be classified as having a high carbon content and large surface area [2] [3]. Carbon content of biochar ranges between 50% and 85% for lignocellulosic feedstocks and 15% and 25% for algal feedstocks and animal waste [4] [5]. Literature shows that carbon content typically increases with reaction temperature [4]-[6].

Biochar resulting from HTL is considered a waste product and an environmental liability, though there have been some studies on potential uses for HTL biochar. Research thus far has focused on investigating biochar for its potential as an adsorbent in wastewater treatment or heavy metal removal [7]-[10], as a catalyst or catalyst support for future HTL reactions [11] [12], or for use in supercapacitors [13].

Biochar resulting from pyrolysis is also considered a waste product; however, there have been significantly more studies on the potential uses of this product. Research has investigated biochar as a composting additive [14]-[16], as a pharmaceutical and heavy metal sorbent in wastewater treatment [17]-[19], as a catalyst in biodiesel production [20] [21], and as an electrode material in supercapacitors and fuel cells [22] [23].

Extensive studies have been conducted on biochar as a potential soil amendment, investigating the potential of biochar to increase the ability of soil to store carbon [24] [25], soil remediation via the adsorption of heavy metals and other pollutants [26] [27], and promoting microbiological activity [28]. Additionally, biochar as a soil amendment has been shown to have some positive impacts on plants, namely decreasing the impacts of drought and salt stress on plants [29]-[31]. Though the literature shows several promising uses for biochar, there are some environmental concerns regarding the usage of biochar, such as biochar used in water treatment leaching polycyclic aromatic hydrocarbons (PAH) or carbon, and biochar produced from wastewater sludge containing heavy metals [5] [32] [33]. Future research is needed into beneficial uses of biochar produced as a by-product of HTL and other thermochemical processes.

This research aims to evaluate the effects of biochar produced via HTL conversion of various food waste feedstocks as a soil additive or amendment. This research includes elemental characterization of HTL-produced biochar and analysis of the effects of the addition of biochar into soil on seedling growth. This research will be instrumental in evaluating a potential use of an HTL by-product that is currently considered a waste product and an environmental liability.

2. Methods and Materials

The presumption of this research is that the utilization of biochar resulting from HTL processing of various food waste feedstocks as a soil additive or amendment would benefit the growth of seedlings used to produce food crops. Biochar was obtained from prior research by Nesheim *et al.*, 2024 [34], which performed HTL of single food waste feedstocks and co-HTL of combined waste feedstocks at 300˚C and 1500 PSI for 30 minutes. The biochar was then analyzed for elemental composition and combined due to limited quantities. In this research, experimental laboratory approaches were used to monitor and evaluate the growth of seedlings in a controlled growth environment. The environmental parameters and experimental procedures of this study were optimized based on available literature and experimental findings. The growth and structural characteristics of the seedlings were used to determine optimal biochar concentration for beneficial soil amendment.

2.1. Experimental Setup

The seedlings grown in this study were grown in a thermally insulated growth tent, equipped with four LED light fixtures and environmental controls that monitored humidity, temperature, and carbon dioxide (CO_2). Three large plastic trays, each containing four biodegradable seed starter trays, were placed approximately 22 inches below the lights, which provided light for 16 hours per day for a total growth period of 3 weeks.

Four mixtures were created with different ratios of HTL processing biochar, consisting of a control tray with no biochar added to the soil mix, then mixtures at 2.5%, 5.0%, and 7.5% biochar (m/m) added to the soil mix in order to detect potential dose-response effects of the addition of the biochar to the soil mix. Previous studies utilizing lettuce and other leafy greens applied rates between 1% and 10% biochar by mass, as these concentrations were shown to allow measurable differences in seedling performance without overwhelming the physical properties of the potting medium [35] [36]. The basis of these mixes was a seed starter mix (Jiffy Natural & Organic Seed Starting Soil Mix, Ohio, USA), which consists of peat moss, vermiculite, and lime. The biochar was obtained through the experimental HTL protocols, then dried in an oven at 105˚C for a period of 24 hours. Each sample was analyzed for elemental composition of percent carbon (C), hydrogen (H), and nitrogen (N) using a CHN Soil Analyzer. All biochar samples were then combined and crushed to prevent large clumps for use in this research.

Nutrient water was created using 3.6 g of "Jack's Nutrients 15-0-0 Calcium Nitrate Part B" and 3.8 g of "Jack's Nutrients 5-12-26 Part A" in 10 L of water. 15-0-0 Part B is composed of calcium nitrate [$Ca(NO_3)_2$] and has a nutrient content of 15% total nitrogen (N) and 18% calcium (Ca). 5-12-26 Part A is composed of potassium nitrate, magnesium sulfate, monopotassium phosphate, iron DTPA, iron EDTA, iron EDDHA, copper EDTA, manganese EDTA, zinc EDTA, boric acid, and ammonium molybdate and has a nutrient content of 5% total nitrogen (N), 12% available phosphate (P_2O_5), 26% soluble potash (K_2O), 6.3% available magnesium (Mg), 8.5% available sulfur (S), 0.05% boron (B), 0.015% copper (Cu), 0.3% iron (Fe), 0.05% manganese (Mn), 0.019% molybdenum (Mo), and 0.015% zinc (Zn).

2.2. Experimental Procedure

For this study, biodegradable seed trays were filled with the soil mixtures described in above and then pre-wetted with 15 mL of the nutrient water. Three trays were filled with each of the four soil mixtures, for a total of twelve trays. Two pelleted seeds of butterhead lettuce (Johnny's Selected Seeds, Maine, USA) were planted ¼ inch down into every spot in the biodegradable seed starter trays to prevent gaps in the trays. In the case of both seeds sprouting, the smaller seedling was removed 1 week after planting. Each spot in the seed starter trays received between 6 and 3.5 mL of nutrient water daily. If any white mold was seen on the soil or seed trays, it was carefully removed with a metal scoopula and discarded.

Seedling studies were continued for a period of 3 weeks. After the duration of the 3-week period, the total leaf area of each plant was measured using the Leafscan app on a mobile device. Three seedling plants from each tray (for a total of 36 plants) were selected, removed from the soil, and had any remaining soil carefully removed from their roots or leaves. The selected seedling plants were weighed, dried in a 50°C oven for a 24-hour period, then weighed again in order to calculate wet and dry weights of the seedling plants. Each study was completed in triplicate to ensure reproducibility.

3. Results and Discussion

This research aimed to experimentally evaluate the effects of utilizing post-HTL processing biochar as a soil additive for food crop growth. This was achieved through experimentally conducting seedling growth studies under a controlled growth environment and assessing the impact of biochar addition on overall seedling plant growth and productivity.

3.1. Post-HTL Processing Biochar Analysis

While there is no existing available literature for the biochar and biochar mixes used in this research, biochar standards do exist. By standards set by the International Biochar Initiative (IBI), all biochar used in this research is Class 1, as it has over 60% carbon by weight (as seen in **Table 1**) [37]. By European Biochar Federation (EBF) standards, the samples have the appropriate carbon content (≥50% [wt%]), but cannot be fully classed as biochar due to the carbon analysis including all carbon, not just organic carbon, as EBF guidelines state that biochar should have a ratio of hydrogen and organic carbon less than 0.7 mol/mol [38]. Additionally, as the analysis did not include oxygen content, the samples are unable to conform to the EBF's standard of a ratio of oxygen and organic carbon being less than 0.4 mol/mol. Elemental analysis was conducted on the biochar produced from the HTL processing of single food waste feedstocks and co-HTL processing of combined food waste feedstocks from Nesheim *et al.*, 2024 [34]. Analysis of the biochar was completed for percent carbon, hydrogen, and nitrogen, as seen in **Table 1**.

Table 1. Elemental analysis of post-HTL biochar for combined food waste feedstocks and three mixed biochar samples, expressed as percent weight (wt%).

Post-HTL Biochar Sample	Carbon (wt%)	Hydrogen (wt%)	Nitrogen (wt%)
Raspberry Puree/Spent Tea Leaves	69.48	6.01	4.11
Raspberry Puree/Spent Grains	72.88	5.96	4.10
Raspberry Puree/Spent Coffee Grounds	73.29	6.17	3.14
Spent Yeast/Spent Tea Leaves	70.03	6.36	4.85
Spent Yeast/Spent Grains	71.03	6.37	5.55
Spent Yeast/Spent Coffee Grounds	72.11	6.84	5.27
Spent Hops/Spent Tea Leaves	69.67	6.17	4.11
Spent Hops/Spent Grains	69.73	6.06	4.42
Spent Hops/Spent Coffee Grounds	73.24	6.58	4.01
Single Feedstock Mix	69.00	5.99	4.44
Combined Feedstock Mix	71.11	6.00	4.26
Total Mix	**72.39**	**6.34**	**4.06**

While it is impossible to speculate on the organic carbon content of any of the biochar or biochar mixes produced from and used in this study, the high carbon content, when paired with low hydrogen and oxygen content, is a strong indicator of carbon stability [5] [33] [39]. As biochar is primarily composed of carbon, carbon stability determines the stability of the overall biochar structure, and thus, low carbon stability can cause unwanted or early degradation of the biochar structure, potentially releasing PAHs or carbon into the environment [32] [33].

3.2. Seedling Plant Growth Analysis

Seedling plant growth studies were conducted with the addition of post-HTL biochar as a soil additive. Grown under controlled environmental conditions, seedling studies utilized the addition of biochar for each study at a percentage added to a seedling soil mixture of 0.0%, 2.5%, 5.0%, and 7.5% (m/m). Seedling studies were carried out over a 3-week growth period. **Figure 1** and **Figure 2** show the progression of seedling plant growth from day 1 to day 21 during the seedling growth studies.

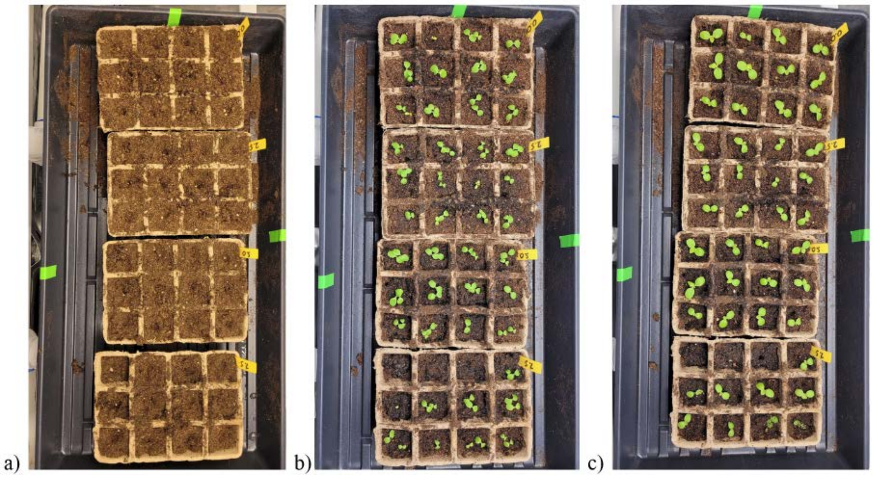

Figure 1. Experimental setup of seedling plant growth studies grown in a controlled environment, settling at (a) day 1, (b) day 7, and (c) day 10. Biochar content equals 0.0, 2.5, 5.0, and 7.5% (m/m), respectively, from top to bottom in each image.

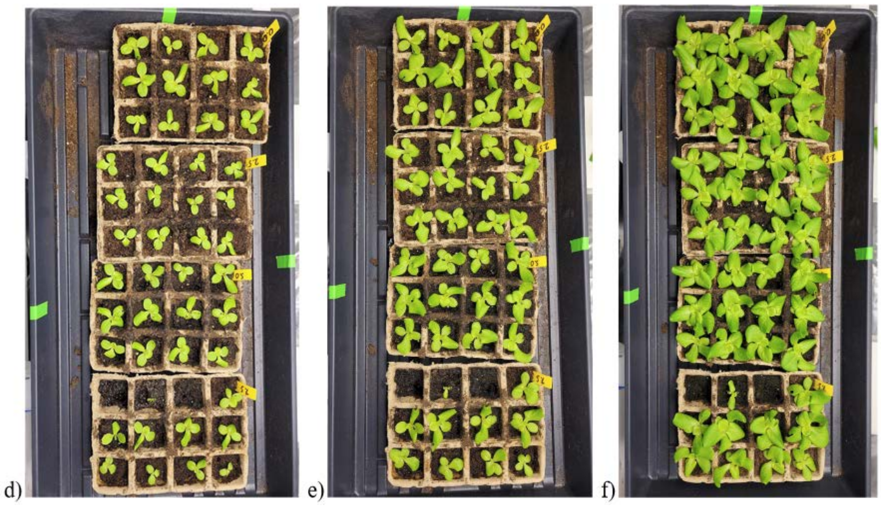

Figure 2. Experimental setup of seedling plant growth studies grown in a controlled environment, settling at (d) day 14, (e) day 17, and (f) day 21. Biochar content equals 0.0, 2.5, 5.0, and 7.5% (m/m), respectively, from top to bottom in each image.

After the duration of the 3-week seedling plant growth studies, wet weight, dry weight, and total leaf area were measured for each of the biochar concentration experiments. Seedling plant growth results are presented in **Table 2**. Overall, seedling plants were measured to have an overall wet weight of 0.65 to 0.73 g/seedling, a dry weight of 0.05 to 0.06 g/seedling, and a total leaf area of 10.6 to 12.4 cm/seedling. The seedlings grown in the soil mixture without the addition of biochar yielded the highest total leaf area, and the seedlings grown in the soil mixture with the addition of 2.5% (m/m) biochar yielded the second highest total leaf area. However, the difference in total leaf area between biochar addition concentrations is not statistically significant.

Table 2. Comparison of wet and dry weight, and leaf area for the four soil mixes, as expressed using grams (g) and square centimeters (cm^2). Confidence intervals correspond to ±1 standard deviation (n = 9 replicates for wet and dry weight, n = 36 replicates for leaf area).

Biochar Content (wt%)	Wet Weight (g/plant)	Dry Weight (g/plant)	Leaf Area (cm^2/plant)
0.0%	0.73 ± 0.10	0.06 ± 0.01	12.4 ± 3.87
2.5%	0.71 ± 0.11	0.06 ± 0.01	11.0 ± 2.64
5.0%	0.65 ± 0.20	0.06 ± 0.02	10.8 ± 2.53
7.5%	0.72 ± 0.14	0.05 ± 0.01	10.6 ± 2.95

The results of the growth studies do not show strong evidence for biochar as a soil amendment having an impact on seedling growth, other than a slight indication that increased biochar content decreases average leaf area per seedling. There is no existing available literature that matches the procedures used in this research, but there are similar studies, typically differing by having the seeds sprout in a controlled starter soil before being moved to biochar soil [35] [36] or the water containing the biochar, rather than the biochar being in the soil [40] [41]. The literature supports the theory of biochar increasing leaf area, leaf size, and leaf number, though there seems to be a point at which any additional biochar seems to stunt plant growth, somewhere between 5% and 15% (wt%) [35]. Elemental (CHN) composition of the biochar was measured for this study; however, accounting for additional characteristics, such as pH, EC, and ash content, could better elucidate mechanisms influencing plant responses to HTL-derived biochar. The HTL-derived biochar used in this research may possess distinct physical and chemical properties—such as higher solubility of certain organic compounds or lower pH, for example—compared to biochar derived from other thermochemical methods. These differences may partly explain the lack of increased leaf area observed in this study.

Additionally, the use of a nutrient-rich watering solution in the experiments may have obscured potential biochar effects on seedling growth. Biochar amendments often influence plant performance by enhancing nutrient retention or supplying mineral nutrients; however, when plants receive a consistent and abundant external nutrient supply, these benefits become less detectable. Under nutrient-rich watering conditions, the soil solution likely remained sufficiently enriched across all treatments such that differences in nutrient availability attributable to biochar were minimized. As a result, any potential positive or negative effects of the HTL-derived biochar may have been masked, both positive and negative effects of the HTL biochar, contributing to the limited differences observed in seedling growth.

4. Conclusion

This study investigated the effects of amending soil with post-HTL biochar on the seedling growth of food crops grown under controlled environmental conditions. Based on experimental results, there is no statistically significant impact on seedling structure in soil containing between 2.5% and 7.5% biochar as compared to seedling growth in soil without the addition of biochar. Although this study did not observe significant biochar-related improvements in seedling growth, further research is warranted to better isolate the effects of HTL-derived biochar. Future experiments should include experiments using a nutrient-poor or water-only watering solution to determine whether fertilizer overshadowed potential biochar influences. Additional characterization of HTL biochar—including pH, EC, and ash content—would also help clarify its interactions with seedling physiology. Longer-term growth studies and testing across multiple crop species could further determine the suitability of HTL-derived biochar as an agricultural soil amendment.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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