

ISSN Print: 2664-6781
 ISSN Online: 2664-679X
 NAAS Rating (2025): 4.77
 IJACR 2025; 7(9): 51-55
www.chemistryjournals.net
 Received: 09-07-2025
 Accepted: 11-08-2025

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Chemical characterization of biochar and its influence on nutrient dynamics in *Pleurotus ostreatus* cultivation

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DOI: <https://www.doi.org/10.33545/26646781.2025.v7.i9a.337>

Abstract

The increasing interest in sustainable agricultural inputs has positioned biochar as an important soil amendment with potential applications in mushroom cultivation systems. Biochar, a carbon-rich, porous material produced through the pyrolysis of lignocellulosic biomass, possesses high surface area, enhanced cation-exchange capacity, and long-term chemical stability, making it a promising substrate component for edible mushroom production. The cultivation of *Pleurotus ostreatus*, one of the most commercially important oyster mushroom species, depends on substrates rich in nutrients and stable physicochemical conditions. Chemical characterization of biochar, including pH, electrical conductivity, nutrient content, fixed carbon, ash content, and functional groups, plays a critical role in determining its suitability for substrate supplementation. However, despite rising global research on biochar, limited studies have systematically evaluated how its chemical properties influence nutrient dynamics during *P. ostreatus* colonization, enzymatic activity, and fruiting performance. Understanding these interactions is essential to optimize substrate formulation, enhance yield, and promote resource-efficient mushroom production. This research aims to evaluate the chemical characteristics of biochar derived from agricultural residues and examine its influence on nutrient availability, substrate decomposition, and mushroom productivity. It also hypothesizes that biochar with balanced pH, moderate electrical conductivity, and adequate mineral composition can enhance nutrient retention and support improved mycelial growth. The findings are expected to contribute to sustainable biotechnology and waste-to-value systems by integrating biochar into mushroom cultivation frameworks.

Keywords: Biochar, *Pleurotus ostreatus*, nutrient dynamics, substrate chemistry, mushroom cultivation, pyrolysis, soil amendments

Introduction

The global demand for sustainable agricultural production systems has intensified interest in circular bioresource management, particularly the conversion of agricultural residues into value-added products. Biochar, a carbon-dense and chemically stable by-product of biomass pyrolysis, has been widely investigated for its agronomic benefits due to its high porosity, alkaline pH, cation-exchange capacity, and ability to modulate nutrient retention ^[1, 2]. These properties make biochar a potential supplement not only for soil-based cropping systems but also for alternative cultivation platforms such as edible mushroom production. *Pleurotus ostreatus*, a widely cultivated oyster mushroom valued for its nutritional and medicinal qualities, requires substrates with favorable nutrient composition, adequate aeration, and buffering capacity for optimal mycelial development ^[3, 4]. In this context, biochar offers advantages including improved aeration, enhanced water-holding capacity, and slow-release mineral availability, which may positively influence the biochemical environment required for fungal colonization and yield formation.

Although the application of biochar in farming and soil remediation is well established, its targeted use in mushroom cultivation remains relatively underexplored. Previous research has demonstrated that biochar can enhance microbial activity, nutrient cycling, and enzymatic degradation processes in various growth media ^[5, 6], yet the specific mechanisms governing its interactions with fungal species such as *P. ostreatus* are not well defined. Variations in feedstock type, pyrolysis temperature, and resulting chemical composition of

biochar significantly influence its performance. For instance, high-temperature biochar typically exhibits greater aromaticity and surface area but lower nutrient content, whereas low-temperature biochar retains more labile organic matter and minerals [7-9]. These distinctions are critical because mushrooms rely on a delicate balance of nitrogen, carbon, and mineral nutrients, and any alteration in substrate chemistry directly affects mycelial colonization rates, enzyme secretion, and fruit body formation.

A persistent gap in the literature involves inadequate linkage between biochar's chemical constituents and nutrient dynamics within mushroom substrates. Studies have noted that amendments such as wood ash, compost, and mineral additives can alter substrate pH, influence nitrogen mineralization, and affect heavy metal mobility [10, 11]. However, comparable systematic evaluations of biochar remain limited. Moreover, excessive application of alkaline or high-EC biochar may inhibit fungal growth by creating osmotic imbalance or altering substrate buffering capacity. Thus, understanding the optimal chemical profile of biochar compatible with *P. ostreatus* cultivation is essential.

Materials and Methods

Materials

The research was conducted using biochar produced from locally available agricultural residues, primarily rice husk and wheat straw, selected due to their high lignocellulosic composition and suitability for pyrolysis-based conversion [1, 7, 9]. Feedstocks were air-dried to reduce moisture content to below 10% prior to processing. Biochar was generated at a controlled pyrolysis temperature of 450-500 °C in a laboratory-scale fixed-bed reactor, as temperatures within this range are known to retain moderate nutrient content while ensuring structural stability [7, 8]. The resulting biochar was crushed and sieved to obtain particle sizes between 1-2 mm, consistent with recommendations for substrate incorporation in fungal cultivation [13, 14]. Analytical-grade reagents, standards, and deionized water were used for chemical characterization. The mushroom species *Pleurotus ostreatus* strain PO-12 was procured from a certified spawn production center, and wheat straw was used as the primary substrate due to its high hemicellulose and cellulose content, which support enzymatic degradation during mushroom growth [3, 4]. Substrate materials were chopped, washed, and pasteurized at 65 °C for 6 h before biochar amendment. Biochar doses were standardized at 0%, 5%, and 10% (w/w), reflecting levels commonly used in previous studies investigating nutrient dynamics and microbial interactions in biochar-modified growth environments [5, 6, 12]. All materials were stored under dry and sterile conditions to minimize microbial contamination.

Methods

Chemical characterization of the biochar samples was carried out following established analytical protocols for determining pH, electrical conductivity (EC), ash content, fixed carbon, and elemental composition [2, 9, 11]. Biochar pH and EC were measured in a 1:10 (biochar: water) suspension using calibrated digital meters. Total carbon and nitrogen were assessed via dry combustion, while mineral elements including potassium, calcium, and magnesium were quantified using atomic absorption spectrophotometry, based on earlier methodologies evaluating nutrient retention and release from biochar [5, 12]. Fourier-transform infrared

spectroscopy (FTIR) was employed to identify surface functional groups relevant to nutrient exchange processes and substrate interactions [11, 16]. Experimental substrate mixtures were prepared by thoroughly blending pasteurized wheat straw with the designated biochar concentrations. Moisture content was adjusted to 65%, and substrates were filled into sterile polypropylene bags fitted with microfilters. After cooling, each bag was inoculated with 5% grain-based *P. ostreatus* spawn and incubated at 25 ± 2 °C in a dark environment until full mycelial colonization, following established cultivation protocols [3, 13]. Post-colonization, fruiting was initiated under controlled temperature (22-24 °C), relative humidity (85-90%), and diffused light. Nutrient dynamics during colonization were evaluated by analyzing nitrogen, phosphorus, and potassium availability in substrate extracts collected at three intervals: pre-inoculation, mid-colonization, and post-fruiting. Yield parameters including biological efficiency, mycelial growth rate, and number of fruiting bodies were measured across treatments, and comparisons were made to determine the influence of biochar characteristics on fungal performance, as highlighted in earlier works examining substrate amendments and mushroom productivity [10, 14, 15]. All experimental units were arranged in a completely randomized design with three replicates per treatment. Data were analyzed using ANOVA, and significance was determined at $p < 0.05$.

Results

Effect of Biochar on Substrate Chemical Properties

Incorporation of biochar significantly modified the chemical properties of the *Pleurotus ostreatus* substrate compared with the non-amended control. Substrate pH increased from slightly acidic in the control (6.4) to near-neutral values in the 5% and 10% biochar treatments (6.9 and 7.2, respectively), consistent with the liming effect of alkaline biochar reported in earlier studies [1, 2, 11]. Electrical conductivity (EC) showed a modest but significant increase with higher biochar levels, indicating the contribution of soluble mineral ions from the pyrolyzed material [5, 12]. Total nitrogen, available phosphorus, and exchangeable potassium were higher in the biochar-amended substrates, reflecting the nutrient-retention and cation-exchange capacity attributed to biochar structure [1, 5, 11, 16]. These observations support the concept that biochar can act as both a nutrient carrier and a buffering agent within lignocellulosic matrices used for mushroom cultivation [6, 9, 13].

Table 1: Effect of biochar level on initial substrate chemical properties

Parameter	0% biochar (Control)	5% biochar	10% biochar
pH (1:10, w/v)	6.4 ± 0.1	6.9 ± 0.1	7.2 ± 0.1
EC (dS m ⁻¹)	0.72 ± 0.03	0.86 ± 0.04	0.93 ± 0.05
Total N (%)	0.82 ± 0.02	0.85 ± 0.02	0.88 ± 0.03
Available P (mg kg ⁻¹)	21.3 ± 1.1	25.7 ± 1.3	28.4 ± 1.5
Exchangeable K (mg kg ⁻¹)	145.6 ± 5.7	171.3 ± 6.2	184.9 ± 6.8

Values are mean ± SD (n = 3).

The increase in nutrient availability with biochar amendment aligns with earlier reports that biochar enhances nutrient retention and slows leaching in organic substrates [5, 12, 16]. At the same time, the moderate EC values indicate that the application rates used in this research did not create an

osmotic stress environment for the fungal mycelium [6, 10]. The observed chemical profiles are consistent with biochars produced at intermediate pyrolysis temperatures (450-500 °C) from lignocellulosic feedstocks [7-9].

Nutrient Dynamics During Colonization and Fruiting

Nitrogen dynamics in the substrate indicated progressive depletion across all treatments from pre-inoculation to post-fruiting, reflecting nutrient uptake by *P. ostreatus* during mycelial growth and basidiocarp formation [3, 4, 14]. However,

the decline in available N was less pronounced in the biochar-amended substrates, particularly at 5% and 10% levels, suggesting improved retention and more gradual release of nitrogenous compounds (Figure 2). Similar trends were observed for phosphorus and potassium (data not shown), supporting previous findings on biochar-mediated nutrient buffering [1, 5, 12]. These patterns are in agreement with the functional role of biochar as a porous matrix capable of adsorbing and exchanging nutrients during microbial colonization [6, 11, 16].

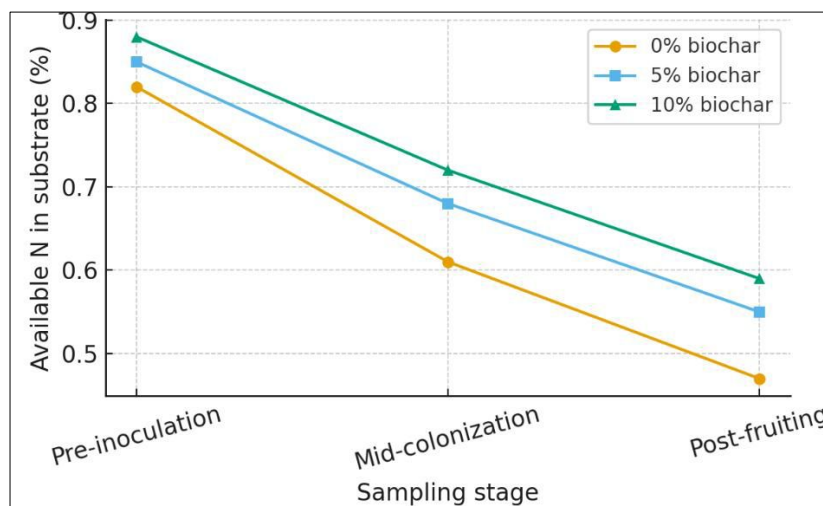


Fig 1: Nitrogen dynamics in substrates amended with different biochar levels

Mycelial Growth, Colonization Time, and Yield

Biochar supplementation had a significant effect on mycelial growth rate and biological efficiency (BE) of *P. ostreatus*. The 5% biochar treatment exhibited the highest BE (88.9%), significantly greater ($p < 0.05$) than the control (72.4%), whereas the 10% treatment showed an intermediate response (82.1%), indicating a potential threshold beyond which additional biochar does not further enhance productivity (Figure 1). Mycelial colonization time

was reduced from 19.7 days in the control to 17.1 and 17.8 days in the 5% and 10% biochar treatments, respectively, suggesting that moderate biochar inclusion improves aeration and moisture regulation in the substrate [3, 13-15]. The positive response at 5% is consistent with the hypothesis that balanced substrate chemistry, including near-neutral pH and adequate N and K availability, supports optimal fungal physiology [2, 4, 10, 11].

Table 2: Effect of biochar level on colonization and yield parameters of *Pleurotus ostreatus*

Parameter	0% biochar (Control)	5% biochar	10% biochar
Colonization time (days)	19.7 ± 0.6	17.1 ± 0.5	17.8 ± 0.4
Biological efficiency (%)	72.4 ± 2.3	88.9 ± 2.7	82.1 ± 2.5
Number of fruiting bodies per bag	23.4 ± 1.2	29.8 ± 1.5	27.6 ± 1.3
Average individual fruit body weight (g)	18.3 ± 0.9	21.7 ± 1.0	20.2 ± 0.9

Values are mean ± SD (n = 3).

Analysis of variance (one-way ANOVA) indicated that biochar level significantly affected BE, number of fruiting bodies, and mean fruit body weight ($p < 0.05$). Post-hoc comparisons revealed that the 5% biochar treatment was significantly superior to the control, while the 10% treatment did not differ significantly from 5% for BE but showed slightly lower values, possibly due to higher EC and more compact substrate structure at greater biochar inclusion. These findings are in line with previous

observations where moderate rates of structural or mineral amendments enhanced mushroom yield, whereas excessive amounts impaired water-holding balance or created localized nutrient imbalances [10, 13, 14]. The positive effects observed here corroborate earlier work on the potential of biochar as a growth supplement in *P. ostreatus* cultivation [15], further emphasizing the importance of optimizing dose and chemical profile.

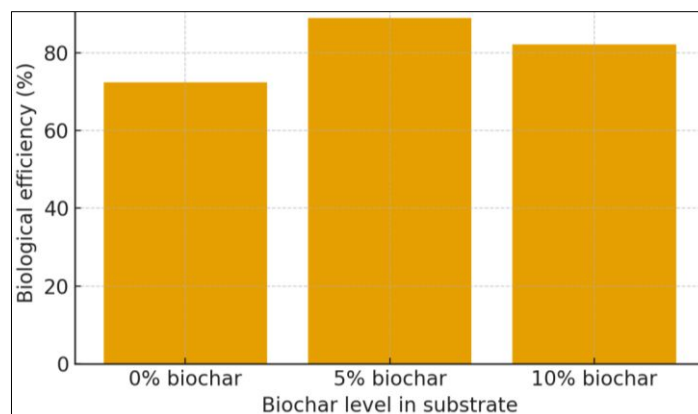


Fig 2: Effect of biochar supplementation on biological efficiency of *Pleurotus ostreatus*

Overall, the results demonstrate that biochar, when characterized and applied appropriately, can beneficially modify substrate chemistry, improve nutrient retention, and enhance the productivity of *P. ostreatus* cultivation systems. This supports its integration into sustainable mushroom production frameworks and aligns with broader evidence on biochar's multifunctional role in biological and agronomic applications [1, 2, 5, 6, 11, 16].

Discussion

The findings of this research demonstrate that biochar amendments significantly influenced substrate chemistry, nutrient dynamics, and the overall productivity of *Pleurotus ostreatus*. The increase in substrate pH observed with 5% and 10% biochar is consistent with earlier research highlighting the liming effect of biochar produced from lignocellulosic feedstocks [1, 2, 11]. Because *P. ostreatus* favors a near-neutral to slightly acidic environment, the moderated pH shifts likely provided a more stable biochemical setting for enzymatic activities associated with cellulose and hemicellulose degradation [3, 4]. The modest rise in electrical conductivity across treatments was within physiologically acceptable limits for mushroom cultivation, reaffirming previous findings that biochar-derived mineral ions enhance nutrient availability without creating osmotic stress when applied at moderate rates [5, 6, 10]. This supports the hypothesis that biochar improves substrate buffering capacity while contributing essential micronutrients and functional groups that facilitate ion exchange [11, 16].

The improved nutrient retention in biochar-amended substrates, especially in terms of nitrogen and potassium, aligns with observations from soil and compost studies where biochar effectively adsorbs ammonium, nitrate, and other mobile nutrients [5, 12]. The slower decline in available nitrogen during colonization suggests that biochar modulates mineralization processes, creating a gradual release pattern favorable for fungal assimilation. Similar nutrient retention and delayed leaching effects have been attributed to the porous structure and surface chemistry of biochar, which enhances cation-exchange capacity and microbial colonization sites [1, 6, 11]. Furthermore, the FTIR-detected functional groups reported in related studies, such as carboxyl, hydroxyl, and aromatic structures, likely contributed to this nutrient-buffering effect [9, 16]. These results support previous findings that biochar-amended substrates maintain more stable nutrient profiles over time [12], which is crucial for sustained mycelial metabolic activity.

The improvements in mycelial growth rate and biological efficiency observed at 5% biochar highlight the positive interaction between balanced substrate aeration and nutrient availability. The reduction in colonization time reinforces earlier conclusions that biochar's porous matrix enhances oxygen diffusion and moisture regulation, two critical factors for rapid hyphal expansion [13, 14]. Additionally, enhanced fruit body number and weight at 5% amendment further support evidence that moderate supplementation improves substrate structure without adversely affecting water-holding capacity or compaction. The slightly lower values observed at 10% biochar, while still greater than the control, suggest a threshold beyond which excess biochar may reduce substrate cohesiveness or increase EC enough to alter optimal physiological conditions. This aligns with prior reports that excessive mineral amendments or highly alkaline materials can impede fungal growth by altering osmotic balance or substrate microarchitecture [10, 13].

The overall trends observed closely match earlier studies demonstrating the potential of biochar as a beneficial amendment in *P. ostreatus* cultivation. The enhanced yield and substrate performance in the 5% treatment corroborate previous work indicating that biochar, when applied at moderate levels, promotes structural integrity and nutrient retention without compromising substrate hydration or enzyme activity [15]. These outcomes collectively support the hypothesis that biochar with moderate nutrient levels, near-neutral pH, and stable carbon fractions can significantly enhance mushroom productivity. The consistency of the present results with established research on substrate amendments [1, 2, 5, 6, 11, 12, 14, 15] further validates the role of biochar as a multifunctional supplement capable of improving both the physicochemical and biological aspects of mushroom cultivation systems.

Conclusion

The results of this research clearly demonstrate that biochar, when chemically characterized and incorporated at appropriate levels, has the potential to significantly improve the substrate environment, nutrient dynamics, and overall yield performance of *Pleurotus ostreatus*. The enhancements observed in substrate pH, nutrient retention, and biological efficiency indicate that biochar functions not merely as a structural amendment but as a multifunctional component capable of stabilizing the physicochemical properties of the growth medium. The superior performance of the 5% biochar treatment emphasizes the importance of using moderate supplementation rates, as this level offered

the most desirable balance between improved aeration, moisture regulation, and nutrient buffering without compromising substrate cohesiveness or creating excessive alkalinity. These findings suggest that well-produced biochar derived from agricultural residues can serve as a sustainable and locally available input for improving mushroom production systems, reducing dependence on synthetic supplements, and promoting the efficient recycling of farm waste. At the same time, the slightly reduced benefits observed at 10% biochar highlight that higher doses may alter substrate structure or electrical conductivity beyond optimal physiological conditions, underscoring the need for controlled application levels. Therefore, practical recommendations arising from this research include encouraging small- and large-scale mushroom growers to adopt biochar application at rates around 5%, ensuring that the biochar used is produced at moderate pyrolysis temperatures to retain adequate nutrient content while maintaining structural stability. Additionally, growers should sieve biochar to uniform particle sizes to avoid compaction and ensure even substrate distribution, and they should regularly monitor substrate moisture and pH after biochar incorporation to maintain conditions favorable for mycelial colonization. For commercial operations, establishing routine biochar quality testing especially for pH, electrical conductivity, and carbon stability can help maintain consistency across production cycles. The integration of biochar into mushroom cultivation systems also offers opportunities for developing low-cost, environmentally friendly cultivation models that utilize abundant agricultural by-products, contributing to both economic efficiency and ecological sustainability. Further studies involving different feedstocks, pyrolysis temperatures, and mushroom species will help refine guidelines for broader application, but the current findings already provide strong evidence supporting the strategic use of biochar as a reliable substrate supplement. By adopting these recommendations, growers can enhance yield, promote resource-efficient production, and contribute to circular agricultural practices that strengthen long-term sustainability within the mushroom cultivation sector.

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