Production of Biochar from Leather Waste and Its Application in Mycelium Growth Enhancement

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Production of Biochar from Leather Waste and Its Application

in Mycelium Growth Enhancement

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Abstract

To achieve high-value utilization of tannery solid wastes, this study use cow hair and trimmings collected from beamhouse process, which are rich in keratin and collagen. By adjusting the proportion of these two waste components, porous carbon was synthesized through KOH activation under anoxic conditions, yielding a high specific surface area of 2893 m^2/g and a total pore volume of 1.88 cm^3/g. The porous carbon was evenly dispersed into the Pleurotus ostreatus substrate to promote mycelial growth. Due to the abundant pore structure acting as an effective growth matrix, the porous carbon significantly improved mycelial network formation, accelerated substrate decomposition and enhanced protease acitivity. This study provides an efficient approach for utilizing the solid wastes generated in leather production, and demonstrates the potential of porous carbon to improve mycelium growth, which will be beneficial for the further development of mycelial leather alternatives production.

Keywords: Solid waste treatment; Porous carbon; Mycelial growth; growth-promoting enzyme acitivityÿMycelial leather alternative

1. Introduction

The tanning process often generates substantial amounts of waste, with approximately 80% of this waste occurring in solid form.Tanning solid waste primarily includes

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materials from depilation, trimming, splitting, edging, and chromium-containing sludge(1).The safe management of solid waste has become an urgent necessity.Due to its high carbon, nitrogen, and oxygen content(2), tanning solid waste can undergo pyrolysis under anaerobic or lowoxygen conditions and subsequently react with activating agents to produce porous carbon materials that exhibit a large specific surface area, well-developed pore structures, and stable physicochemical properties(3).Current research indicates that porous carbon can be incorporated into culture substrates as a growth medium and exogenous nutrient source, promoting the growth of basidiomycetes such as Pleurotus ostreatus and Ganoderma lucidum(4).Given Pleurotus ostreatus cultivation’s advantages—short growth cycle, strong adaptability, rich enzyme systems, suitability for large-scale cultivation, and efficient degradation of lignocellulosic waste(5)-this study focuses on Pleurotus ostreatus.The growth and development of Pleurotus ostreatus involves mycelium and fruiting body stages. During the mycelium stage, extracellular enzymes are secreted to decompose macromolecules in the substrate, synthesizing polysaccharides, proteins, lipids, nucleic acids, and other substances essential for mycelium growth and development.Among these, protease synthesis facilitates the hydrolysis of macromolecular proteins in the substrate into oligopeptides and free amino acids, promoting protein synthesis, energy metabolism, and the production of secondary metabolites(6).

This study utilized cow hair and trimming waste from the pre-tanning preparation stage as raw materials.Through

pyrolysis and activation reactions, a keratin-collagen-based porous carbon material was prepared, and its structure and morphology were characterized. The porous carbon material was then mixed with culture medium substrate for fungal growth cultivation, and the protease activity of the mycelium was evaluated.FTIR

characterization was further employed to validate the experimental conclusions.

2. Methodology

2.1 Preparation and Structural Analysis of Porous Carbon Materials

Leather waste and hair waste were pulverized into fine powder and mixed at a 1:1 ratio to obtain LW. LW was carbonized at 6000C under a nitrogen atmosphere for 3 hours. It was then mixed with KOH at a 1:4 ratio and activated at 8000C for 1.5 hours. After washing and drying, the material was designated as ALW.

2.2 Preparation and Characterization of Mycelium

Corn cobs were crushed to a diameter of less than 0.5 cm. A substrate mixture was prepared with corn cobs, bran, soybean meal powder, and corn flour. Lime water (1%) was added at a 1:1 ratio to the mixture.The mixture was sealed in bags with three replicates per group. Mix porous carbon with the substrate mixture at 10% dry

Figure 2 (A), (B) SEM images of hyphae in the blank group (C), (D) SEM images of hyphae in the ALW group

weight. The control group omitted porous carbon. Inoculate, and incubate in a 240C dark chamber for 20 days. System mass change was expressed as the mass difference between control and experimental samples after 20 days. Substrate decomposition rate was expressed as the ratio of mass change to incubation days.Protease activity was assessed per GB/T 23527.1-2023(7) using the Forin method. Absorbance at 680 nm was measured with a UV spectrophotometer and plotted against a standard curve to calculate protease activity.

3. Results & Discussion

3.1 Microscopic Morphology Analysis of Mycelium Fibers

Under microscopic examination, oyster mushroom mycelium fibers exhibit a tightly packed arrangement with a curved and folded morphology (Fig. 2A,B). Their surfaces display fine wrinkles, and diameters typically range from 2 to 10 μm. Mycelium fibers grown on ALW exhibit diameters of approximately 0.5-2 μm, primarily extending in straight lines with minimal curling or entanglement, and adhere tightly to the ALW surface (Fig. 2C,D). Porous carbon materials possess a large specific surface area (2893 m2/g) and pore volume (1.88 cm3/g), provides an efficient growth scaffold for the mycelium, facilitating its spread throughout the culture medium.

3.2 Analysis of Substrate Degradation Rate in Mycelium

This study characterizes the decomposition rate of the matrix by measuring mass changes within the system(8),which indirectly reflects mycelium growth rates. Following inoculation, mycelium initially forms primary hyphae.Through heterokont synergy, genetically compatible mononuclear hyphae undergo syngamy, forming secondary binuclear hyphae with clamp

Figure 3 (A) Changes in the system mass of mycelium (B) Substrate degradation rate of mycelium

3.3 Analysis of Mycelial Protease Activity and Mechanism Investigation

Protease activity can to some extent reflect the utilization efficiency of organic nitrogen sources by Lentinula edodes mycelium(10). By synthesizing proteases,the mycelium hydrolyzes macromolecular proteins in the substrate into oligopeptides and free amino acids,thereby promoting protein synthesis, energy metabolism, and the production of secondary metabolites(11). As shown in Figure 4A, adding ALW porous carbon to the substrate significantly increased the protease activity of the mycelium.In FTIR,the absorption peak at 2921 cm-1 indicates the stretching vibration of CH groups, associated with aliphatic synthesis(12).The higher absorption peak after ALW addition indicates elevated lipid content in the surface mycelium and well-developed cell membrane systems.The peak at 1650 cm-1 corresponds to the C=O bond stretching vibration (amide I band).The peak at 1322 cm-1 can be attributed to the coupled vibration

connections that spread throughout the culture medium(9). Consequently, mycelium growth exhibits an initial slow phase followed by accelerated expansion (Figure 3A).The addition of ALW reduced substrate weight from 200 g to 182.8 g, a significant improvement compared to the 185.7 g observed in the control group.Concurrently, the substrate decomposition rate during the growth cycle increased from 0.72 g/d to 0.86 g/d (Figure 3B), indicating that ALW promotes mycelium growth.

of C-N stretching and N-H bending (amide III band).Weakened absorption peaks in the amide I and amide III bands indicate the breakage of exogenous protein peptide chains, with protein macromolecules being degraded into oligopeptides or amino acids.This further demonstrates high protease activity and efficient utilization of organic nitrogen sources(13),thereby validating the conclusion.

4. Conclusion

After high-temperature carbonization and activation treatment of leather waste, a porous carbon material (ALW) with high specific surface area (2893 m2/g) and high pore volume (1.88 cm^3/g) was produced.When incorporated into corn cob-based culture media, ALW significantly accelerated substrate decomposition by Lentinula edodes mycelium. This enhancement stemmed from ALW’s ability to increase the utilization rate of organic

Figure 4 (A) Protease activity of mycelium (B) FTIR characterization of mycelium

nitrogen sources in the substrate, thereby boosting mycelial protease activity by 91%. Simultaneously, the porous carbon material, acting as an efficient growth scaffold due to its abundant pore structure, transformed the curved mycelial fibers into extended linear forms, significantly altering the formation of the mycelial network. This study successfully establishes an efficient resource utilization pathway for tanning solid waste while introducing a novel carrier material for oyster mushroom mycelium cultivation. It holds positive implications for advancing the development of mycelium-based leather alternatives.

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