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PROXIMATE ANALYSIS OF THE GROWTH OF ORGANIC GREY OYSTER MUSHROOMS ON BIOCHAR FROM AGRICULTURAL WASTE

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A B S T R A C T

Agricultural waste is a major problem in Thailand. To eliminate waste and constrain global warming, waste should be renewed for other purposes by thermochemical processes. Pyrolysis is a process that produces biochar, which is useful for amending soil, improving soil fertility, and creating microorganism habitats. This research aimed to produce biochar from agricultural wastes for use as a mushroom substrate and to increase the nutrient consumption by the mushrooms. Sugarcane leaf biochar had the lowest C:N ratio and neutral pH, which are important factors for *Pleurotus pulmonarius* mycelium growth and the formation of fruiting bodies. Moreover, this biochar has micropores suitable for fungal growth. According to the nutrient measurements, the proximate analysis showed that 1% sugarcane leaf biochar produced a higher yield and protein content than the others, whereas the biochar reduced the fat content but increased the moisture in the fruiting body, including aeration increasing. Thus, 1% sugarcane leaf biochar was suitable as an extra substrate for growing grey oyster mushrooms. From the result of this research, biochar production is an excellent process to convert agricultural wastes to be a renewable resource for improving the efficiency of crop production.

Keywords: oyster mushroom, biochar, agricultural waste, SEM, XRD.

INTRODUCTION

Thailand is a land of agriculture that also produces agricultural wastes and residues. Normally, agriculturists dispose of waste by burying or burning it, which emits greenhouse gases. To minimize global warming, the waste needs to be reused, recycled, or renewed: thermochemical processing is a method to terminate and renew waste. Previous studies have shown that thermochemical processing produced in a closed-system tank does not produce greenhouse gases; instead, it produces hydrogen gas, a clean energy source, from the gasification process (Cay *et al*., 2019). Moreover, pyrolysis, a thermochemical process, converts biomass to organic carbon matter (Cay *et al*., 2019; Panwar *et al*., 2019).

Biochar is produced using the pyrolysis process, which consists of burning the raw materials at high temperatures without oxygen. The resulting biochar is high in carbon and porous, depending on the raw material (Phuong *et al*., 2015). Biochar can be produced from various raw materials, such as wood, agricultural waste, leaves, and manures. Biochar is used for soil improvement through acidity adjustment, soil bulk density reduction, and enhanced water retention (Singh *et al*., 2010; Nidiate *et al*., 2022). Moreover, carbon from biochar is slowly released into the soil for plant uptake. The porosity of biochar provides a habitat for soil microorganisms; however, the inhabitant depends on the size of the biochar pores: bacteria (0.3 to 3 µm), protozoa (7 to 30 µm), and fungi (2 to 80 µm) (Lu and Zong, 2018; Leng *et al*., 2021). Previous studies have used biochar to increase the growth and yields of crops such as wheat, rice, ginger, soybeans, and even mushrooms (Saxena *et al*., 2014; Hamzah and Shuhiami, 2018; Jabborova *et al*., 2020).

The oyster mushroom, *Pleurotus ostreatus* and *Pleurotus pulmonarius*, is an economical and favored edible mushroom. Previous studies have shown that the

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Pleurotus spp. have therapeutic properties, such as antioxidant, antidiabetic, and lipid reduction. Moreover, they are high in protein and carbohydrate content. Due to consumer demand, cultivation has increased annually (Ashraf *et al*., 2013; Shalahuddin *et al*., 2018; Nguyen and Ranamukhaarachchi, 2020; Inyod *et al*., 2022). Various studies have cultivated the oyster mushroom on different materials, such as sawdust, corn cobs, rice bran, spent mushroom substrate, tea waste, and even biochar from waste recycling and at a reduced cost (Aslam *et al*., 2021; Asraf *et al*., 2013; Nam *et al*., 2017; Nguyen and Ranamukhaarachchi, 2020). A previous study showed that palm shell waste biochar produced by microwave vacuum pyrolysis reduced the time needed for mycelium growth and improved the yield (Nam *et al*., 2017). In addition, as a predominantly agricultural nation, Thailand generates a great deal of agricultural waste. Lopburi province produces corn and sugarcane, and as a result, there are many sugarcane leaves and corn stalks available after harvesting. There are also community enterprises that cultivate mushrooms. Therefore, this study aimed to renew agricultural wastes to produce biochar for oyster mushroom cultivation, including determining the nutritional content.

MATERIALS AND METHODS

Agricultural waste biochar production: Two agricultural wastes, sugarcane leaves and corn stalks, and spent mushroom substrate, were collected and pyrolyzed at 400°C for 4 hours. At this temperature, cellulose and hemicellulose are destroyed by burning. The small-scale pyrolysis tank is composed of inner and outer tanks with diameters of 11 and 20 cm, respectively. The corresponding tank heights are approximately 23 and 30 cm. During biochar production, the raw material was placed in the inner tank, while the space between the inner and outer tanks was filled with firewood.

Biochar characterization: After pyrolysis, the basic chemical properties of the biochar samples were measured, such as the pH (Singh *et al*., 2010) and electrical conductivity of 1:5 biochar: water (EN 13651-2002). The total carbon and total nitrogen were determined with CHNS/O analyzers (Thermo Scientific™ FLASH2000). The Barton method was used to determine total phosphorus (Barton and Embse, 1998). The total potassium and sodium were measured by a flame photometer (Fawcett and Wynn, 1961). The total calcium and magnesium were analyzed using an atomic absorption spectrophotometer (Isaac and Kerber, 1971). Moreover, the biochar samples were imaged with scanning electron microscopy (SEM): the samples were coated with platinum, and cross-sections were prepared. The samples were observed using SEM (JEOL JSM-6610LV, Japan), and the biochar surface morphology was analyzed.

A multipurpose X-ray diffraction (XRD) system SmartLab® SE (Rigaku, Japan) was used for XRD analysis. The biochar samples were prepared by crushing until they were less than 50 microns. Then, 20 mg of sample was measured over two ranges, 10°–120°, using a step size of 0.02°.

Mushroom production: Biochar samples were ground into small pieces approximately (2–4 mm) and mixed with other materials to form the mushroom cultivation medium. This experiment was divided into four groups of each biochar sample: the control, 1% biochar (w/w), 3% biochar (w/w), and 5% biochar (w/w). The basic mushroom cultivation medium consists of rice bran (0.6 g), sawdust (1 g), Epsom salts (0.02 g), gypsum (0.1 g), dolomite (0.2 g), sticky rice flour (0.1 g), and 60% of water. After mixing these components with the biochar, 800 kg of the mixture was packed into a cylindrical polyethylene bag. Subsequently, this baglog was sealed with Polyvinyl Chloride (PVC), and the bags were sterilized at 121°C for 3 hours and allowed to cool to room temperature. The oyster mushroom spawn was inoculated into the baglogs after they were moved to a storage room for 30 days to grow mycelium. The baglogs were transferred to a mushroom house with controlled moisture and temperature on the thirty-first day.

The biochar treatments: The biochar treatments were divided into nine treatments that followed the various biochar composition

T_0: control
T_1: 1% corn biochar
T_2: 3% corn biochar
T_3: 5% corn biochar
T_4: 1% sugarcane leaf biochar
T_5: 3% sugarcane leaf biochar
T_6: 5% sugarcane leaf biochar
T_7: 1% spent mushroom waste biochar
T_8: 3% spent mushroom waste biochar
T_9: 5% spent mushroom waste biochar

Proximate analysis: The nutritional value of the oyster mushrooms was determined, including ash content, water content, crude fiber, protein, and fat. For the ash

content, 1 g of oyster mushrooms from each treatment was placed into a crucible and heated at 500°C for 4 hours. Following cooling, the sample was weighed, and the ash content was calculated as follows:

$$\text{Ash (\%)} = \frac{\text{weight of sample after ashing}}{\text{weight of fresh sample}} \times 100$$

Porcelain dishes were dried at 105°C for 3 hours, then cooled in a desiccator and weighed. After that, 1 g of mushroom samples was placed in the porcelain dishes. These samples were dried at 105°C for 3 hours and cooled in a desiccator before they were weighed. Subsequently, the water content was calculated according to the formula of the Association of Official Analytical Chemists (AOAC):

$$\text{Moisture (\%)} = \frac{(\text{weight of fresh sample} - \text{weight of dried sample})}{\text{weight of fresh sample}} \times 100$$

Filter papers and 1 g of oyster mushroom sample from each treatment were dried at 105°C for 3 hours and cooled in a desiccator before they were individually weighed. Each sample was added to a flask, and then 200 mL of 1.25% HCl was added into the flasks and boiled for 30 min. Next, the samples were rinsed with distilled water three times. Two hundred milliliters of 1.25% NaOH was added to the samples and boiled for 30 min. After that, the solution was released and washed three times with distilled water. The samples were placed on filter papers. The samples and crucibles were dried at 105°C for 3 hours and cooled in a desiccator before weighing. The sample was placed in a crucible and weighed before burning at 500°C for 4 hours. After cooling, the crucibles were placed in a desiccator before they were re-weighed. The fiber content was calculated as follows:

$$\text{Crude fiber content (\%)} = \frac{(W4 - W3 - W2) - (W5 - W3)}{W1} \times 100$$

W1 = fresh sample weight (gram)
W2 = filter paper weight (gram)
W3 = crucible weight (gram)
W4 = (crucible weight + filter paper weight + fiber weight after drying)
W5 = (crucible weight + fiber weight after burning)

The fat determination followed the Soxhlet method: 1 g of oyster mushrooms was extracted with petroleum ether using a Soxhlet extractor for 5 hours. Then, the samples were dried in a fume hood until the petroleum ether was completely evaporated. Subsequently, the samples were dried at 105°C for 3 hours, cooled in a desiccator, and the fat content was measured.

The protein content analysis followed the Kjeldahl method. One gram of fresh oyster mushrooms was placed into a digestive glass tube, and the catalyst, consisting of 6.65g of K_2SO_4 and 0.35g of Cu_2SO_4, was added into the tube before adding 20 ml of 100% sulfuric acid. The samples were placed into a Kjeldahl digester and heated for 2 hours until the solution was clear and homogenous. After cooling, the digested sample was placed in a Kjeldahl distillation system, and about 50 ml of 4% boric acid was added to the sample, followed by 70 ml of 50% NaOH, until the solution turned black. The distillation process was performed until the sample solution was dropped into an Erlenmeyer flask. The solution was titrated with 4 N sulfuric acid, using methylene red as the indicator, until the color changed from green to pink or red. The total nitrogen of the sample was compared to a blank, and the protein content was calculated as follows:

$$\text{Nitrogen content (\%)} = \frac{(A - B) \times C \times 0.014}{D} \times 100$$

A = milliliter of 0.1 N sulfuric acid of sample
B = milliliter of 0.1 N sulfuric acid of blank
C = sulfuric concentration
D = sample mass

And the protein content was converted following the formula:

$$\text{Protein (\%)} = N\% \times 6.25$$

RESULTS AND DISCUSSIONS

Biochar is usually used for soil amendment because it improves soil bulk density and increases porosity and water retention (Yang and Lua, 2003). This research produced biochar from agricultural wastes in Thailand: sugarcane leaves, corn stalks, and spent mushroom substrate. These wastes were pyrolyzed at 400°C for 4 hours; this temperature maintains the structure of raw material without producing polycyclic aromatic hydrocarbons (PAHs) or carcinogenic gases (Phuong *et al*., 2015).

Carbon and nitrogen are required for the mycelium and the fruiting body of mushroom growth (Lu *et al*., 2021). However, previous studies have stated that the C:N ratio is an important factor for production yield because the nitrogen content is one factor that affects protein production (Osunde *et al*., 2019; Shalahuddin *et al*., 2018; Lu *et al*., 2021). Table 1 shows that the corn stalk biochar showed the highest carbon content; sugarcane leaf biochar had the lowest C:N ratio.

Moreover, moisture is a factor in mycelium growth, especially in the fruiting stage. This stage requires 80%–95% humidity to form a mushroom layer (Navarro *et al*., 2021). Because of its high porosity, the corn stalk biochar contained the highest moisture (Table 1). Biochar porosity is vital for water retention, microorganism habitats, and chemical element adsorption (Lu and Zong, 2018).

The pH is another important factor for fungal mycelium growth. A previous study has shown that oyster mushroom fungi grow well near neutral or slightly basic pH (Khan *et al*., 2013). Table 1 shows that sugarcane leaf biochar was neutral to slightly basic, whereas the other biochars were strongly basic.

After mixing the biochar with the mushroom substrate, the properties of the treatment groups were determined, and the results are given in Table 2. Group T_4 had the highest nitrogen percentage, lowering the C:N ratio, which is necessary for mycelium growth and protein production. Groups T_4 and T_5 had the highest moisture levels and suitable pH for fungal growth. In contrast, group T3 showed 0.54% nitrogen, similar to T4, but the moisture and the pH were not appropriate for mycelium growth.

In addition, the amount of calcium carbonate ($CaCO_3$) is a factor in mycelium growth and mushroom yield (Khan *et al*., 2013). The SEM results showed the biochar porosity of each sample, and the XRD results identified the inorganic compounds present in the biochar. The XRD pattern of the corn stalk biochar showed two peaks assigned to $CaCO_3$ and KCl in the main structure of the biochar. The sugarcane leaf biochar contained only one inorganic compound, $CaCO_3$. In contrast, the spent mushroom substrate biochar showed several peaks indicating various inorganic compounds: $CaCO_3$, KCl, quartz, and silicate because this biochar was produced from mushroom substrate, which contained many raw materials.

The SEM results showed that the corn stalk biochar had an average pore diameter of nearly 100 µm, whereas the pore sizes of the other biochars were less than 50 µm. The micropores are vital for water retention and providing habitats for microorganisms, such as bacteria (0.3 to 3 µm), protozoa (7 to 30 µm), and fungi (2 to 80 µm) (Lu and Zong, 2018; Leng *et al*., 2021). Moreover, biochar porosity increases aeration during mycelium growth by absorbing oxygen and releasing carbon dioxide that retards mycelium growth (Jang *et al*., 2009; Jung and Son, 2021).

After inoculation, the treatment added to the sugarcane biochar showed growth from the top to the bottom of the baglog within 20 days (Figure 3). That was in agreement with the mycelium growth factors discussed above, so the sugarcane leaf biochar was suitable for *Pleurotus pulmonarius* mycelium growth and reduced the time required for growth.

Table 1. Chemical properties of biochars

	pH (1:8)	EC (dS /m)	C (%)	N (%)	C:N	P_2O_5 (%)	K_2O (%)	CaO (%)	MgO (%)	S (%)	Na (%)	Fe (mg/ kg)	Zn (mg/ kg)	Mn (mg/ kg)	Cu (mg/ kg)	Moisture (%)
Corn stalk biochar	9.84	7.07	66.63	0.77	86.53	0.20	3.07	0.98	0.42	1.60	0.05	276	36	4	4	1.82
Sugarcane leaf biochar (S)	7.95	2.20	57.35	0.92	62.34	0.14	0.43	1.03	0.30	0.29	0.05	1398	25	277	7	1.52
Spent mushroom substrate biochar (M)	9.27	2.81	50.00	0.29	172.41	0.58	1.39	13.36	1.70	1.01	0.05	1895	84	33	10	1.12

Table 2. Chemical properties of the mushroom substrates

Sample	Nitrogen (%)	P_2O_5 (%)	K_2O (%)	Moisture	pH
T_0	0.44	0.14	0.43	34.31	8.15
T_1	0.42	0.12	0.49	34.1	8.3
T_2	0.48	0.11	0.49	35.28	8.18
T_3	0.54	0.14	0.8	35.46	8.36
T_4	0.56	0.13	0.62	55.19	6.77
T_5	0.54	0.15	0.62	55.99	7.14
T_6	0.48	0.16	0.93	51.14	7.63
T_7	0.47	0.11	0.43	34.96	8.09
T_8	0.51	0.22	0.55	53.84	7.82
T_9	0.52	0.15	0.49	51.92	7.89

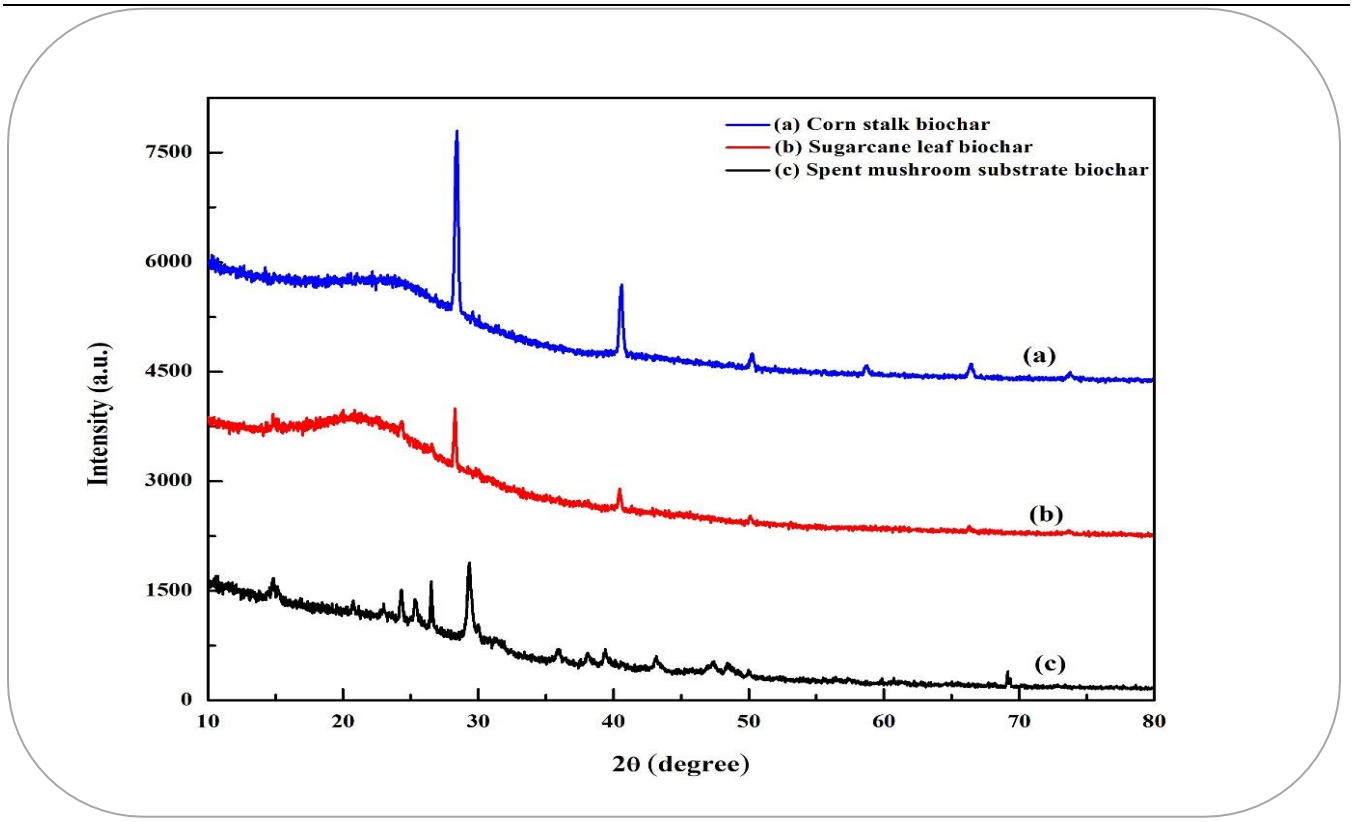
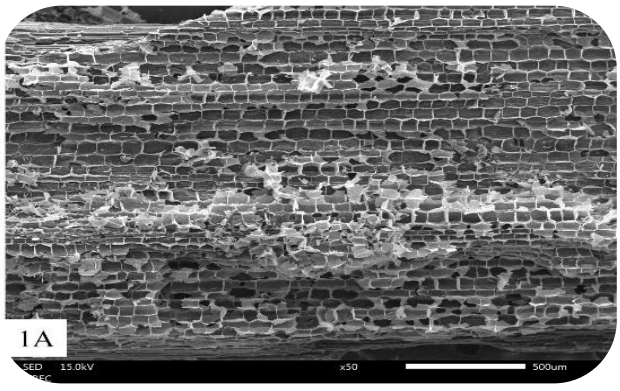
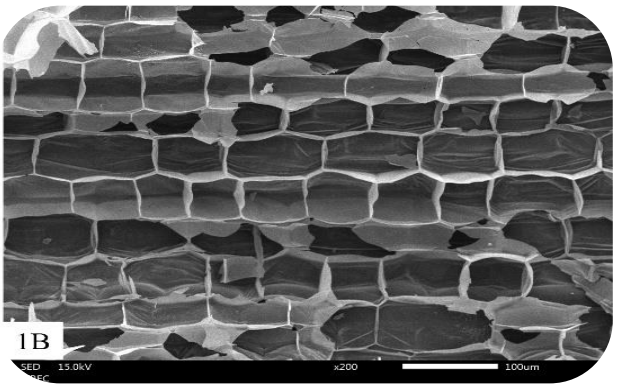


Figure 1. X-ray diffractograms of a. corn stalk biochar, b. sugarcane leaf biochar, and c. spent mushroom substrate biochar





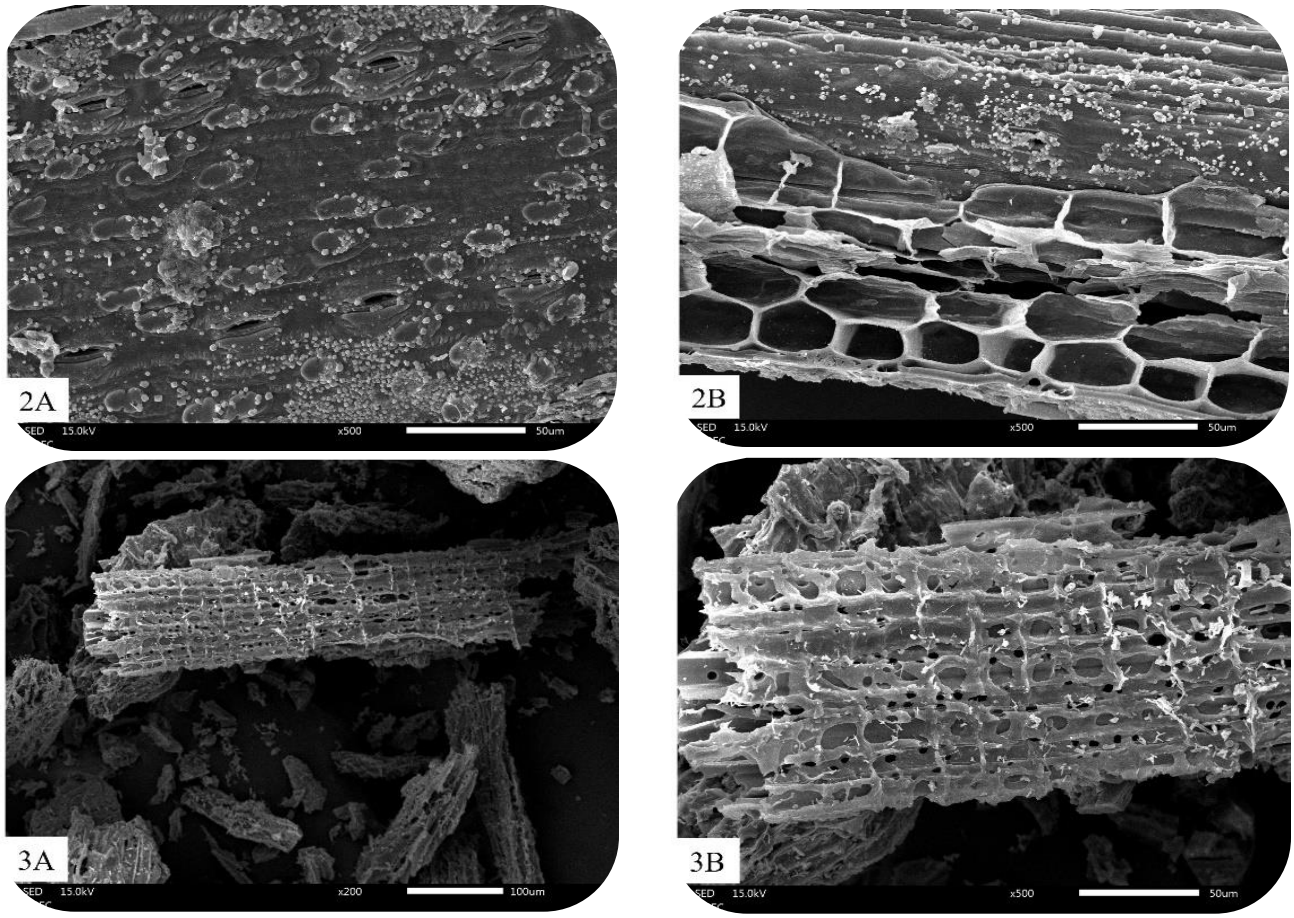


Figure 2. Surface porosity of biochar after pyrolysis at 400°C for 4 hours. A. corn stalk biochar, B. sugarcane leaf biochar, and C. spent mushroom substrate biochar

Figure 3. The aging of mycelium during mycelium growth stages within 20 days

Table 3. Proximate contents of oyster mushrooms in renewable agriculture wastes and spent mushroom substrate.

Biochar treatment	Mass (g)	Crude fiber (g)	Protein (g)	Lipids (%)	Moisture (%)	Ash (%)
T_0	45.41±0.63	60.57±2.02	40.83±0.006	6.41±0.474	95.33±0.58	1.33±0.58
T_1	50.99±0.36	57.95±0.00	45.52±0.096	3.75±0.608	97.50±0.87	3.17±1.04
T_2	50.54±0.49	68.89±3.50	42.00±0.014	3.88±1.211	98.33±0.58	1.17±0.29
T_3	52.51±1.25	60.73±2.31	39.67±0.046	5.29±0.512	98.17±0.29	1.93±0.12
T_4	54.23±2.12	61.68±4.04	57.17±0.073	3.91±0.738	98.33±0.58	1.03±0.05
T_5	52.08±1.11	62.36±4.04	53.67±0.050	4.35±0.155	97.83±0.76	1.03±0.05
T_6	56.35±1.58	58.35±3.50	45.50±0.055	3.84±0.165	98.17±0.29	1.97±0.06
T_7	56.06±4.56	60.73±2.02	33.83±0.022	2.48±0.036	98.17±0.29	1.33±0.58
T_8	53.67±2.44	64.30±2.02	32.67±0.022	3.82±0.059	98.50±0.50	1.02±0.03
T_9	53.24±3.82	66.65±2.02	29.17±0.106	2.08±0.008	97.50±0.50	1.33±0.58

Proximate analysis: Mushrooms are a source of plant-based protein. This research tested the effects of the different C:N ratios, pore sizes, and inorganic matter associated with biochars from different raw materials and analyzed the consumption value of the resulting mushrooms. Mushroom fungi are aerobic fungi that use oxygen and emit carbon dioxide that can inhibit mushroom growth (Jung and Son, 2021). This experiment showed that biochar could have high porosity for fungi habitat, but the size of the pores is critical for aeration for oxygen consumption and carbon dioxide release (Hu *et al*., 2022).

Table 3 shows that when the mushrooms were flushed ten times, and the average mass was calculated, group T_6 had the highest mass. A previous study showed that larger biochar porosity had an anti-aging effect on mycelium growth. This effect meant the mycelium at the bottom of the baglog was weaker than that at the top because the mycelium degraded, causing small fruiting bodies that took longer to form (Hu *et al*., 2022).

Furthermore, the mushroom weight depended on the amount of lignin, which inhibits fungus enzyme secretion, in the substrate. For example, sawdust has a high lignin component that is resistant to degradation. The activity of the lignin degradation enzyme could be stimulated by less amounts of nitrogen in the substrate (Bellettini *et al*., 2019; Osunde *et al*., 2019). Because the C:N ratio of the sugarcane leaf biochar was the lowest, it may have stimulated the lignin degradation enzyme and produced more fruiting bodies in each flush. Many factors can cause low yields, such as moisture, pH, and nitrogen levels (Ashraf *et al*., 2013), and these relate to the yield of the groups containing spent mushroom substrate and corn stalks.

The C:N ratio is an important factor for forming fruiting bodies; previous studies have shown that raw materials with a low C:N ratio, similar to the T_4 treatment (Table 3), produced a high protein content (Shalahuddin *et al*., 2018). However, excess nitrogen has been demonstrated to be a limiting factor for mycelium growth (Boddy and Watkinson, 1995; Zaghi *et al*., 2010). Groups T_5 and T_6 agreed with this trend, even though they contained increased concentrations of biochar. Furthermore, oyster mushrooms are a carbohydrate source beneficial to heart patients (Ashraf *et al*., 2013): group T_2 produced the most carbohydrates compared to the other treatments, including the control (T_0).

Generally, the lipid content in mushrooms is low, approximately 1.75% to 15.5%, depending on species (Paul *et al*., 2016), so their consumption is beneficial for heart disease patients. The control mushroom had a high lipid content of approximately 6.14%, whereas the lipid content of the mushrooms grown on the treated substrate was lower. The biochar could inhibit lipid production and promote water retention. The moisture content in oyster mushrooms is over 90%, increasing the mushroom yield in agreement with a previous study that showed moisture was an important factor in mushroom layer formation (Navarro *et al*., 2021). This work showed that the moisture content of the treatments was slightly higher than the control, whereas the ash content did not differ from the control.

CONCLUSION

Oyster mushroom production depends on various factors, such as substrate, pH, temperature, and moisture. However, this research showed that porosity was one of the most important factors for mushroom production. Porosity is a special characteristic of biochar that affects water retention, soil amendment, and aeration. In this research, porosity was useful for providing fungal habitat,

maintaining moisture, and promoting ventilation. It also improved the antiaging of mycelium growth. Moreover, the C:N ratio of biochar improved the protein content.

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