STRUCTURAL CHARACTERIZATION AND SUGAR RECOVERY FROM SPENT MUSHROOM COMPOST AND SAWDUST VIA ACID PRETREATMENTS FOR POTENTIAL LACTIC ACID BIOCONVERSION

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Agricultural residues, such as spent mushroom compost (SMC) and sawdust (SD), are promising lignocellulosic biomasses for sustainable biochemical production. This study evaluates the structural modification and sugar recovery efficiency from SMC derived from *Pleurotus* and *Schizophyllum* species, as well as sawdust, following pretreatment with sulfuric acid and lactic acid. Structural changes were assessed using Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD), while sugar content was quantified via high-performance liquid chromatography (HPLC). Pretreatments were conducted at 105 °C for 60, 120, and 180 minutes. Sulfuric acid was more effective than lactic acid in enhancing cellulose accessibility and removing hemicelluloses and lignin. The results showed that the highest cellulose content (61.63%) was observed in sulfuric acid-treated SMCS at 180 minutes, while the maximum total sugar yield (80%) was obtained from *Pleurotus* SMC. These findings highlight the potential of acid-pretreated SMC and sawdust as viable substrates for lactic acid bioconversion, laying the groundwork for optimizing pretreatment strategies in biorefineries.

Keywords: spent mushroom compost, sawdust, lactic acid, acid pretreatment, FTIR, XRD, sugar recovery, lignocellulosic biomass

INTRODUCTION

The growing demand for sustainable and renewable feedstocks for industrial bioprocessing has driven interest in lignocellulosic biomass (LCB), particularly agricultural and forestry residues.[1] LCB, derived from plant-based materials, such as agricultural waste, forestry by-products, and grasses, has emerged as a significant source of renewable energy due to its abundance, low cost, and renewability.[2] These attributes make LCB a strong candidate for sustainable energy solutions, contributing to energy diversification and reductions in greenhouse gas emissions. According to the International Energy Agency (IEA, 2023),[3] bioenergy, including energy derived from LCB, accounts for approximately 55% of global renewable energy consumption (excluding traditional biomass use) and over 6% of the total global energy supply, making it the leading source of renewable energy worldwide.

Sawdust (SD), a major by-product of the timber industry,[4,5] and spent mushroom compost (SMC), the residual substrate left after mushroom cultivation,[6,7] are both rich in lignocellulose and represent underutilized materials with high valorization potential. Improper disposal of these materials can contribute to environmental pollution; however, they hold promise as feedstocks for the production of value-added biochemicals, such as biofuels, organic acids, and bioplastics.[8]

LCB typically consists of three primary components: cellulose (35–50%), hemicelluloses (20–35%), and lignin (5–30%).[9,10] These components are tightly bound within the plant cell wall, contributing to the structural rigidity and natural resistance (recalcitrance) of the biomass to enzymatic or microbial degradation. To overcome this recalcitrance and release fermentable sugars, pretreatment methods are required to alter the

physical and chemical structure of the biomass.[11] Among various pretreatment techniques, acid hydrolysis, using either strong mineral acids like sulfuric acid or milder organic acids, such as lactic acid, is commonly employed due to its ability to disrupt hemicelluloses and partially remove lignin, thereby enhancing cellulose accessibility.[12-14]

One of the key emerging applications of lignocellulosic hydrolysates is the production of lactic acid, a versatile platform chemical widely used in food processing, pharmaceuticals, and the manufacture of biodegradable polymers. The economic feasibility of lactic acid production, however, is heavily influenced by the cost and supply of raw materials, which may account for up to 70% of the overall production cost.[15,16] Therefore, exploring cost-effective lignocellulosic feedstocks is crucial for improving the sustainability and scalability of lactic acid production.

The valorization of SMC, particularly from *Pleurotus* (oyster mushroom) and *Schizophyllum* (split-gill mushroom) cultivation, has recently attracted attention due to its rich composition of carbohydrates, proteins, phenolic compounds, and minerals.[17-19] However, there is still a paucity of studies that comprehensively evaluate the structural changes and sugar release efficiency of SMC under various acid pretreatment conditions. While substantial research has been conducted on other lignocellulosic materials, such as sugarcane bagasse,[20,21] specific investigations focused on SMC remain limited.

Thus, this study seeks to address this gap by comparing the effects of sulfuric and lactic acid pretreatments on the structural characteristics and sugar recovery potential of SMC and sawdust. Through the use of Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and high-performance liquid chromatography (HPLC), we aim to evaluate changes in crystallinity, chemical structure, and sugar composition. The findings will contribute to the development of optimized pretreatment strategies for biorefineries focused on sustainable lactic acid bioproduction.

EXPERIMENTAL

Biomass collection and preparation

Raw sawdust, spent mushroom compost used to cultivate *Schizophyllum* (SMCS), and spent mushroom compost used to cultivate *Pleurotus* (SMCP) were sourced from Glami Lemi Biotechnology Research Center (PPGBL), Jelebu (2º 56’ O’’ N, 102º 5’ O’’ E), Negeri Sembilan, Malaysia. The collected biomasses were air-dried to a moisture content below 10%, ground using a Wiley mill to pass through a 1 mm sieve, and stored in airtight plastic bags at room temperature until further use.

Chemical pretreatment

Acid pretreatment was conducted using sulfuric acid (H_2SO_4) and lactic acid ($C_3H_6O_3$) to enhance the digestibility of lignocellulosic components. For each biomass type, 10 g of dry sample was mixed with 100 mL of 1% (v/v) sulfuric acid or lactic acid solution in a 250 mL Erlenmeyer flask. The mixture was autoclaved at 105 °C for varying durations (60, 120, and 180 minutes). After the treatment, the slurry was cooled to room temperature, filtered through Whatman No. 1 filter paper, and the solid residue was washed with distilled water until a neutral pH was achieved. The pretreated solids were oven-dried at 70 °C for 24 hours and stored for subsequent FTIR and XRD analyses.[23,23]

Structural characterization (FTIR and XRD)

Subsequently, the dried samples were ground into fine powders and sieved through a 500 µm to ensure uniform particle size, facilitating consistent spectral acquisition. FTIR spectra were acquired using a PerkinElmer Spectrum 400 FT-IR/NIR spectrometer, which is capable of collecting high-resolution spectral data across a broad wavenumber range. The instrument was configured to record spectra within the 400–5000 cm^{-1} range, encompassing the mid-infrared region pertinent to the identification of functional groups in lignocellulosic materials. Each spectrum was obtained by averaging multiple scans to enhance the signal-to-noise ratio, ensuring accurate and reliable identification of characteristic absorption bands associated with cellulose, hemicelluloses, and lignin components. The resulting spectra were analyzed to determine the presence and alterations of functional groups induced by the pretreatment processes, providing insights into the structural modifications of the biomass constituents.[24,25]

For X-ray diffraction (XRD) analysis, both untreated and pretreated lignocellulosic biomass samples were finely ground and passed through a 500 µm sieve. The sieved material was further refined to obtain particles smaller than 20 µm, which were electrostatically dispersed onto glass Petri dishes. Larger particles were discarded, and the finest particles adhering to the Petri dish surface were collected for analysis.

XRD measurements were then conducted to assess the crystallinity of the samples. The relative degree of crystallinity (CrI) was calculated using the empirical method proposed by Barnette *et al.*[26] and El Hajam *et al.*,[27] which is widely adopted for evaluating cellulose crystallinity in lignocellulosic materials.

Acid hydrolysis and sugar quantification

The pretreated biomass (5 g) was subjected to acid hydrolysis using 100 mL of 1% (v/v) sulfuric acid and lactic acid at 105 °C for 1 hour. The hydrolysate was

cooled, neutralized with calcium carbonate, and filtered. The filtrate was analyzed for cellulose, hemicelluloses, and lignin content using a High-Performance Liquid Chromatography (HPLC) Agilent 1260 System, equipped with a refractive index detector, utilizing a Bio-Rad Aminex HPX-87H column and a carbohydrate analysis column, according to National Renewable Energy Laboratory (NREL) protocols.[28] The mobile phase consisted of deionized water at a flow rate of 0.6 mL/min, and the column temperature was maintained at 65 °C. Calibration curves were prepared using standard solutions of glucose, galactose, mannose, and xylose.

Statistical analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation. Statistical analyses were performed with GraphPad Prism (version 9.0) using ANOVA to determine significant differences among treatment groups, with a significance level set at $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the sample denotations and the treatment conditions, while Figure 1 presents a visual progression of three lignocellulosic biomasses: sawdust, spent mushroom compost from *Pleurotus* species (SMCP), and spent mushroom compost from *Schizophyllum* species (SMCS), as they undergo pretreatment processes, culminating in Figure 1d after sieving, drying, and grinding. This sequence showed the transformation of raw materials into forms more amenable to biochemical analyses and subsequent applications. Sawdust, a by-product of wood processing, is rich in cellulose, hemicelluloses, and lignin. Its fine particulate nature facilitates uniform pretreatment and analysis.

Fourier-transform infrared (FTIR) spectroscopy is a powerful analytical technique used to elucidate the distinct changes in functional groups, chemical structure, and composition of lignocellulosic biomass. In this study, FTIR analysis was employed to assess the structural changes in sawdust, spent mushroom compost from *Pleurotus* species (SMCP), and spent mushroom compost from *Schizophyllum* species (SMCS) before and after acid pretreatment. The FTIR analysis of spent mushroom compost from *Pleurotus* species (SMCP) revealed distinct spectral bands indicative of lignocellulosic and fungal-derived functional groups.

Table 1
Samples denotation and treatment conditions

Raw material	Treatment condition	Sample
Sawdust	Untreated sawdust	USD
	Pretreated with sulfuric acid for 60 min	SDH60
	Pretreated with sulfuric acid for 120 min	SDH120
	Pretreated with sulfuric acid for 180 min	SDH180
	Pretreated with lactic acid for 60 min	SDL60
	Pretreated with lactic acid for 120 min	SDL120
	Pretreated with lactic acid for 180 min	SDL180
Spent mushroom compost used to cultivate *Pleurotus*	Untreated	USMCP
	Pretreated with sulfuric acid for 60 min	SMCPH60
	Pretreated with sulfuric acid for 120 min	SMCPH120
	Pretreated with sulfuric acid for 180 min	SMCPH180
	Pretreated with lactic acid for 60 min	SMCPL60
	Pretreated with lactic acid for 120 min	SMCPL120
	Pretreated with lactic acid for 180 min	SMCPL180
Spent mushroom compost used to cultivate *Schizophyllum*	Untreated	USMCS
	Pretreated with sulfuric acid for 60 min	SMCSH60
	Pretreated with sulfuric acid for 120 min	SMCSH120
	Pretreated with sulfuric acid for 180 min	SMCSH180
	Pretreated with lactic acid for 60 min	SMCSL60
	Pretreated with lactic acid for 120 min	SMCSL120
	Pretreated with sulfuric acid for 180 min	SMCSL180

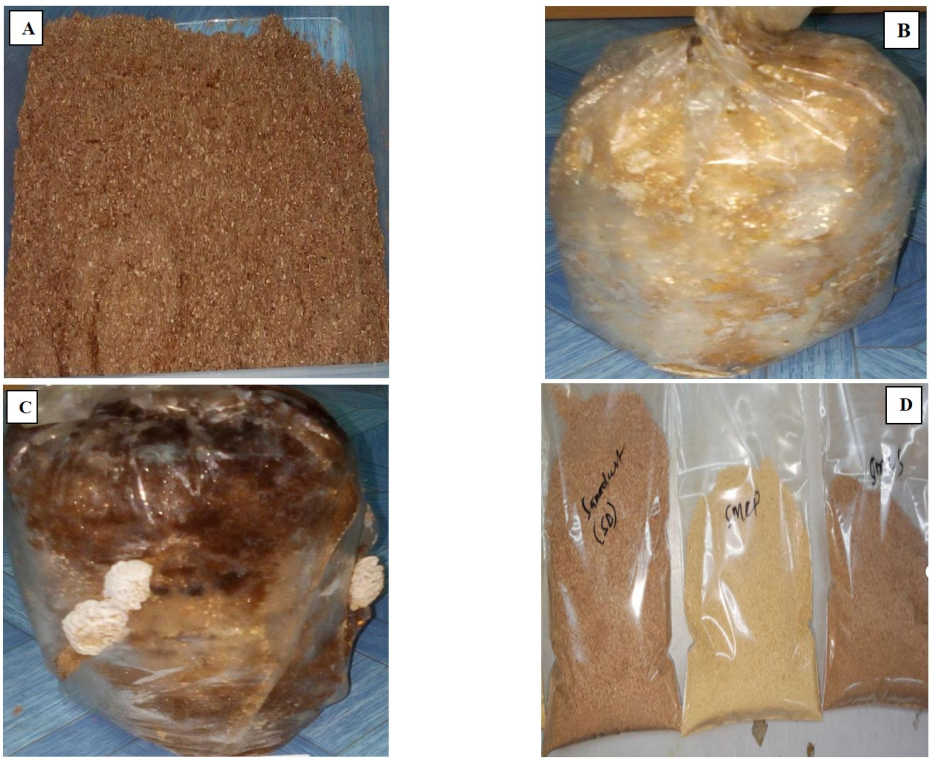

Figure 1: Pictorial representation of (a) untreated sawdust, (b) untreated spent mushroom compost *Pleurotus* (SMCP), (c) untreated spent mushroom compost *Schizophyllum* (SMCS), (d) treated biomasses after sieving, drying, and grinding

The spectrum displayed a broad O–H stretching vibration around 3419 cm^{-1}, consistent with hydroxyl groups found in cellulose and hemicelluloses. A notable peak at 2929 cm^{-1} corresponded to C–H stretching vibrations of aliphatic $–CH_2$ groups. Additionally, a prominent absorption band at 1690 cm^{-1} was attributed to C=O stretching, suggesting the presence of carbonyl groups likely arising from hemicelluloses and lignin degradation. Minor bands observed near 1230 cm^{-1} and 1040 cm^{-1} were associated with C–O and C–C stretching or C–OH bending, further supporting the presence of carbohydrate structures. These results collectively confirm the partial decomposition of lignocellulosic material and the integration of fungal biomass in SMCP (Fig. 2b, Tables 2-4).

Following acid pretreatment, significant reductions in the intensity of the bands at 2929 cm^{-1} and 1690 cm^{-1} were observed, indicating the removal of methylene groups and partial degradation of hemicelluloses and lignin components. The shift and broadening of the O–H band also reflected chemical modification of hydroxyl-rich polysaccharides. These findings align with previous FTIR characterizations of untreated lignocellulosic biomass. For instance, untreated sawdust typically exhibits broad O–H stretching near 3400 cm^{-1}, C–H stretching around 2925 cm^{-1}, and aromatic skeletal vibrations between 1400–1600 cm^{-1}, indicative of lignin content. Post-pretreatment reductions in these peaks, especially at 2925 cm^{-1}, signify the removal of methylene groups and breakdown of structural polysaccharides.[29] FTIR spectra of SMCP reported by Sunkar and Bhukya[30] similarly revealed O–H stretching near 3419 cm^{-1}, C–H stretching at 2929 cm^{-1}, and C=O stretching at 1690 cm^{-1}, affirming the presence of hydroxyl, aliphatic, and carbonyl functional groups, characteristics of cellulose, hemicelluloses, and lignin.

In addition, spent mushroom compost from *Schizophyllum* species (SMCS) contains decomposed lignocellulosic material and fungal residues. Although fewer studies specifically analyze SMCS, FTIR spectra of spent mushroom substrates (SMS) from various fungi, such as those presented by Singh *et al*.,[31] revealed functional groups at 3423 cm^{-1} (O–H), 2921 cm^{-1} (C–H), and 1044 cm^{-1} (C–O/C–C/C–OH), all indicative of lignocellulosic structures (Fig. 2c, Tables 2-4). These bands are also expected in SMCS, although

differences in fungal enzymatic activity and substrate metabolism may result in variations in intensity or peak sharpness.[32-34]

The broad O-H stretching vibration at ~3300 cm^{-1} in all samples indicates the presence of hydroxyl groups in cellulose and hemicelluloses. The intensity and position of this band can provide insights into the hydrogen bonding environment and the degree of crystallinity of the cellulose. After pretreatment, the shifts in C–O–C stretching (~1030 cm^{-1}) and the disappearance of lignin-related peaks at ~1730 cm^{-1} confirm partial removal of lignin and hemicelluloses. The band around 1030 cm^{-1} corresponds to the C–O–C stretching vibrations in cellulose and hemicelluloses. Shifts or changes in the intensity of this band after pretreatment suggest alterations in the polysaccharide backbone, such as the cleavage of glycosidic linkages or changes in the crystallinity of cellulose. These modifications can enhance the accessibility of cellulose to enzymatic hydrolysis, thereby improving the efficiency of biofuel production processes.[35] The absorption band near 1730 cm^{-1} is attributed to the C=O stretching vibrations of acetyl and uronic ester groups in hemicelluloses and the ester linkages of lignin. A decrease or disappearance of this band after acid pretreatment indicates the removal of hemicelluloses and the cleavage of ester bonds in lignin, leading to partial delignification. This observation aligns with previous studies that reported the reduction of hemicelluloses content and lignin disruption upon acid treatment of lignocellulosic biomass.[35]

Notably, the appearance of new peaks near 897 cm^{-1} suggests an increase in amorphous cellulose, corroborating enhanced enzymatic digestibility as discussed in Yao *et al*.[36] This band is associated with the C–H deformation vibrations in β-glycosidic linkages of cellulose and is more prominent in amorphous regions than in crystalline ones. An increase in this band after pretreatment suggests a disruption of the crystalline structure of cellulose, increasing in amorphous regions that are more susceptible to enzymatic attack. This structural change is beneficial for subsequent enzymatic hydrolysis and fermentable sugar production.[37,38]

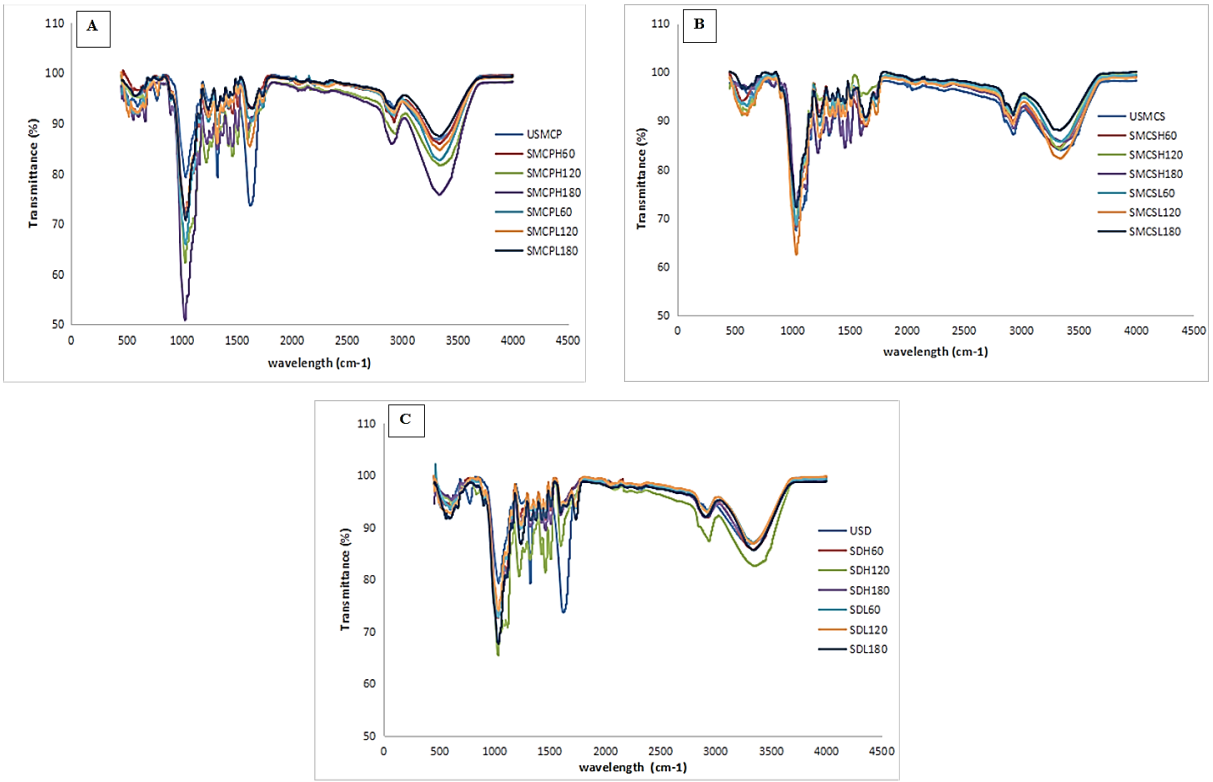

Figure 2: FTIR spectra of untreated and pretreated (a) sawdust, (b) spent mushroom compost used to cultivate *Pleurotus*, (c) spent mushroom compost used to cultivate *Schizophyllum*, with sulfuric acid and lactic acid

Table 2
Changes in absorption bands of untreated and H_2SO_4 and lactic acid treated sawdust

№	Assignment	Vibrations	IR Bands						
			Untreated	SDH60	SDH120	SDH180	SDL60	SDL120	SDL180
1	Cellulose	O-H stretching of hydrogen bonds	3322.77	3334.03	3354.74	3336.45	3336.79	3331.79	3338.57
2	Cellulose	Methylene C-H stretching	2923.65	2915.11	2932.11	2905.11	2911.90	2917.28	2908.32
3	Lignin	Hemicellulose-lignin complex	1732.13	-	-	-	1731.97	1731.49	1732.40
4	Lignin	Aromatic ring vibration	-	1503.54	1502.76	1503.42	-	1505.19	1504.55
5	Cellulose I & II	C-O-C stretching vibration of β (1-4) glycosidic linkage	1426.32	1422.36	1421.39	1421.81	1423.33	1423.46	1423.69
6	Xylan (hemicelluloses)	Acetylated hemicelluloses	1237.41	1225.78	1266.09	1221.76	1235.57	1236.20	1235.57
7	Hemicelluloses	C-O-C asymetric stretching	-	1154.67	-	-	1156.82	-	1156.94
8	Hemicelluloses	C-O stretching	1026.80	1026.60	1027.73	1034.69	1033.01	1032.84	1033.61
9	Amorphous cellulose	C-O-C stretching vibration of β (1-4) glycosidic linkage	-	-	-	894.50	897.55	897.75	897.45

ND (not detected)

Table 3
Changes in absorption bands of untreated and H_2SO_4 and lactic acid treated *Pleurotus* spent mushroom compost

№	Assignment	Vibrations	IR Bands						
			Untreated	SMCPH60	SMCPH120	SMCPH180	SMCPL60	SMCPL120	SMCPL180
1	Cellulose	O-H stretching of hydrogen bonds	3315.17	3328.03	3348.69	3334.03	3329.80	3333.06	3327.03
2	Cellulose	Methylene C-H stretching	2922.82	2922.30	2926.65	2904.82	2910.97	2918.52	2912.50
3	Lignin	Hemicellulose-lignin complex	-	-	-	-	1731.34	1730.29	1727.73
4	Lignin	Aromatic ring vibration	-	1506.06	1504.20	1504.22	1505.32	1506.28	1505.68
5	Cellulose I & II	C-O-C stretching vibration of β (1-4) glycosidic linkage	1418.18	1422.83	1420.67	1421.40	1422.42	1423.48	1424.10
6	Xylan (hemicelluloses)	Acetylated hemicelluloses	1242.31	1224.08	1265.45	1224.97	1239.16	1240.55	1237.09
7	Hemicelluloses	C-O-C asymetric stretching	-	1155.34	-	1156.55	1157.34	1158.28	1158.16
8	Hemicelluloses	C-O stretching	1035.07	1027.32	1031.50	1028.48	1031.78	1032.07	1035.03
9	Amorphous cellulose	C-O-C stretching vibration of β (1-4) glycosidic linkage	-	-	-	895.98	895.98	896.36	-

ND (not detected)

Table 4
Changes in absorption bands of untreated and H_2SO_4 and lactic acid treated *Schizophyllum* spent mushroom compost

№	Assignment	Vibrations	IR Bands						
			Untreated	SMCSH60	SMCSH120	SMCSH180	SMCSL60	SMCSL120	SMCSL180
1	Cellulose	O-H stretching of hydrogen bonds	3313.63	3323.12	3346.39	3353.32	3328.93	3328.86	3319.30
2	Cellulose	Methylene C-H stretching	2922.55	2904.74	2925.98	2929.05	2921.44	2921.10	2923.19
3	Lignin	Hemicellulose-lignin complex	-	-	-	-	1730.10	1730.22	1730.33
4	Lignin	Aromatic ring vibration	1506.31	1505.31	1503.38	1503.73	1505.92	1506.66	1509.48
5	Cellulose I & II	C-O-C stretching vibration of β (1-4) glycosidic linkage	1422.80	1422.59	1421.64	1420.90	1422.15	1423.06	1422.77
6	Xylan (hemicelluloses)	Acetylated hemicelluloses	1235.35	1265.59	1220.75	1220.12	1234.70	1235.80	1232.92
7	Hemicelluloses	C-O-C asymetric stretching	-	1158.33	-	-	-	1154.57	1155.60
8	Hemicelluloses	C-O stretching	1030.81	1027.29	1028.99	1027.72	1032.74	1032.61	1034.11
9	Amorphous cellulose	C-O-C stretching vibration of β (1-4) glycosidic linkage	-	897.95	-	-	896.93	898.16	-

ND (not detected)

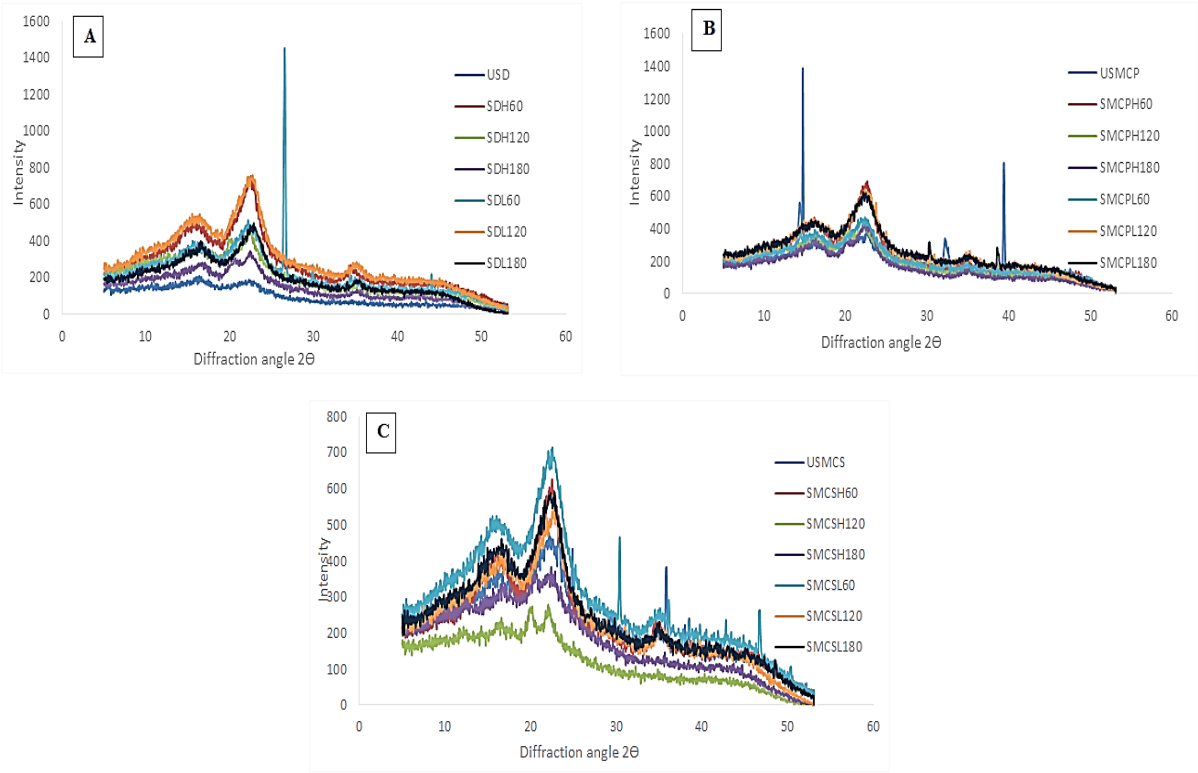

Figure 3: XRD patterns of untreated and pretreated (a) sawdust, (b) spent mushroom compost used to cultivate *Pleurotus*, (c) spent mushroom compost used to cultivate *Schizophyllum*, with sulfuric acid and lactic acid at 105 °C for 60, 120, and 180 min

Additionally, the persistence of aromatic ring vibrations (~1500 cm^{-1}) in acid-treated samples indicates incomplete lignin removal, consistent with findings by Yao *et al*.,[36] Bekiaris *et al*.[37] and Asim *et al*.[39]

X-ray diffraction (XRD) results show a notable increase in crystallinity index (CrI%) following sulfuric acid pretreatment across all three biomass types: sawdust, SMCP, and SMCS. Sawdust pretreated with H_2SO_4 for 60 min (SDH60) showed the highest CrI% (44.45%) compared to the untreated sample (1.34%) (Fig. 3, Table 5). Similar trends were observed for SMCP and SMCS, indicating that acid pretreatment removes amorphous components, such as hemicelluloses and lignin, thereby enriching crystalline cellulose fractions. This is supported by Zhang *et al*.,[40] who reported that dilute acid treatment effectively removes amorphous regions, enhancing cellulose crystallinity in lignocellulosic biomass.

However, lactic acid pretreatment showed a more moderate increase in CrI%, suggesting milder delignification and hemicelluloses removal, which is in line with Liu *et al*.,[41] who found that organic acid pretreatment preserves more hemicelluloses compared to inorganic acids.

Acid pretreatments, as shown in Table 6, significantly ($p<0.05$) increased cellulose content (up to ~60% in SDH120 and SMCPH180), while hemicelluloses decreased significantly ($p<0.05$), especially in H_2SO_4-treated samples. This supports effective hydrolysis and solubilization of hemicelluloses during pretreatment.[42] Cellulose increased from 39.49% (USD) to 60.77% (SDH120), and hemicelluloses dropped from 6.60% to 1.01%. These results align with the findings of Kumar *et al*.,[43] who reported the selective ability of sulfuric acid to break down hemicelluloses. Conversely, lactic acid pretreatment was less effective at hemicelluloses removal, retaining values close to the untreated samples (9%), corroborating the findings of Liu *et al*.,[44] who reported that lactic acid pretreatment, which is selective and environmentally friendly, but less aggressive than inorganic acids, may lead to partial delignification and moderate hemicelluloses reduction.

Table 5
Percentage crystallinity index of untreated and pretreated biomass

Sample	Crystallinity index (CrI%)
USD	1.34
SDH60	44.45
SDL60	30.79
SDH120	27.41
SDL120	37.77
SDH180	26.41
SDL180	19.57
USMCP	23.49
SMCPH60	44.05
SMCPL60	22.25
SMCPH120	30.73
SMCPL120	36.60
SMCPH180	39.57
SMCPL180	38.30
USMCS	22.65
SMCSH60	40.95
SMCSL60	30.85
SMCSH120	26.79
SMCSL120	23.13
SMCSH180	18.76
SMCSL180	33.80

Table 6
Chemical composition of various untreated and pretreated biomass samples used in this study

Sample	Cellulose (%)	Hemicelluloses (%)	Lignin (%)	Others (%)
USD	39.49 ± 1.39^{b}	6.60 ± 0.21^{c}	23.61 ± 1.33^{a}	30.24 ± 0.31
SDH60	56.41 ± 0.62^{c}	1.22 ± 0.52^{b}	31.26 ± 0.05^{b}	10.56 ± 1.31
SDH120	60.77 ± 1.53^{c}	1.01 ± 0.09^{b}	18.52 ± 4.78^{a}	17.55 ± 3.13
SDH180	60.64 ± 1.54^{c}	0.89 ± 0.12^{a}	35.22 ± 4.32^{c}	30.05 ± 5.97
SDL60	38.58 ± 0.22^{b}	7.99 ± 0.60^{c}	27.49 ± 0.12^{a}	25.91 ± 0.68
SDL120	38.08 ± 0.93^{b}	8.63 ± 0.82^{c}	27.58 ± 0.00^{a}	25.63 ± 1.75
SDL180	40.64 ± 0.65^{b}	8.99 ± 0.16^{c}	27.12 ± 0.30^{a}	23.17 ± 0.91
USMCP	29.73 ± 0.89^{a}	6.85 ± 0.77^{c}	23.68 ± 4.26^{a}	38.56 ± 6.09
SMCPH60	56.47 ± 1.10^{c}	1.44 ± 0.24^{b}	29.91 ± 0.25^{b}	11.55 ± 1.45
SMCPH120	60.39 ± 0.40^{c}	1.19 ± 0.01^{b}	29.25 ± 1.26^{b}	8.76 ± 1.66
SMCPH180	61.63 ± 1.75^{c}	1.12 ± 0.16^{b}	30.44 ± 0.25^{b}	6.08 ± 2.25
SMCPL60	37.69 ± 1.48^{b}	7.54 ± 0.04^{c}	22.88 ± 2.01^{a}	30.36 ± 1.83
SMCPL120	35.17 ± 3.22^{b}	8.86 ± 1.04^{c}	23.96 ± 0.16^{a}	$31.2\ 0 \pm 2.08$
SMCPL180	40.73 ± 1.29^{b}	8.89 ± 0.13^{c}	24.59 ± 0.09^{a}	25.74 ± 1.38
USMCS	25.99 ± 2.31^{a}	6.98 ± 0.93^{c}	28.70 ± 0.06^{b}	37.94 ± 3.20
SMCSH60	49.40 ± 1.38^{c}	1.45 ± 0.38^{b}	38.09 ± 0.15^{c}	9.72 ± 1.89
SMCSH120	54.41 ± 0.19^{c}	1.37 ± 0.38^{b}	38.59 ± 0.37^{c}	3.74 ± 0.74
SMCSH180	56.24 ± 0.14^{c}	1.04 ± 0.05^{b}	38.81 ± 0.67^{c}	2.50 ± 0.85
SMCSL60	32.98 ± 2.17^{a}	8.17 ± 1.66^{c}	29.55 ± 0.91^{b}	27.54 ± 3.22
SMCSL120	35.63 ± 4.27^{b}	8.84 ± 0.60^{c}	33.17 ± 0.21^{b}	21.74 ± 3.41
SMCSL180	35.38 ± 0.41^{b}	10.12 ± 0.13^{c}	32.24 ± 0.00^{b}	20.87 ± 0.81

Table 7
Sugars produced before and after the pretreatment of biomass with sulfuric or lactic acid

Biomass sample	Glucose (%)	Xylose (%)	Galactose (%)	Mannose (%)
USD	39.49	6.59	0.00	ND
SDH60	56.41	ND	1.22	ND
SDH120	60.77	ND	1.01	ND
SDH180	60.64	ND	0.00	0.89
SDL60	38.58	ND	ND	ND
SDL120	38.08	ND	8.63	ND
SDL180	40.64	ND	8.99	ND
USMCP	29.73	6.85	ND	ND
SMCPH60	56.47	ND	1.44	ND
SMCPH120	60.39	ND	ND	1.19
SMCPH180	61.63	ND	ND	1.12
SMCPL60	37.69	7.54	ND	ND
SMCPL120	35.17	ND	8.86	ND
SMCPL180	40.73	ND	8.89	ND
USMCS	25.99	6.97	ND	ND
SMCSH60	49.40	ND	1.45	ND
SMCSH120	54.41	ND	1.37	ND
SMCSH180	56.24	ND	ND	1.04
SMCSL60	32.98	8.17	8.84	ND
SMCSL120	35.63	ND	10.12	ND
SMCSL180	56.24	ND	ND	1.04

*ND = not detected

The analysis of sugar produced before and after the pretreatment of biomass with sulfuric and lactic acid in Table 7 showed that pretreatment with H_2SO_4 significantly ($p<0.05$) increased glucose yield, with SDH120 (60.77%) and SMCPH180 (61.63%) outperforming untreated and lactic acid-treated samples. The absence of xylose in most H_2SO_4-treated samples confirms extensive hemicellulose degradation, consistent with the findings of the chemical composition. Meanwhile, galactose and mannose were more prominent in lactic acid-treated samples, especially in SDL120 and SMCPL120, suggesting lactic acid preserves or selectively hydrolyzes certain polysaccharides. This nuanced sugar profile is essential for tailored bioconversion pathways.[45,46]

CONCLUSION

This study demonstrates that sulfuric acid pretreatment significantly enhances cellulose accessibility, increases crystallinity, and improves sugar yield across all tested biomass types, especially *Pleurotus* and *Schizophyllum* spent mushroom composts. The amount of cellulose obtained and lignin removed from the biomasses pretreated with the two treatments increased as the time of pretreatment increased from 60 to 180 minutes, with SMCPH180 having the highest cellulose percentage of 61.63% and SMCSH180 with the highest lignin percentage of 38.81% removed. In contrast, lactic acid pretreatment retains more hemicelluloses, with SMCSL180 having the highest content (10.12%). The HPLC analysis also revealed that the maximum conversion of the carbohydrates in biomass to glucose, xylose, galactose, and mannose was obtained from SMCPH180. These results provide critical insights into how acid pretreatments influence structural and compositional changes in lignocellulosic biomass and lay a solid foundation for their utilization in downstream microbial fermentation processes.

Consequently, all the results obtained indicated that both types of spent mushroom compost biomass are highly viable, sustainable, and cost-effective raw materials for producing lactic acid due to their high lignocellulosic content. Also, spent mushroom compost is a by-product of mushroom cultivation, often available in large quantities, which makes it an excellent candidate for agricultural waste valorization and better potential substrate for sustainable lactic acid production compared to sawdust. However, more environmentally friendly and effective biomass pretreatment technologies that could bring the notion of sustainable biorefineries to reality and overcome the resistant nature of lignocellulosic biomass, and guarantee the highest recovery of

sugars and other bioproducts with commercial relevance should be developed.

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