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Research Article

Approach to Sustainable Fibers from Spent Mushroom Substrate for Future All-Natural-Materials

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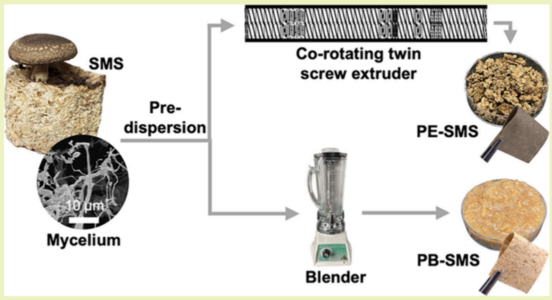
ABSTRACT: Spent mushroom substrates (SMS), a lignocellulosic residue from mushroom cultivation, represent a promising raw material for the valorization of nontoxic materials supporting the circular bioeconomy. The inherent biological pretreatment of the birch wood substrate during shiitake cultivation reduces the need for chemicals prior to fibrillation. SMS was fibrillated using an extruder and a blender at high (28 wt %) and low (5 wt %) solid contents, respectively, with and without a predispersion step. Extrusion proved to be the most energy-efficient method, requiring only 11 kWh/t, compared with 417 kWh/t for blending. When combined with predispersion, extrusion is the second most energy-efficient fibrillation method (789 kWh/t), compared to blending with predispersion (1195 kWh/t). Microscopy and fiber fractionation confirmed fibrillation into microfibers after extrusion and the presence of residual mycelium. Sheet formation by vacuum filtration over a coarse mesh significantly lowered the filtration time compared to a fine filter. Sheets produced from fibrillated SMS possessed tensile strength up to 7.5 times higher than commercial birch kraft pulp sheets prepared under the same conditions. The improved tensile strength is due to the presence of mycelial fibrils, which enhanced fiber−fiber bonding. Overall, extrusion provides a scalable, energy-efficient route for SMS fibrillation for the production of future all-natural materials without the need for chemical modification.



KEYWORDS: *spent mushroom substrate, biological pretreatment, efficient fibrillation, energy consumption, sheet properties*

INTRODUCTION

The circular bioeconomy integrates economic, social, and environmental goals to reduce reliance on fossil resources, promoting a sustainable, carbon-neutral future.[1] The utilization of spent mushroom substrate (SMS) − lignocellulosic biomass residues after mushroom cultivation − for sustainable material development aligns with circular bioeconomy. Prior to mushroom cultivation, the substrate typically comprises wood residues such as sawdust, with smaller amounts of wheat bran and grains serving as a nutrient source.[2,3] Approximately 5 kg of SMS is generated per kg of mushrooms produced.[4] The growing demand for mushrooms, as evidenced by 14% increased European mushroom production from 2017 to 2021,[5] will lead to much greater volumes of SMS that require proper resource management. Current disposal methods for SMS include composting and mulch production for soil quality enhancement.[6,7] Higher value alternatives for SMS utilization have since been explored, which include energy production[2,3] and as a raw material source; fibrillation of SMS into micro- or nanosized wood fibers presents as a promising strategy to develop sustainable materials from mushroom production side streams.[8] Lignocellulosic biomass is typically fibrillated by mechanical means exerted in a blender,[9] ultrafine grinder,[10] or high-pressure homogenizer.[11] These fibrillation methods often process only low solid contents (around 2 wt %) and are more effective when combined with chemical and/or enzymatic pretreatments of the raw material.[12] Uetani and Yano[9] showed that lignin removal from softwood pulp followed by fibrillation in a blender for 30 min at low solid content (0.1 wt % to 1.5 wt %) yielded quality cellulose nanofibrils (CNF) with a comparable level of fibrillation to an ultrafine grinder (Masuko) but with less fibril damage.[9] SMS has also been used as a raw material for fibrillation; Konno et al.[13] used 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation as pretreatment of

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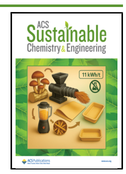

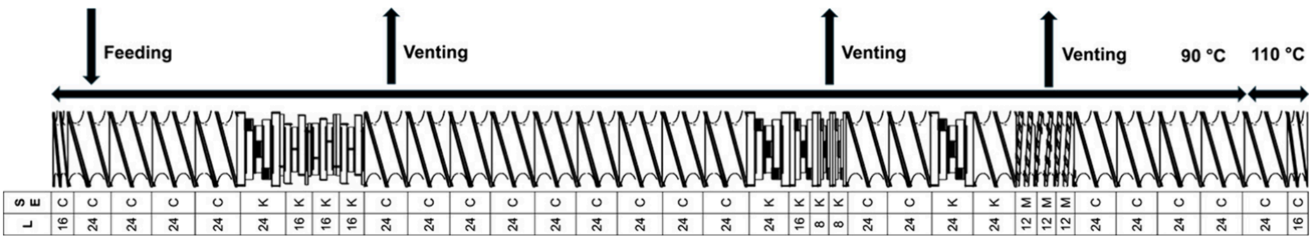

Figure 1. Screw configuration of the corotating twin-screw extruder, showing the arrangement of screw elements (SE) comprising conveying (C), kneading (K), and mixing (M) sections with their respective lengths (L). The temperature was set to 90 °C in the first eight zones and 110 °C in the final zone. SMS was continuously fed into the extruder, and the venting zones were used for moisture removal.

SMS to produce cellulose nanofibrils by sonication. Li et al.[14] extracted chitin/cellulose nanofibrils using elaborate chemical pretreatments prior to ultrafine grinding (Masuko) of SMS.

In contrast to fibrillation methods for processing of low solid content raw materials, extrusion has been shown to be an energy-efficient method to fibrillate high solid content (>25 wt %) wood particles,[15] cellulose pulp,[16] sawdust,[17] and SMS[18] into micro/nanofibrils. By applying shear and compressive forces via rotating screws, extrusion not only breaks down the raw material but also facilitates fibrillation through a combined mechanical and thermal action. Hietala et al.[15] demonstrated that twin-screw extrusion effectively fibrillated spruce wood chips into cellulose microfibrils when using reverse screw elements and higher screw speeds. Building on this, Taheri et al.[19] demonstrated the feasibility of one-step fibrillation of 40 wt % cellulose pulp using hydroxyethyl cellulose as a processing aid during extrusion. Later, Taheri et al.[17] reported enhanced fibrillation of wood sawdust pretreated with deep eutectic solvent (DES) when compared to untreated wood sawdust. However, chemical pretreatments add cost and an environmental burden.

Aim of the Work

We aim to leverage the inherent biological pretreatment of birch wood particles during shiitake production to develop future all-natural materials without using either chemicals or polymers that cause disposal issues. We explore energy-efficient fibrillation of a spent mushroom substrate (SMS) into a mix of wood fibers and mycelial fibrils combined with a rapid sheet-forming process as a model approach for production of novel molded fiber products. SMS fibrillation is performed with high solid throughput in a twin-screw extruder and compared with fibrillation in a blender. Additionally, we investigated whether the wood mycelial fiber mix results in improved sheet properties over those of commercial birch kraft pulp. In our previous studies,[18,20] we used the same raw material for fibrillation by ultrafine grinding at solid content of 6.5 wt % and by extrusion at a solid content of 28 wt %. We demonstrated superior life-cycle performance compared with commercial birch kraft pulp (BKP) and confirmed that fibrillation in an extruder is both feasible and promising; however, both studies were focusing on ultrafine ground materials. The novelty of the present work lies in the optimization of the fibrillation process of the SMS using an extruder and a blender with and without a predispersion process-step. In addition, we investigated the associated energy consumption and developed a sheet formation process that could potentially be extended to molded fiber products.

■ EXPERIMENTAL SECTION

Materials

Spent mushroom substrate (SMS) originally consisting of birch (*Betula pubescens*) particles (<5.0 mm in size) after cultivation and harvesting of shiitake fruiting bodies (*Lentinula edodes*) after 74−81 days was provided by the Mushroom Laboratory, Swedish University of Agricultural Sciences, Umeå, Sweden. The SMS was collected and stored at −18 °C prior to use. The chemical composition of SMS was disclosed in Xiong et al.:[2] lignin content of 10.6 ± 0.1 wt % and polysaccharide content comprising cellulose, hemicellulose, and glucan of 68.1 ± 0.8 wt %. As reference material, we used a commercial birch kraft pulp (BKP) kindly supplied by SCA (Munksund, Sweden). The chemical composition of BKP was disclosed in Berglund et al.:[20] lignin content 5.0 ± 0.1 wt % and polysaccharide (here only cellulose and hemicellulose) content 91.0 ± 0.9 wt %. Distilled water was used in all of the processing steps.

SMS Fibrillation

The SMS was defrosted at room temperature and manually disintegrated. The solid content of SMS determined using a moisture analyzer (HR83 Halogen, Mettler Toledo, Ohio, USA) was found to be 45 wt %. SMS was soaked in warm water (50 °C) for 2 d. Excess water was manually squeezed out from the material and the solid content determined to be 25 wt %. Four different fibrillation processes were investigated: extrusion and blending with and without predispersion. First, fibrillation of SMS was carried out in a corotating twin-screw extruder (ZSK-18 MEGALab, Coperion W&P, Stuttgart, Germany) using the screw configuration shown in Figure 1 at a throughput of 7 kg/h. This material is hereafter referred to as E-SMS. Prior to extrusion, the solid content of SMS was adjusted to 28 wt % in an oven at 90 °C. The extruder was operated at a screw speed of 120 rpm, with the temperature profile set to 90 °C in the first eight zones and to 110 °C in the final zone to remove moisture and increase the solid content. The residence time of E-SMS during fibrillation was approximately 3 min. Extrusion was performed without a die to minimize the back pressure and prevent clogging from dried wood fibers, and moisture was removed through three atmospheric vents.

Second, SMS was fibrillated using a mechanical blender (WARING Laboratory Blender, VWR International AB, Stockholm, Sweden) hereafter referred to as B-SMS. During blending, the solid content was maintained at approximately 5 wt %, and the material was processed for 10 min at 7000 rpm.

Moreover, SMS was predisintegrated using a high-speed disperser (Ultra-Turrax, IKA T25 digital, Staufen, Germany) for 10 min at 13000 rpm. The predispersed SMS was fibrillated in a corotating twin-screw extruder at solid content of 28 wt % (referred to as PE-SMS) and in a blender at a solid content of 5 wt % (referred to as PB-SMS). All fibrillated SMS materials were held at 8 °C after adding a few drops of acetic acid to prevent mold growth.

Sheet Formation

Sheets with a target grammage of 150 g/m^2 (gsm) were prepared by vacuum filtration (VCP 80, VWR International AB, Stockholm, Sweden) at 900 mbar using three different filters: Durapore PVDF filter with a pore size of 0.1 μm (Duran, Millipore, Darmstadt, Germany), cellulose filter with a pore size of 7 μm (Whatman grade

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52, Sigma-Aldrich, Darmstadt, Germany; unfortunately it failed (see Figure S6 in Supporting Information)) and nylon mesh with 140 US mesh. The materials were diluted to the same solid contents of 0.3 wt % and stirred for 60 min prior to vacuum filtration. Three sheets were produced, and the filtration time was measured. After filtration, wet filter cakes were carefully peeled from the filter, sandwiched between metal meshes and tissue papers to remove excess water, and then placed between aluminum plates (300 mm × 300 mm). The sandwiched sheets were placed in a vacuum oven (NSV 9000, SVENSKA LABEX AB, Helsingborg, Sweden) under a load of 50 N for 35 min at 80 °C to remove water. The partially dried sheets were placed between BoPET films (100 MICRON MYLAR, Lohmann Technologies, Milton Keynes, UK) and aluminum plates for compression molding (LabEcon 300, Fontijne Press, Vlaardingen, The Netherlands) at a pressure of 4 MPa at 100 °C for 10 min and cooled down to 20 °C under applied pressure. The final grammage of the prepared sheets was measured, and the average with standard deviations was reported.

Viscosity Measurements

The efficiency of fibrillation was indirectly evaluated using a Vibro-Viscometer (SV-10, A&D Company, Oxford, UK) at a concentration of 5 wt % and at temperature of 21 °C. Measurements were performed in triplicate over 2 min until a viscosity plateau was reached, and the average viscosity values were reported with standard deviations.

Fiber Size Measurement

To determine the fiber and fibril dimensions, all samples were diluted in distilled water to a concentration of 0.3 wt % and dispersed using a magnetic stirrer. Prior to fractionation, all suspensions were passed through a 2.5 mm sieve to remove large particles that could potentially clog the fractionator. The procedure was performed twice: first to determine the dry mass of the material retained on the sieve and second to analyze the dimensions of the retained larger fibers on the sieve using stereomicroscopy (SMZ 1270, BergmanLabora, Danderyd, Sweden). Each filtrate was analyzed using a tube flow fractionator (Metso, Valmet Automation Oy, Kajaani, Finland), measuring approximately 20000 fiber fragments per sample. Five fiber length fractions were obtained: F1 (3.2−2.0 mm), F2 (2.0−1.2 mm), F3 (1.2−0.6 mm), F4 (0.6−0.2 mm), and F5 (<0.2 mm), all with a fiber width greater than 10 μm. All fiber length measurements were conducted in accordance with TAPPI T271 and ISO 16065 standards. Fines were further categorized into five different fractions: A1 (0.20−0.16 mm), A2 (0.16−0.12 mm), A3 (0.12−0.08 mm), A4 (0.08−0.04 mm), and A5 (0.04−0.00 mm).

Polarized Optical Microscopy (POM)

The fiber morphology of fibrillated SMS and BKP was examined using polarized optical microscopy (Nikon Eclipse LV100N POL, BergmanLabora AB, Danderyd, Sweden), using Nikon imaging software (NIS)-Elements D 4.30). Samples were analyzed at a concentration of 0.25 wt %.

Scanning Electron Microscopy (SEM)

The morphology of the fibrillated SMS was examined using scanning electron microscopy (JEOL JSM-IT300LV, JEOL Nordic AB, Sollentuna, Sweden) operated at an acceleration voltage of 15 kV. The sample preparation was carried out as follows: fibrillated SMS dispersions were diluted to 0.1 wt %, and a drop of the dispersion was pipetted onto carbon tape mounted on a metal stub. The samples were immediately frozen in liquid nitrogen and subsequently freeze-dried (MARTIN CHRIST Alpha 1−4 LSC plus, SVENSKA LABEX AB, Helsingborg, Sweden). Freeze-drying was carried out in two stages: initially at −55 °C and 1 mbar for 40 h followed by further drying at 0.1 mbar for 1 h. For cross-sectional analysis, sheets were frozen at −24 °C for 24 h and manually fractured. Prior to imaging, all samples were sputter-coated with a 15 nm platinum layer by using a sputter coater (EM ACE200, Leica, Wetzlar, Germany).

Envelope Density

The density ρ_e of the fibrillated SMS sheets and BKP sheets was calculated for five tensile specimens by dividing sheet mass m by volume of the sheets:

$$\rho_e = \frac{m}{Ad} \tag{1}$$

where A is the specimen area for 50 × 5.9 mm^2 and d is their thickness (indicated in Table 1). The average envelope density is reported with the standard deviation.

Table 1. Average Sheet Grammage Γ, Thickness *t*, and Envelope Density ρ_e of Sheets Prepared by Vacuum Filtration from Predispersed SMS Fibrillated in an Extruder (PE), in a Blender (PB), and BKP Suspensions Produced by Filtration over a PVDF Filter (*) and Nylon Mesh

materials	Γ [gsm]	t [μm]	ρ_e [g cm^{-3}]
PE-SMS*	144 ± 1	205 ± 23	0.71 ± 0.09
PE-SMS	137 ± 3	280 ± 20	0.61 ± 0.02
PB-SMS*	136 ± 2	240 ± 11	0.52 ± 0.04
PB-SMS	131 ± 2	310 ± 10	0.39 ± 0.20
BKP*	149 ± 1	214 ± 3	0.53 ± 0.02
BKP	143 ± 2	248 ± 21	0.51 ± 0.02

Fourier-Transform Infrared (FTIR) Spectroscopy

The chemical composition of fibrillated SMS and BKP sheets was analyzed using attenuated total reflectance FTIR (Bruker Tensor II, Ettlingen, Germany). Measurements were performed 32 times in the wavenumber range 400−4000 cm^{-1} at a resolution 4 cm^{-1}.

Mechanical Properties

The tensile properties of the compression-molded sheets were measured by using a universal testing machine (Shimadzu AGX-V, BergmanLabora AB, Danderyd, Sweden) equipped with a 500 N load cell. Test specimens (50 × 5.9 mm^2) were cut from sheets using a precision punch, and the gauge length of the test specimens was 30 mm. All samples were conditioned at 22 °C and 50% RH for 48 h prior to testing. Thickness was measured at three different locations using a micrometer gauge (Digital Outside Micrometer 0−25 mm, AnyiMeasuring, Guilin City, P.R. China). Tensile tests were performed at a strain rate of 3 mm min^{-1}, and stroke-to-failure for all samples was calculated from the grip displacement. Young's modulus was determined from the initial linear region of the stress−strain curve (0.01 and 0.2% strain), and the ultimate tensile strength (UTS) was recorded. Results are reported as averages and standard deviations from up to 10 individual specimens obtained from three different sheets. Specimens fractured within the grips were excluded from the analysis.

Specific Energy Consumption (SEC)

The specific energy consumption was quantified differently for twin-screw extrusion, blending, and predispersion. The SEC [kWh/t] for twin-screw extrusion was determined as follows:[21]

$$\mathrm{SEC} = \frac{N \times \tau \times P_{max}}{N_{max} \times Q} \tag{2}$$

where N is the screw speed (120 rpm), N_{max} is the maximum screw speed (1200 rpm), τ is the torque (%), which was around 8%, P_{max} is the maximum motor power (10 kW), and Q is the mass throughput (7 kg dry matter/h). As per the manufacturer's specifications, a factor of 0.95 is typically applied to P_{max} to account for gearbox losses. For the predispersion step and blending, the *SEC* was determined using eq 3:

$$\mathrm{SEC} = \frac{\mathrm{PR} \times t}{m_d} \tag{3}$$

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where PR is the power rating (0.375 kW for the blender and 0.7 kW for homogenizer), t is the blending time for both processes (10 min), and m_d is dry weight of the material (0.15 kg). The SEC for fibrillation of PE-SMS (predispersion step followed by extrusion) and PB-SMS (predispersion step followed by blender) was calculated by adding the corresponding SEC for the fibrillation processes.

■ RESULTS AND DISCUSSION

Fibrillation of SMS

Shiitake cultivation on a birch wood particle substrate followed by harvesting mushrooms and utilization of SMS is illustrated in Figure 2a,b. The appearance of SMS after fibrillation is shown in Figure 2d−e'. The different appearance was caused by actual solid contents at the end of the fibrillation process (38 wt % for extrusion versus 5 wt % for blending); extrusion permitted processing of higher solid content materials. SMS fibrillated in an extruder without and with a predispersion step (E-SMS and PE-SMS) had a dark brown appearance (Figure S1 in Supporting Information), while that fibrillated in a blender (B-SMS and PB-SMS) had a similar color to the birch wood substrate with distributed pigment particles (Figure 2d−e'). The color change is caused by improved disintegration and dispersion of the melanin-rich dark brown mycelium regions[22] (see Figure 2a,b) in the extruder.

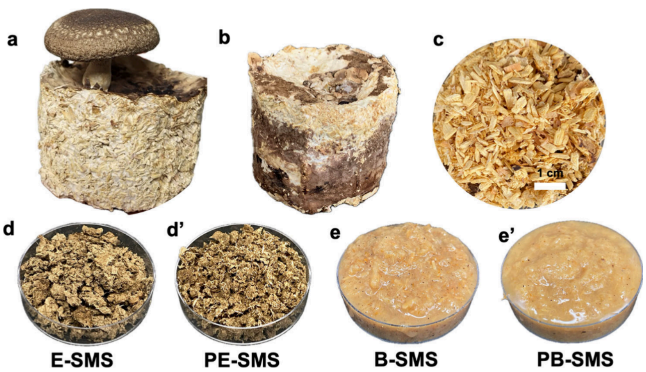

Figure 2. (a) Shiitake grown on a birch wood particle substrate, (b) SMS after shiitake harvest, (c) manually SMS disintegrated, (d) appearance of fibrillated SMS after extrusion (E-SMS), (d') after predispersed and extrusion (PE-SMS) both with a solid content of 38 wt %, (e) after blending (B-SMS), and (e') after predispersion and blending (PB-SMS) both with a solid content of 5 wt %.

Commonly, viscosity is measured as an indirect indicator of the degree of fibrillation: a higher viscosity typically suggests a greater degree of fibrillation and corresponding network formation in dispersion.[8,20,23] We measured the viscosity of extruded (E and PE) and blended (B and PB) materials; the extruded E and PE fiber dispersions possessed a slightly higher viscosity of 79 ± 10 and 90 ± 20 mPa•s, respectively, when compared to blended B and PB fiber dispersions with viscosities of 47 ± 2 and 60 ± 5 mPa.s, respectively. These measured viscosities are more than 1 order of magnitude lower compared to nanofibrillated SMS,[20] indicating no network formation. However, the viscosities show that the extrusion process combined with a predispersion step resulted in a higher degree of fibrillation as compared to the blending process.

The fibrillation efficiency of SMS was characterized by fiber size measurements following fractionation and compared to commercial birch kraft pulp (BKP). Prior to fractionation of SMS materials, larger wood shives (>2.5 mm) were removed (see Figure S2 in Supporting Information). Extruded SMS materials contained a much smaller fraction of large shives compared to blended SMS (4 vs 14 wt %), while a predispersion step significantly reduced the fraction of large shives by a factor of 4. The results shown in Figure 3a show that fibers can be found in all fractions. The fraction F1 containing the longest fibers shows that the fiber dimensions after SMS extrusion are comparable to those of BKP but in lower amounts (Figure 3b), while SMS fibrillated in a blender appeared to be bundles. Fraction F3 is dominated by BKP fibers, while F4 and F5 are dominated by SMS fibers. Further analysis of the fines in fraction F5 is shown in Figure 3c. The fine fraction is completely dominated by the SMS material compared to BKP, and this is expected to be due to the presence of mycelium fibrils. The average fiber width of different fractions is summarized in Table S1 (Supporting Information). Notably, extruded SMS (E and PE) had a smaller fiber width in the larger fractions (F1−F4) compared to blended materials but significantly larger than the BKP, except for the fines fraction.

Both extruded SMS (E and PE) materials had a higher fines fraction (Figure 3c, A4 and A5) compared to the blended ones (B and PB), indicating a higher degree of fibrillation. In contrast to the SMS fibrillated by extrusion, the blended SMS (B and PB) still contains larger particles as seen in polarized optical microscopy images (Figure 4a). In addition to the higher degree of disintegration caused by extrusion, SEM micrographs (Figure 4b) confirmed the presence of thread-like filaments making up the mycelium. Higher magnification SEM images in the Supporting Information (Figure S3) confirm the presence of hyphae from mycelium present in these materials.

SMS Sheets Prepared from Fibrillated Fibers

We tested different filters for sheet formation. The average filtration time, sheet grammage, envelope density, and mechanical properties of the sheets were evaluated. The average filtration time of fibrillated SMS and BKP suspensions during sheet formation over a PVDF filter was 186 ± 16 min. To reduce filtration times, a Nylon mesh was selected, resulting in significantly reduced average filtration times of 30 ± 12 s. The fibrillated SMS sheets had hues of brown as compared to the pure white BKP sheets due to the presence of residual lignin and fungal pigments remaining in the SMS after shiitake cultivation (Figure 5). Notably, only the sheets produced from fibrillated SMS by filtration over a PVDF filter exhibited two different sides; the darker one corresponded to the surface in contact with the filter (Figure S4a in the Supporting Information), while the opposite one had a lighter hue (Figure S4b in the Supporting Information). The fiber suspension is filtered against the filter medium − larger fibers initially form the filter cake, while smaller fibrils penetrate the filter cake formed and were retained on the filter surface, producing a smoother haptic. The darker color was likely caused by the fibrils from the mycelium. In contrast, filtration through the coarser Nylon mesh causes partial loss of fine fibers and mycelium (Figure S5 in the Supporting Information), resulting in a more homogeneous optical appearance but coarser touch. Material loss − fines, finer fibers, and mycelium − during filtration through the coarser Nylon mesh resulted in a reduced grammage of the produced sheets as compared to those prepared using the PVDF filter (Table 1).

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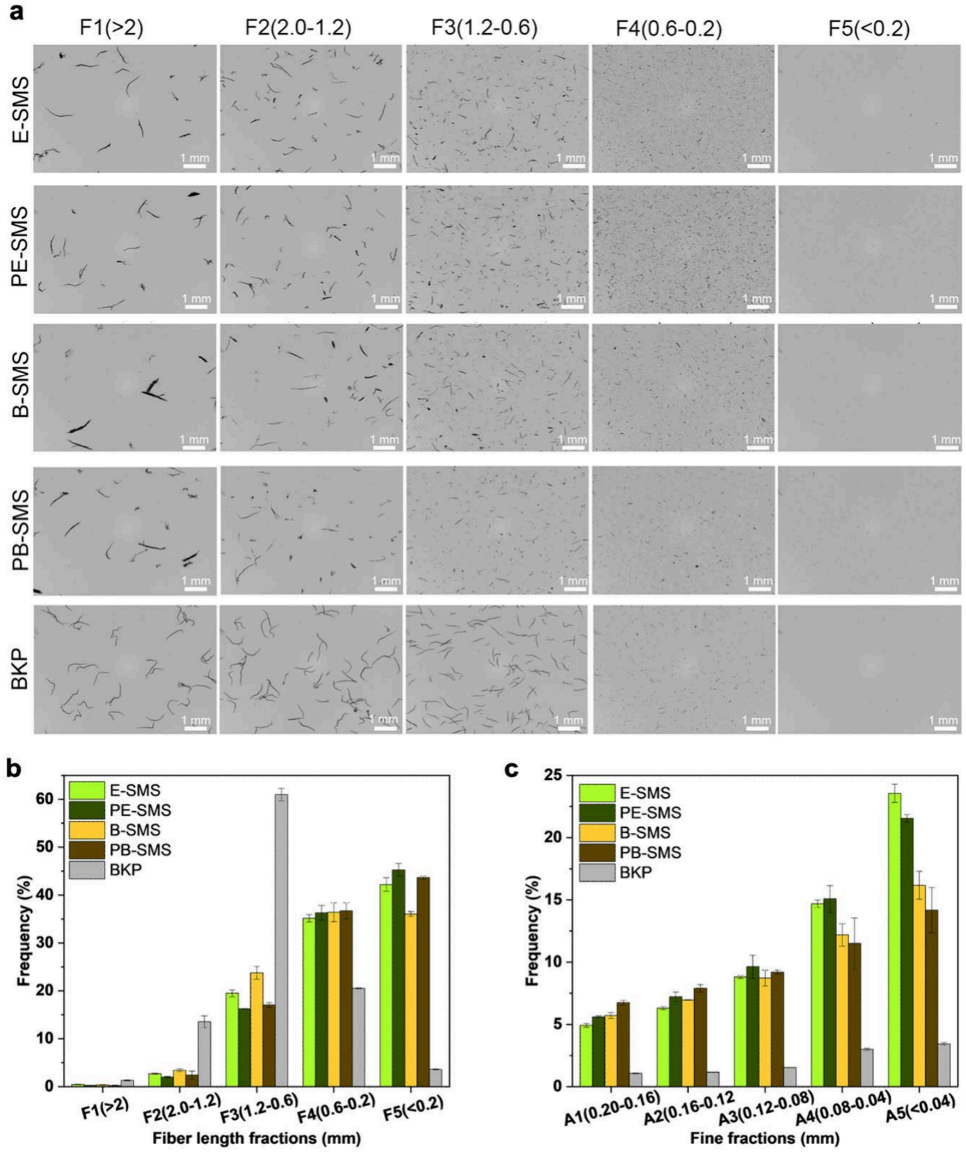

Figure 3. Fiber fraction analysis of fibrillated SMS determined at 0.3 wt % solid content in five different fractions: (a) images of fiber lengths and fines, (b) frequency of fiber length presented with standard deviations, and (c) frequency of fines presented with standard deviations.

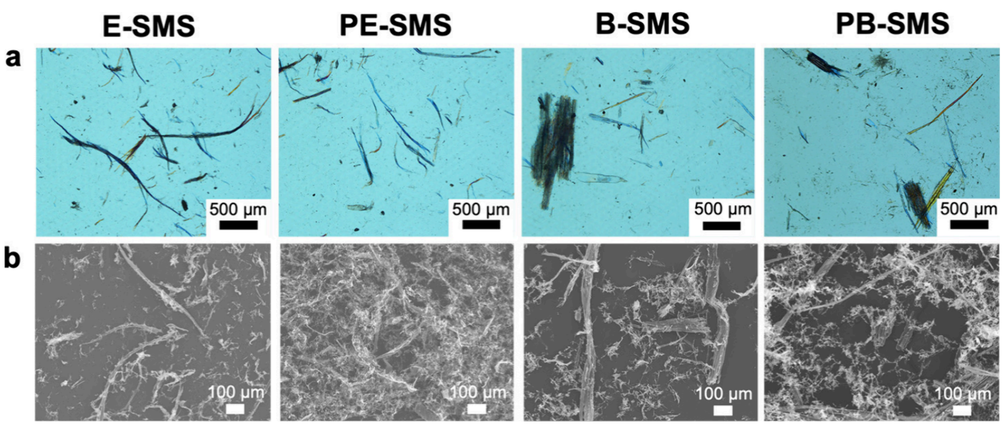

Figure 4. (a) Polarized optical microscopy showing the fiber size reduction and the effect of a predispersion step prior to extrusion and blending and (b) scanning electron microscopy images of fibrillated SMS showing the effect of a predispersion step and the presence of mycelial fibrils.

The presence of fungal chitin in fibrillated SMS sheets was confirmed by FTIR spectroscopy (Figure 6). As reference, bleached BKP still contained 5 ± 0.1 wt % lignin;[24] characteristic bands in the range of 1029−1160 cm^{-1}

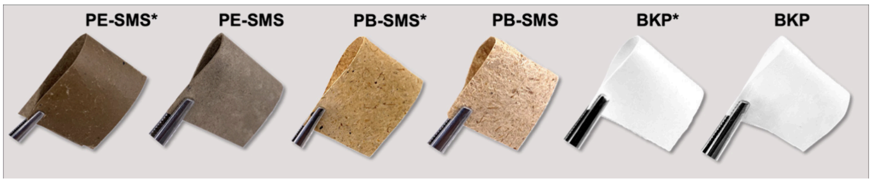

Figure 5. Appearance of compression-molded sheets produced from fibrillated SMS prepared using extrusion (PE) and blending (PB) and of bleached kraft pulp (BKP) by fibrillation using a PVDF filter (highlighted by asterisks) and Nylon mesh.

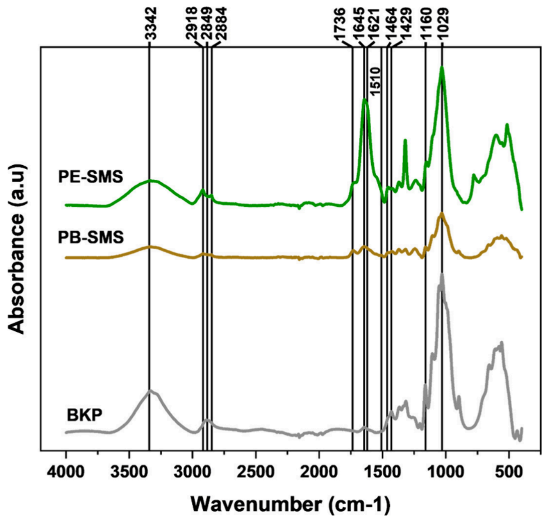

Figure 6. FTIR spectra of predispersed and extruded (PE) and blended (PB) fibrillated SMS, compared to BKP, showing differences in their chemical composition, especially the characteristic bands of amide I and II at 1645 and 1621 cm^{-1}, respectively, present in SMS.

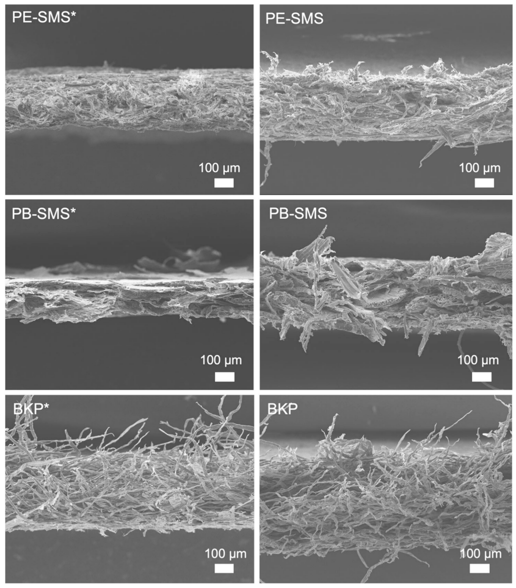

Figure 7. Scanning electron microscopy images of cross sections of fibrillated SMS sheets produced after predispersion and extrusion (PE), predispersion and blending (PB), and BKP sheets as reference. The sheets were produced by vacuum filtration over a PVDF filter (*) and a Nylon mesh.

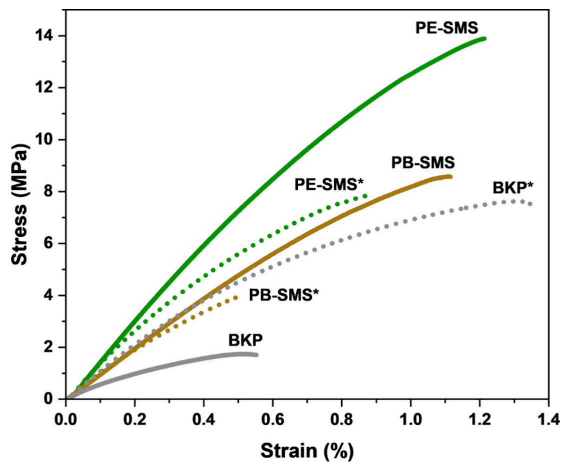

Figure 8. Typical stress−strain curves are shown for fibrillated SMS sheets produced by predispersion followed by extrusion (PE), predispersion followed by blending (PB), and BKP. The sheets were vacuum filtered over a PVDF filter (*) and a nylon mesh.

correspond to C−O−C stretching vibrations from the anhydroglucose units of cellulose, while that at 1736 cm^{-1} to C═O stretching (typically from residual hemicellulose), 3342 cm^{-1} to −OH stretching, and bands between 2980 and 2820 cm^{-1} to C−H stretching all present in cellulose.[24] The bands at 1429, 1464, and 1510 cm^{-1} are assigned to aromatic rings and skeletal vibrations, indicating the presence of residual birch lignin.[24,25] Besides the bands corresponding to cellulose, distinct bands were notable between 1645 and 1621 cm^{-1}, corresponding to amide I and II,[26] respectively, confirming the presence of fungal chitin within the fibrillated SMS sheets.

SEM images of cross sections of fibrillated SMS and BKP sheets prepared by filtration using a PVDF filter and Nylon mesh are shown in Figure 7. The fibers comprising all sheets are much better compacted when using a PVDF filter as compared to those produced using a Nylon mesh because of the higher pressure drop across the PVDF filter and wet filter cake during filtration. The better compaction of the sheets produced using PVDF filters resulted in lower average sheet thicknesses and higher envelope densities (Table 1). Sheets manufactured from SMS fibrillated in an extruder contained finer fibers and significantly less wood shives as compared to those produced from material fibrillated in a blender. Compared to BKP sheets comprising long fibers about ∼20 μm in diameter, the fibers within PE-SMS sheets were shorter but of similar diameter, whereas those in PB-SMS sheets were coarser, corroborating the fiber size analysis (see Figure 4 and Table S1).

The stress−strain curves of fibrillated SMS and BKP sheets are presented in Figure 8. Sheets prepared from fibrillated SMS (PE and PB) using the coarser Nylon mesh had higher tensile properties as compared with BKP sheets. Interestingly, the BKP sheets prepared using the finer PVDF filter had much higher tensile properties (Table S2 in Supporting Information) than those prepared using the Nylon mesh, which was due to the higher compaction, i.e., higher envelop density, causing

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better fiber−fiber connectivity. In contrast, for fibrillated SMS sheets, the use of a coarser filter mesh during filtration resulted in the loss of a significant fraction of sheet-like wood shives (Figure S6 in Supporting Information), which in turn reduced stress concentrations in the sheet, delaying crack formation.[27,28] The notably higher tensile strength (12.5 MPa) of extruder fibrillated SMS sheets produced using the coarser mesh is due to the higher degree of fibrillation of the wood fibers and the presence of a significant fibril-like fines fraction comprising mycelium fibrils and/or cellulose fines binding the disintegrated wood fibers allowing for better stress transfer[29] as compared to all other produced sheets, including BKP sheets using the fine filter (7.2 MPa). The mechanical properties of BKP sheets were in the range of values reported in the literature.[10] The tensile moduli of extruder fibrillated SMS sheets were significantly higher (1.7 GPa) than those determined for all other sheets, including BKP, which were in the range of 1 GPa. As expected for sheets comprising short cellulose fibers, the strains to failure were rather low, around 1%.

Green Aspects of SMS as a Raw Material for Fibrillation

SMS is an underutilized byproduct of mushroom production. We demonstrated in our previous study[20] that birch wood SMS used from shiitake cultivation has a substantially lower environmental burden than BKP as a source for fibrillation.[18,20] In particular, we showed that the biological pretreatment process of wood during fungal cultivation allowed fibrillation without the use of any chemicals, which contributed significantly to reduced impacts across ecotoxicity categories − minimizing toxicity to aquatic organisms, terrestrial life, plants, and humans due to fewer emissions of harmful substances.[20] The SMS coproduct of shiitake production used by us does serve as a model system for the utilization of other commercially relevant mushroom substrates after cultivation,[32,33] such as those used for cultivation of king trumpet mushrooms (*Pleurotus eryngii*), oyster mushrooms (*Pleurotus ostreatus*), or enoki (*Flammulina velutipes*) grown on lignocellulosic residues, which could be potentially fibrillated into a valuable fibers.

However, nanofibrillation processes are energy intensive; therefore, we explored fibrillation of a spent mushroom substrate comprising birch wood particles and residual mycelium into lignocellulose fibers with dimensions comparable to pulp fibers using less energy intensive processes (Table S3 in Supporting Information). Fibrillation of SMS by extrusion required the lowest specific energy (11 kWh/t) compared to the blender (417 kWh/t). Meanwhile, fibrillation of predispersed SMS − resulting in sheets with the highest mechanical properties − by extrusion required a specific energy of 789 kWh/t much lower than those prepared in a blender (1195 kWh/t), which was still much lower than using an ultrafine grinder (1700 kWh/t).[20] Extrusion allows for continuous operation, shorter residence times of approximately 3 min, and processing of high solid contents (28 wt %), resulting in high material throughput. We showed that the predispersion step required more energy than the fibrillation step (Table S3 in Supporting Information), indicating significant potential energy savings. The advantage of the fungal pretreatment a birch biomass experienced during shiitake production was highlighted by Berglund et al.;[20] ultrafine grinding of SMS required much lower specific energy (1700 kWh/t) as compared to commercial BKP requiring 8000 kWh/t. It is worth noting that our specific energy values are calculated based on laboratory equipment, which usually results in higher bounds when compared to optimized industrial processes.

Our work aligns with a number of the 12 Principles of Green Chemistry[30] and Engineering.[31] The use of inherently biologically pretreated wood present in SMS eliminates the need for toxic chemical pulping, thus aligning with Principle 3 (Less Hazardous Chemical Syntheses) of Green Chemistry.[30] The fungal pretreatment of SMS resulted in significant reductions of lignin (74−77%) and xylan (62−72%) of the material (SMS compared to birch wood) postharvesting.[2] Furthermore, mechanical fibrillation without added chemicals supports safer processing (Principle 5: Safer Solvents and Auxiliaries).[30] The observed reductions in energy demand for the fibrillation of SMS into a mix of short lignocellulosic fibers and mycelium fibrils that can be processed further using papermaking into sheets support Principle 6 (Design for Energy Efficiency) of Green Chemistry and align with Principle 1 (Inherent Rather Than Circumstantial) and Principle 2 (Prevention Instead of Treatment) of Green Engineering.[31] The use of SMS − a renewable, biodegradable, and nontoxic agricultural byproduct − allows for the elimination of hazardous raw materials (Principle 1) and reduces waste generation (Principle 2). The integration of upgraded residual biomass (SMS) into a potential industrial process for molded fiber/pulp products, shown here in sheet form, aligns with Principle 10 (Integrate Material and Energy Flows).[31]

CONCLUSIONS

Fibrillation of spent mushroom substrate (SMS) after shiitake cultivation was carried out using a corotating twin-screw extruder with and without a predispersion step and compared to simple disintegration of SMS in a blender. Extrusion enables the processing of high solid contents of 38 wt %, resulting in a high throughput of 7 kg/h and relatively low specific energy consumption of 11 kWh/t as compared to blending or ultrafine grinding, permitting only processing of dilute fiber suspensions (5−6.5 wt %) and requiring high specific energy input (417 and 1700 kWh/t, respectively). The fibrillated SMS materials varied in appearance; extruded SMS fibers had a darker brown hue with respect to the fungal pigments remaining on the substrate. The size reduction of SMS fibrillated in an extruder was more prominent compared with those fibrillated in a blender, which was confirmed by fiber fractionation and microscopy analysis. SEM analysis confirmed the presence of mycelium fibrils on fibrillated SMS. Sheet formation from fibrillated SMS by vacuum filtration over a Nylon mesh was faster than that over a PVDF filter. After compression molding, sheets produced from fibrillated SMS over a Nylon mesh possessed higher tensile strength than sheets from birch kraft pulp because of the removal of shives and the presence of fibril-like fines, particularly mycelial fibrils, which promoted stronger fiber−fiber interactions. Overall, our results demonstrate that extrusion-based fibrillation offers a scalable and energy-efficient approach for converting common shiitake SMS into pulplike materials, potentially suitable for molded fiber products.

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■ ASSOCIATED CONTENT

Supporting Information

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> Appearance of fibrillated E-SMS and PE-SMS; fiber analysis of fibrillated SMS through a sieve; fractionation data for width of fibrillated SMS and BKP; high magnification SEM images of fibrillated SMS; filtrate images of fibrillated SMS and BKP from PVDF filter and Nylon mesh; fibrillated SMS and BKP sheet appearance; mechanical properties for fibrillated SMS sheets and BKP sheets; image of B-SMS attempt to filter through Whatman filter paper; SEM image of BKP cross-section (PDF)

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Notes

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