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Comparative Study on Quality of Fuel Pellets from Switchgrass Treated with Different White-Rot Fungi

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Abstract: Fungal pretreatment of switchgrass using *Phanerochaete chrysosporium* (PC), *Trametes versicolor* 52J (TV52J), and the *Trametes versicolor* mutant strain (m4D) under solid-state fermentation was conducted to improve its pellet quality. For all three fungal strains, the fermentation temperature had a significant effect ($p < 0.05$) on pellet unit density and tensile strength. The *p*-values of the quadratic models for all the response variables showed highly significant regression models ($p < 0.01$) except for dimensional stability. In addition, 3.1-fold and 2.8-fold increase in pellet tensile strength were obtained from *P. chrysosporium- and T. versicolor* 52J-treated materials, respectively. Microstructural examination showed that fungal pretreatment reduced pores in the pellets and enhanced pellet particle bonding. Among the fungal strains, PC had the shortest optimum fermentation time (21 d) and most positive impact on the pellet tensile strength and hydrophobicity. Therefore, switchgrass pretreatment using PC has the potential for resolving the challenges of switchgrass pellet transportation and storage and reducing the overall pelletization cost. However, a detailed comparative technoeconomic analysis would be required to make definitive cost comparisons.

Keywords: fungal pretreatment; pelletization; pellet quality; solid-state fermentation; switchgrass

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1. Introduction

The transportation and storage of biomass are prime concerns in its supply chain and logistics due to the cost implication of these operations. Densification of biomass is often employed as a strategy to reduce the cost of transportation and storage of biomass by increasing its bulk density. Pelletization, a form of densification, primarily aims at compacting small particles into larger particles using mechanical processes along with moisture, heat, and pressure [1]. The quality of fuel pellets is evaluated by measuring the pellet's mechanical strength, physical properties, chemical properties, calorific value, and hydrophobicity [2,3]. The heating system and handling properties of pellets specified by the end-users can also be used to determine pellet quality [4]. According to Picchio et al. [5] and Gilbert et al. [6], pellet quality metrics are influenced mainly by factors associated with the feedstock properties such as biomass type, moisture content, particle size, and chemical composition, and factors associated with the quality of management of the manufacturing process such as operational conditions, pelletizer type, and binding agents. Several researchers investigated the impacts of these factors and their interactions on fuel pellet quality. An increase in pelletization pressure from 55.2 to 552 bar resulted in an increase in the bulk density of cut switchgrass pellets from 55 to 400 kg/m^3 [6]. High process temperature, low moisture content, and reduced particle sizes were noted as major factors for obtaining good quality fuel pellets, and particularly, improved mechanical properties of pellets [7]. However, Tilay et al. [8] reported that a 20 °C rise in process temperature led

to a small increase in pellet unit density (1.4%) and durability (2.1%) in canola meal pellets. A similar trend was also seen in the pelletization of maize residues [9]. On the other hand, the addition of bio-based additives was shown to influence the mechanical properties and energy density of canola hull fuel pellets [10]. In another study on camelina straw, changes in the proportion of lignocellulosic component of camelina straw improved the durability and dimensional stability of its pellet [11].

Generally, the pelletization of biomass, especially agricultural residues and energy grasses, produces poor quality pellets in terms of durability and bulk density [12], limiting their utilization as fuel pellets. In addition, the recalcitrant nature of the plant cell wall due to the integral structural complexity of lignocellulosic components of biomass restricts the use of lignocellulosic biomass as feedstock for energy generation via the biochemical or thermochemical route [13]. In order to enhance biomass pellet quality, binders and some kinds of pretreatment are applied, which negatively affect the overall cost of densification [13,14]. The goal of biomass pretreatment is to modify the structure of the biomass feedstock to make it more suitable for pelletization. A variety of biomass pretreatment options have been employed to facilitate the pelletization of agricultural biomass. Lam et al. [15] subjected Douglas fir particles to high pressure saturated steam treatment to enhance its pellet quality. The findings of their study showed that moisture adsorption rate of pellets produced from Douglas fir reduced from 0.0152 to 0.0125 min^{-1}, which was indicative of improved storability. Ultrasonic irradiation was employed by Zhang et al. [16] to modify biomass properties and improve the quality of pellets. The torrefaction of biomass at lab-scale was conducted prior to its pelletization to enhance the quality of pellets [17]. The study demonstrated that torrefaction of canola hull at temperature of 300 °C resulted in an increase of approximately 43.5% in heating value and significantly elevated the fuel ratio. Physicochemical and thermochemical pretreatment options are the most investigated pretreatment strategies for improving biomass fuel pellet quality. Although some of the pretreatment methods improved the quality of the pellets, they have adverse economic and environmental impacts [18,19].

Biological pretreatment, in contrast to the thermochemical and physicochemical pretreatment methods, is an energy-saving and environmentally benign process, which breaks down the lignocellulosic matrix using microorganisms (fungi or bacteria) under liquid-state or solid-state fermentation (SSF) [20,21]. Fungi, including white-, brown-, and soft-rot fungi, possess highly efficient enzymatic systems that are responsible for the degradation of lignocellulosic materials. This enzymatic system consists of two groups of extracellular enzymatic systems: the hydrolytic system, which produces hydrolases that are responsible for polysaccharide degradation; and a unique extracellular oxidative ligninolytic system that degrades lignin and opens phenyl rings [21]. The application of lignin-degrading fungi in biofuel production has focused chiefly on enhancing enzymatic digestibility for cellulosic ethanol production [22–24] and anaerobic biogas production [25,26]. Studies have shown the potential of fungal pretreatment of biomass under solid-state fermentation in terms of enhancing fuel pellet quality. The tensile strengths of fuel pellets from wheat straw and oat straw underwent 2.5-fold and 2.1-fold increases, respectively, after subjecting the raw materials to solid-state fermentation using the wild-type strain of *Trametes versicolor* [27,28]. Gao et al. [28] also reported that fermentation time had a highly significant impact on the pellet unit density. Minimal research has been reported on the applications of fungal pretreatment of biomass for improved fuel pellet quality, hence the need to conduct more research on the fungal pretreatment of agricultural biomass to enhance its pelletization potential. This study investigated the SSF of switchgrass using lignin-degrading white-rot fungi to facilitate pellet production. The effects of different white-rot fungi and pretreatment conditions, including fermentation time, fermentation temperature, inoculum concentration, and hammer mill screen size, on pellet quality metrics were evaluated.

2. Materials and Methods

2.1. Feedstock

Switchgrass (*Panicum virgatum* L.) of the variety "Cave-in-rock" used in this study was acquired from a farm in the Nappan area (45.77° N, 64.24° W) of Nova Scotia, Canada, in February of 2019. The switchgrass was harvested during the month of October 2018 and was swathed and air-dried in a storage building for two months before collection. The switchgrass samples collected were pre-chopped and stored in plastic bags. The switchgrass samples were comminuted using a hammer mill to three different screen sizes (6.4, 3.2 and 1.6 mm) and then stored in air-tight polyethylene bags as described in our previous study [29].

2.2. Fungal Growth and Inoculation Preparation

A cellobiose dehydrogenase-deficient strain (mutant) of the Basidiomycete *Trametes versicolor* (m4D), the wild-type strains of *Trametes versicolor* 52J (TV52J) (ATCC 20869) [30], and *Phanerochaete chrysosporium* (PC) were used in this study. The fungal strains were maintained in the laboratory of the Agriculture and Agri-Food Canada, Saskatoon Research Centre, as glycerol stocks stored at −80 °C. The fungal strains were cultured on Difco malt extract agar (MEA) (Benton Dickenson, Sparks, MD, USA) in agar plates. Inoculation cultures were prepared by transferring 10 agar plugs (5 mm diameter) of the fungal mycelium from the solid culture to malt extract broth (MEB) using the wide edge of a sterilized Pasteur pipette. The mixture was blended in an Eberbach blender cup at full speed on a Waring blender (Model 38BL54, Waring Laboratory Science, Torrington, CT, USA) base for 30 s (3 pulses of 10 s) and thereafter incubated at room temperature (21–23 °C) for 4 days at 150 rpm.

2.3. Solid-State Fermentation of Substrate

The substrate was sterilized using an autoclave at 121 °C for about 20 min prior to inoculation to suppress the growth of endogenous microorganisms. Twenty grams of the sterilized switchgrass were loaded in a plastic vented bag, which provided room for gas exchange. Homogenized liquid fungal cultures of 5-, 10-, and 15-mL inoculum volumes were inoculated over the surface of the sterilized switchgrass grinds using a pipette to uniformly spread the inocula over the entire substrate. The moisture content of the cultures was adjusted to 80% (w.b.) using sterile water. Approximately 73 mL of sterile water was added to the control substrate to adjust the moisture content to 80% (w.b.). The bags were sealed using a manual bag sealer and incubated at 22, 28, and 34 °C for 21, 28, and 35 days. A tray containing water was placed into the incubator to maintain a stable ambient humidity. The fungal-pretreated switchgrass samples were dried at 45 ± 3 °C for 24 h after incubation, and thereafter densified to investigate the effect of the fungal treatment on switchgrass pellet quality.

2.4. Experimental Design

A four-factor, three level Box–Behnken Design (BBD) was used to optimize the selected fungal pretreatment variables for producing good quality switchgrass pellets. The selected independent variables were fermentation time, fermentation temperature, inoculum concentration, and hammer mill screen size, while the response variables included pellet unit density (density immediately after pelleting), dimensional stability, and pellet tensile strength. The effects of the individual factor and their interactions on the response variables were evaluated using analysis of variance (ANOVA) and response surface methodology using the Design Expert version 7.1. software (Stat-Ease, Inc., Minneapolis, MN, USA). Supplementary Material Table S1 presents the actual and coded factor values of the independent variables in the fungal pretreatment. The response variables (y_n) were fitted to a second-order polynomial model, as shown in Equation (1).

$$y_n = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j \ (n=1,2,3) \quad (1)$$

where y_1 = pellet unit density (kg m^{-3}); y_2 = dimensional stability (%); y_3 = pellet tensile strength (MPa); x_1 = fermentation temperature (°C); x_2 = fermentation time (d); x_3 = inoculum concentration (mL); x_4 = hammer mill screen size (mm); β_0, β_i, β_{ii} and β_{ij} = the regression coefficients of intercept terms, linear terms, quadratic terms, and linear interaction terms in the equation, respectively.

2.5. Pelletization of the Switchgrass

The untreated and fungal-treated switchgrass samples were pelleted using a single pelleting unit (SPU) attached to the Instron universal tester (Model No. 3366, Instron Corp., Norwood, MA, USA) with control software as described in previous studies [11]. The SPU consists of a cylindrical steel die of diameter 6.4 mm and a plunger attached to the upper moving crosshead of the Instron. A heating element connected to the cylindrical die generated the required heat (95 ± 0.3 °C) for the compaction process, while the plunger provided a compressive pressure of about 128 MPa at a rate of 50 mm/min. Approximately 0.5–0.55 g of the samples, at a moisture content of 8.5% (w.b.), were fed into the cylindrical die to produce each pellet. An average of eight pellets (replicates) was produced for each pretreatment condition, and they were stored individually in an air-tight polyethylene bag at room temperature for further analysis.

2.6. Pellet Unit Density and Dimensional Stability

The unit density of pellet is the mass of a single pellet over its volume. In this study, the length and diameter of each pellet were measured using a digital vernier caliper with an accuracy of ±0.01 mm, while a digital weighing balance (with an accuracy of ±0.001 g) was used to measure each pellet mass. The pellet unit density was determined immediately after pelleting. The volumetric changes in percent immediately after pelleting (V_0) and after 14 d relaxation (V_{14}) were used to compute the dimensional stability as given in Equation (2).

$$\text{Dimensional stability } (\%) = \frac{V_0 - V_{14}}{V_0} \times 100 \quad (2)$$

2.7. Pellet Tensile Strength

Pellet tensile strength was determined in place of durability due to the limited number of pellets produced in single pelleting experiments. Pellet tensile strength is relevant during the storage and transportation of pellets. Although this test does not give a mechanical stress similar to that of the tumbling test method for durability [31], it provides useful information on the internal strength of pellets. The diametral compression test, performed using the Instron universal tester, described by Kashaninejad and Tabil [32], was used to evaluate the pellet tensile strength. Prior to the diametral compression test, the pellets were cut diametrically into samples of approximately 2.0–2.5 mm using a 0.5 mm circular disc mounted on a portable drilling machine. The cutting was conducted gently and the pellet face was carefully trimmed to prevent having a ragged pellet face. The diameter and thickness of each specimen were measured before being tested. Equation (3) was used to evaluate the pellet tensile strength.

$$\delta_x = \frac{2F}{\pi dl} \quad (3)$$

where δ_x = tensile strength (MPa); F = load at fracture (N); d = diameter of the specimen (mm); l = thickness of the specimen (mm).

2.8. Moisture Absorption Isotherm of Pellets

The method described by Lam et al. [15] was adopted in this study. Before conducting the moisture absorption test in a humidity chamber (Espec SH-641, ESPEC Corp, Osaka, Japan), the untreated and fungal-treated switchgrass pellets were dried in a convection oven at 103 °C for 24 h according to ANI/ASAE S358.3 [33]. The dried pellets were kept in a small aluminum dish and placed in the controlled environment chamber (25 °C and 90% relative humidity) for 72 h. The sample weight was taken at 10 min intervals

for the first hour and 30 min for the next 4 h. For the rest of the time, the sample weight was taken three times at equal time intervals to evaluate the equilibrium moisture content (EMC) of the samples. The aluminum dish was covered with an aluminum tray to avoid moisture loss during sample weight measurement. Caution was taken to keep the time the chamber was opened during measurement as short as possible to minimize alteration to chamber conditions. The relative humidity of the chamber dropped to about 85% during the course of measuring the sample weight; however, the fluctuation in the relative humidity was considered negligible. The moisture adsorption test was performed in triplicate for each pellet sample.

2.9. Raw Material and Pellet Characterization

The untreated and fungal-treated switchgrass grinds and their pellets were characterized to assess the effects of each of the fungal strains on pellet quality. The fungal-treated samples that produced pellets with the highest tensile strength and unit density were used for all the analyses.

2.9.1. Ultimate Analysis

The elemental compositions of the untreated and fungal-treated switchgrass grinds were analyzed using an Elemental Analyzer (Vario EL III Elementar, Analysensysteme GmbH, Hanau, Germany). The instrument was first calibrated with a known standard (Sulfanilic acid). About 4–6 mg of the sample was carefully packed in a thin aluminum foil sheet and combusted explosively in an oxygenated environment to liberate carbon, hydrogen, nitrogen, and sulphur (CHNS). Each test was replicated three times. The percentage oxygen content in the sample was obtained by subtracting the sum of C, H, N, S, and ash content wt % dry basis from 100%. We performed a preliminary test using pellets and the ground switchgrass (data not shown) and obtained similar results. Consequently, the raw material was preferred for the ultimate analysis because it requires no further preparation.

2.9.2. Higher Heating Value and Ash Content

The higher heating value (HHV) of the untreated and fungal-treated switchgrass pellets was measured using a bomb calorimeter (6400 Automatic Isoperibol, Parr Instrument Company, Moline, IL, USA). The procedure involves burning approximately 0.5 g of a sample in an oxygen-filled metal cylinder submerged in a measured quantity of water, all held within a thermally insulated chamber. The test was performed in duplicate for each sample.

2.9.3. Thermogravimetry Analysis (TGA)

The TGA of untreated and fungal-treated switchgrass grinds was carried out in a TGA/DTA Q500 model instrument (TA Instruments, New Castle, DE, USA). The thermal properties of 10–20 mg of the samples were investigated from ambient temperature to 600 °C at a temperature ramping rate of 10 °C/min in a nitrogen environment. The flow rate of nitrogen was 60 mL/min.

2.9.4. Proximate Analysis

Moisture contents of the pellets were analyzed according to the ANI/ASABE S358.3 [33] standard. The total ash content was measured according to the NREL standard method (NREL/TP-510-42622). Quantities of 0.5–2 g of the sample were heated at 575 ± 20 °C in a preheated muffle furnace (model no. F-A1730; Thermolyne, Dubuque, IA, USA) in triplicate [34]. The volatile matter was determined from the TGA curve as the weight loss due to heating from 105 to 400 °C in a nitrogen atmosphere. The percentage fixed carbon (FC) content was determined as the difference of 100 and the sum of the percentage moisture, ash, and volatile matter contents of the samples.

2.9.5. Chemical Composition

Lignocellulosic components of the untreated and fungal-treated samples were quantified using a modified NREL protocol [35]. The retentate was used to determine the quantity of acid insoluble lignin by gravimetric analysis, while the total acid soluble lignin (ASL) was measured using the absorbance of the acid hydrolysate determined with ultraviolet visible light spectrophotometer (UV mini 1240, Shimadzu Corp., Kyoto, Japan) at a wavelength of 240 nm. The acid hydrolysate fraction was neutralized with calcium carbonate and centrifuged prior to liquid chromatography analysis. Cellulose and hemicellulose contents were evaluated by determining the concentration of the individual sugar monomers detected with an Agilent HPLC (Agilent 1100, Agilent Technologies, Santa Clara, CA, USA).

2.9.6. Microstructural Examination

The surface morphology of the pellet samples was examined using a Field Emission Hitachi SU8000 Scanning Electron Microscope (Hitachi High-Tech Corp., Tokyo, Japan). The samples were dissected longitudinally using a surgical knife and coated with gold to provide a gold layer with a thickness of 10 nm using a vacuum sputter coater (Q150T ES, Quorum Technologies, Sussex, UK). The images were collected at an accelerating voltage of 3.0 kV and a magnification of 50×.

3. Results and Discussion

3.1. Switchgrass Pellet Produced

Figure 1 depicts the fuel pellets produced from the untreated and fungal-treated switchgrass ground with hammer mill screen size of 3.2 mm. The diameter and length of the pellets produced ranged from 6.60 to 6.67 mm and from 12.0 to 14.0 mm, respectively. The fuel pellets from the untreated switchgrass had conspicuous cracks and appeared weaker than the pellets from the fungal-treated switchgrass.

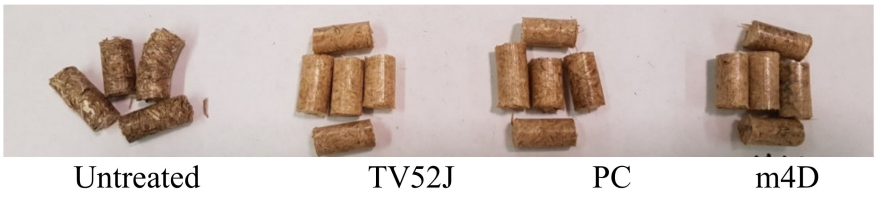

Figure 1. Untreated, *T. versicolor* 52J (TV52J), *P. chrysosporium* (PC), *T. versicolor* m4D (m4D)-treated switchgrass pellets.

3.2. Properties of the Switchgrass Pellets

The pellet unit density, dimensional stability, and pellet tensile strength of the fuel pellets from the untreated sample were 1068.76 ± 31.53 kg m^{-3}, 2.03%, and 0.751 ± 0.19 MPa, respectively. The unit pellet density obtained in this study is slightly different from the value (1016 ± 20 kg m^{-3}) reported by Mani et al. [36] for switchgrass ground using a hammer mill screen size of 3.2 mm and a single pelleting unit. The variation in the results could be related to the differences in moisture content, cultivar, and pelletization conditions.

Table S2 summarizes the physical and mechanical properties of the fuel pellets from the fungal-treated switchgrass grinds. From the results of the 29 experimental runs conducted for each of the fungal strains, the mean pellet tensile strength, pellet unit density, and dimensional stability for the PC-treated switchgrass pellets ranged between 2.3 and 0.805 MPa, 1171.10 and 981.93 kg m^{-3}, and 2.02 and 0.13%, respectively. Similarly, the mean values obtained for m4D-treated switchgrass pellet were 1.745–0.866 MPa, 1168.08–1115.04 kg m^{-3}, and 1.08% to −0.42%, respectively, while those of the TV52J-treated switchgrass pellet were 2.069–0.879 MPa, 1176.34–1111.67 kg m^{-3}, and 0.85% to −0.29%. PC treatment resulted in an approximately 3.1-fold increase in pellet tensile strength, while TV52J and m4D treatment led to 2.8- and 2.3-fold increases in pellet tensile strength, respectively. The pellet unit density of the fuel pellets from the fungi-treated switchgrass recorded in this present study had no significant difference from the pellet unit density of the untreated switchgrass

pellets; however, the values were significantly higher than previously reported values (700 ± 20 kg m^{-3}) for switchgrass pellets made using ultrasonic-vibration-assisted single pelleting units [37].

3.3. Effects of Independent Variables and Their Interactions on the Switchgrass Pellet Quality

The analysis of variance (ANOVA) results of all the response variables based on the influence of the fermentation temperature, fermentation time, inoculum concentration, and hammer mill screen size after stepwise multivariate regression are presented in Tables 1–3. The *p*-values of the quadratic regression models for all the response variables studied showed highly significant regression ($p < 0.01$) except for the dimensional stability of TV52J-pretreated pellets (significant at $p < 0.05$) and m4D-pretreated pellets (significant at $p < 0.1$). The significant models indicate that the variation in the independent variables affected the values of the pellet quality parameters in the multivariate quadratic models (Equations (4)–(12)) developed in this study. The ANOVA results also showed a statistically insignificant lack of fit for all the response variables, which corroborates the reliability of the models developed.

Table 1. Analysis of variance showing the effect of fungal pretreatment on pellet tensile strength.

Fungal Strain	**Analysis of Variance (ANOVA)**					
	Source	Sum of Squares	df	Mean Square	F value	ρ value
m4D	Model	0.71	4	0.18	6.87	0.0008 ***
	x_1	0.42	1	0.42	16.38	0.0005 ***
	x_2	0.09	1	0.091	3.55	0.0716 *
	x_2x_3	0.15	1	0.150	5.83	0.0238 **
	Lack of Fit	0.46	20	0.023	0.60	0.8051
TV52J	Model	1.33	6	0.22	5.91	0.0009 ***
	x_1	0.16	1	0.16	4.36	0.0487 **
	x_2	0.68	1	0.68	18.05	0.0003 ***
	x_3	0.16	1	0.16	4.35	0.0488 **
	x_1x_3	0.15	1	0.15	3.86	0.0622 *
	x_3x_4	0.17	1	0.17	4.65	0.0423 **
	Lack of Fit	0.74	18	0.04	1.83	0.2965
PC	Model	1.97	7	0.28	4.87	0.0022 ***
	x_1	0.78	1	0.78	13.50	0.0014 ***
	x_2	0.32	1	0.32	5.46	0.0295 **
	x_2x_3	0.25	1	0.25	4.28	0.0511 *
	x_4^2	0.36	1	0.36	6.16	0.0216 **
	Lack of Fit	1.02	17	0.060	1.22	0.4712

m4D = *T. versicolor* m4D; TV52J = *T. versicolor* 52J; PC = *P. chrysosporium*; x_1 = fermentation temperature (°C); x_2 = fermentation time (d); x_3 = inoculum concentration (mL); x_4 = hammer mill screen size (mm); *** highly significant ($p < 0.01$); ** significant ($p < 0.05$); * slightly significant ($p < 0.1$).

For all three fungal strains, fermentation temperature had a significant effect ($p < 0.05$) on all the response variables studied except dimensional stability. Fermentation time had a significant impact on the tensile strength of pellets pretreated with the wild-type fungi (PC and TV52J), but only a slightly significant ($p < 0.1$) effect on the tensile strength of pellets pretreated with the mutant strain (m4D). A statistically significant effect of inoculum concentration was seen on the tensile strength of the pellets pretreated with TV52J and the dimensional stability of the PC-pretreated pellets. Among the independent variables considered in this study, the hammer mill screen size showed no significant impact on any of the response variables. This could be connected to the slight differences in the geometric mean diameter of the switchgrass grind milled with the different hammer mill screen sizes selected in this study, as reported in a previous study [29]. However, the interaction effect of the hammer mill screen size and inoculum concentration on the pellet unit density and tensile strength of pellets from PC- and TV52J-pretreated switchgrass was statistically

significant. Gao et al. [28] studied the physical quality of pellets from fungal-pretreated wheat straw and reported that fermentation temperature and time had significant effects on the pellet tensile strength and pellet unit density, which buttresses the findings of this present study. A significant effect of fermentation time on the tensile strength and unit density of pellets from TV52J- and PC-pretreated biomass was also recorded in similar studies by Gao et al. [38] on oat straw and Gao et al. [27] on corn stover.

Table 2. Analysis of variance showing the effect of fungal pretreatment on pellet unit density.

Fungal Strain	**Analysis of Variance (ANOVA)**					
	Source	Sum of Squares	df	Mean Square	F-value	*p*-value
m4D	Model	4485.54	10	448.55	7.57	0.0001 ***
	x_1	2019.43	1	2019.43	34.06	<0.0001 ***
	x_2	786.35	1	786.35	13.26	0.0019 ***
	x_1x_3	265.20	1	265.20	4.47	0.0486 **
	x_2x_3	306.25	1	306.25	5.17	0.0355 **
	x_3x_4	355.32	1	355.32	5.99	0.0248 **
	x_2^2	399.13	1	399.13	6.73	0.0183 **
	Lack of Fit	940.59	14	67.19	2.12	0.2436
TV52J	Model	4670.60	7	667.23	6.57	0.0003 ***
	x_1	1345.78	1	1345.78	13.25	0.0015 ***
	x_2	1174.14	1	1174.14	11.56	0.0027 ***
	x_3x_4	1027.84	1	1027.84	10.12	0.0045 ***
	x_4^2	771.01	1	771.01	7.59	0.0119 **
	Lack of Fit	800.59	17	47.09	0.14	0.9985
PC	Model	30,946.79	4	7736.70	4.24	0.0097 ***
	x_1	22,356.88	1	22,356.88	12.27	0.0018 ***
	x_2x_4	5364.10	1	5364.10	2.94	0.0991 *
	Lack of Fit	32,577.3407	20	1628.87	0.58	0.8135

m4D = *T. versicolor* m4D; TV52J = *T. versicolor* 52J; PC = *P. chrysosporium*; x_1 = fermentation temperature (°C); x_2 = fermentation time (d); x_3 = inoculum concentration (mL); x_4 = hammer mill screen size (mm); *** highly significant ($p < 0.01$); ** significant ($p < 0.05$); * slightly significant ($p < 0.1$).

Table 3. Analysis of variance showing the effect of fungal pretreatment on dimensional stability.

Fungal Strain	**Analysis of Variance (ANOVA)**					
	Source	Sum of Squares	df	Mean Square	F-value	*p*-value
m4D	Model	0.50	2	0.25	3.25	0.0550 *
	x_1	0.26	1	0.26	3.32	0.0798 *
	x_2	0.24	1	0.24	3.17	0.0865 *
	Lack of Fit	1.63	22	0.07	0.81	0.6748
TV52J	Model	1.09	7	0.16	2.63	0.0409 **
	x_1x_2	0.42	1	0.42	7.09	0.0146 **
	x_4^2	0.45	1	0.45	7.47	0.0125 **
	Lack of Fit	0.90	17	0.05	0.59	0.8004
PC	Model	11.99	5	2.40	4.01	0.0092 ***
	x_3	2.84	1	2.84	4.75	0.0398 **
	x_2^2	6.50	1	6.50	10.87	0.0032 ***
	x_3^2	1.79	1	1.79	2.99	0.0972 *
	Lack of Fit	11.69	19	0.62	1.19	0.4821

m4D = *T. versicolor* m4D; TV52J = *T. versicolor* 52J; PC = *P. chrysosporium*; x_1 = fermentation temperature (°C); x_2 = fermentation time (d); x_3 = inoculum concentration (mL); x_4 = hammer mill screen size (mm); *** highly significant ($p < 0.01$); ** significant ($p < 0.05$); * slightly significant ($p < 0.1$).

Figures 2–4 illustrate the response surface curves for changes in the response variables as a function of the interaction of two independent variables with the other two

independent variables at a constant level. All the interaction effects on the pellet quality presented in Figures 2–4 were statistically significant. The effect of the interaction of fermentation time and inoculum concentration on the tensile strength of pellets produced from PC-treated switchgrass is shown in Figure 2. An increase in inoculum concentration up to 13 mL and an increase in fermentation time up to 31 d resulted in an increase of about 2.3-fold in pellet tensile strength, whereas a further increase in inoculum concentration and fermentation time led to a reduction in the tensile strength value of the pellet. However, high pellet tensile strengths were obtained at the lowest and highest inoculum concentration and fermentation time, respectively, and vice versa. *P. chrysosporium* is a known non-selective lignin-degrading fungus, and was reported to degrade both lignin and holocellulose progressively with increasing fermentation time [39]. This peculiar attribute of *P. chrysosporium* could be linked to the changes in tensile strength at varying fermentation time and inoculum concentration observed in this present study, which was likely due to the relative proportion of inherent binders (lignin and hemicellulose) in the fungus-treated materials. Figure 3a–d depict the response surface plots for the pellet quality of TV52J-pretreated switchgrass pellets. The interaction between the inoculum concentration and the hammer mill screen size (Figure 3a) showed that the pellet unit density decreased with the increasing of the hammer mill size and inoculum concentration. The inverse relationship between the hammer mill screen size and the pellet density observed in this work agrees with the report of the study on the effect of particle size on the mechanical properties of biomass pellets [40]. The impact of the fermentation temperature interaction with inoculum concentration on pellet unit density presented in Figure 3b indicated that the rise in fermentation temperature above 25 °C with the decreasing of the inoculum concentration caused an increase of 4.9–7.2% in pellet unit density. This result supports the assertion by Reid [41] that a minimum amount of inoculum is generally needed for the effective colonization and subsequent delignification of biomass. Hence, the solid-state fermentation of biomass using low inoculum concentrations at above room temperature favors high pellet density. Long fermentation time and high fermentation temperature resulted in higher dimensional stability (Figure 3c), whereas more dimensionally stable pellets could be obtained at the midpoint values of fermentation temperature and time studied. The impacts of the interaction of the inoculum concentration with fermentation temperature and hammer mill screen size with inoculum concentration on pellet tensile strength are shown in Figure 3d,e, respectively. An increase in inoculum concentration and fermentation temperature had a positive impact on the tensile strength, while a decrease in hammer mill screen size and inoculum concentration led to an increase in the pellet tensile strength. The effect of the interaction of factors on the pellet quality for M4D-pretreated switchgrass was recorded for only the pellet unit density and tensile strength (Figure 4a–d). As shown in Figure 4a, a low pellet unit density was recorded at a high inoculum concentration and a small hammer mill screen size, whereas a decreasing inoculum concentration and an increasing fermentation time resulted in a high pellet unit density (Figure 4b). A similar trend was observed for the interaction between inoculum concentration and fermentation temperature (Figure 4c). Unlike the pellet unit density, the interaction effect of fermentation time and inoculum concentration on tensile strength presented in Figure 4d shows that the highest tensile strength value was obtained at the midpoint of the two factors with the range of values evaluated in this study.

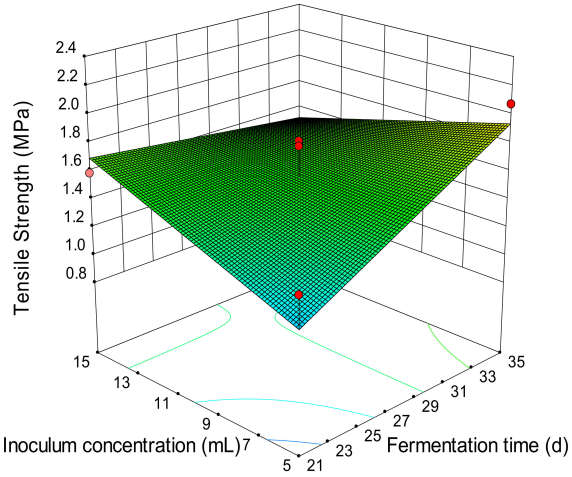

Figure 2. Interaction effect of inoculum concentration and fermentation time on the tensile strength of *Phanerochaete chrysosporium*-pretreated switchgrass pellet.

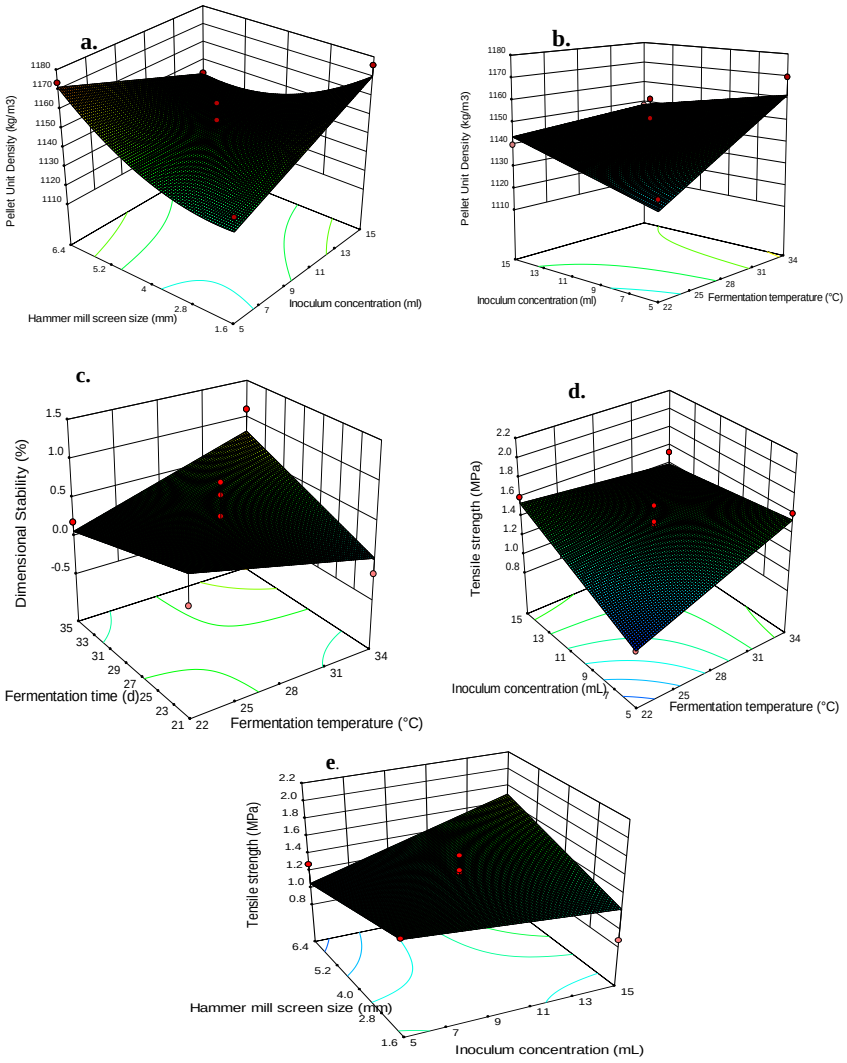

Figure 3. Response surface and contour plots for all responses of *T. versicolor* 52J-pretreated switchgrass pellets: (**a**) hammer mill screen size vs. inoculum concentration on pellet unit density; (**b**) inoculum concentration vs. fermentation temperature pellet unit density; (**c**) Fermentation time vs. temperature on dimensional stability; (**d**) inoculum concentration vs. fermentation temperature on tensile strength; (**e**) hammer mill screen size vs. inoculum concentration on tensile strength.

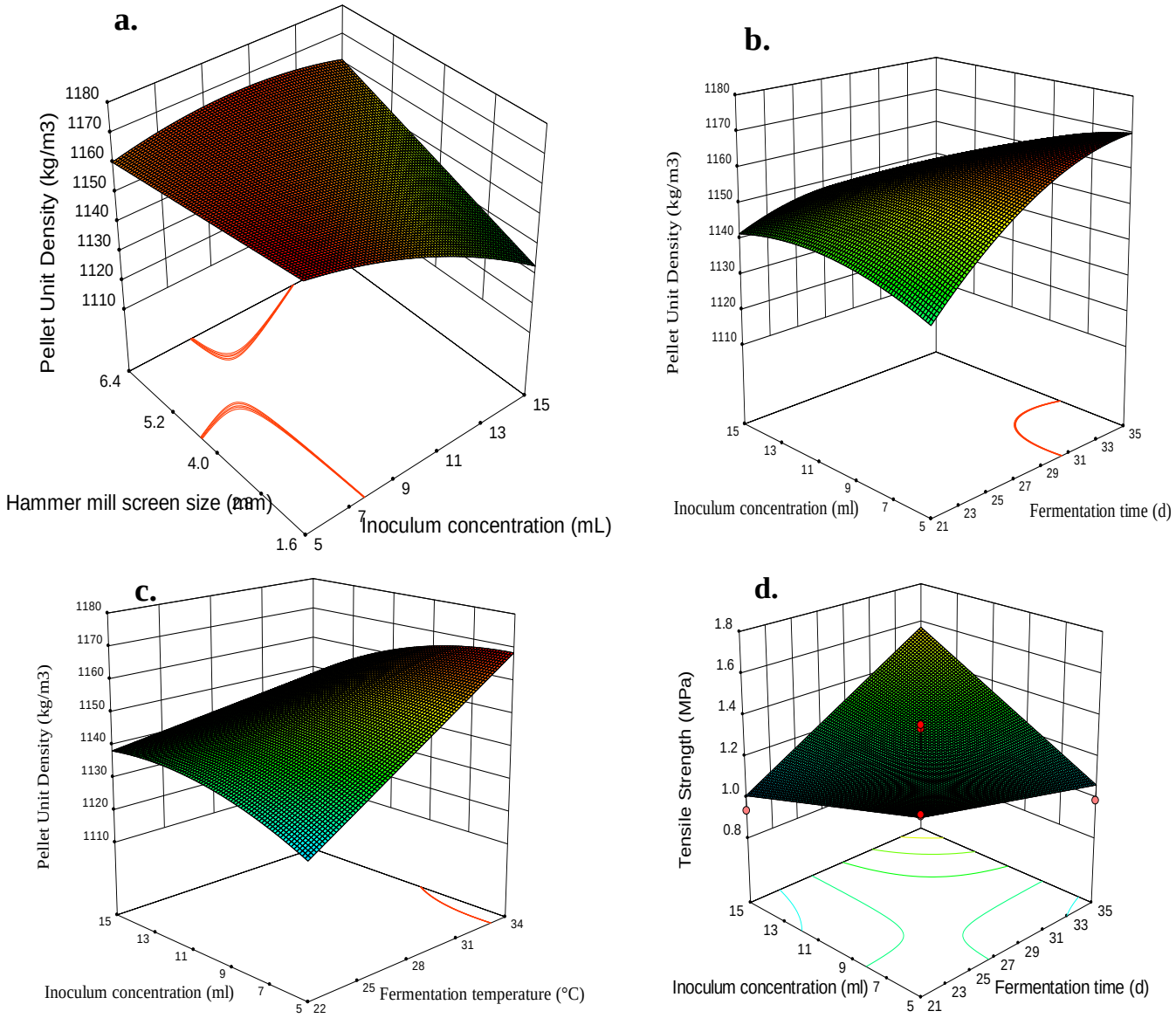

Figure 4. Response surface and contour plots for all responses of *T. versicolor* m4D-pretreated switchgrass pellets: (**a**) hammer mill screen size vs. inoculum concentration on pellet unit density; (**b**) hammer mill screen size vs. inoculum concentration pellet unit density; (**c**) inoculum concentration vs. temperature on pellet unit density; (**d**) inoculum concentration vs. fermentation time on tensile strength.

Quadratic models for switchgrass pellets pretreated with *T. versicolor* m4D:

$$\begin{aligned}\text{Pellet unit density} = 835.23 + 4.88x_1 + 10.841x_2 + 15.72x_3 - 17.40x_4 \\ -0.271x_1x_3 - 0.25x_2x_3 + 0.376x_2x_4 + 0.785x_3x_4 - 0.155x_2^2 - 0.203x_3^2\end{aligned} \quad (4)$$

$$\text{Pellet tensile strength} = 1.44 + 0.031x_1 - 0.043x_2 - 0.143x_3 + 0.0055x_1x_3 \quad (5)$$

$$\text{Dimensional stability} = 0.132 + 0.024x_1 - 0.020x_2 \quad (6)$$

Quadratic models for switchgrass pellets pretreated with *T. versicolor* 52J:

$$\text{Pellet unit density} = 948.72 + 4.46x_1 + 1.41x_2 + 13.20x_3 - 0.26x_4 - 0.269x_1x_3 - 1.34x_3x_4 + 1.82x_4^2 \quad (7)$$

$$\text{Pellet tensile strength} = -1.50 + 0.083x_1 + 0.034x_2 + 0.131x_3 - 0.163x_4 - 0.0064x_1x_3 + 0.017x_3x_4 \quad (8)$$

$$\text{Dimensional stability} = 4.67 - 0.209x_1 - 0.208x_2 + 0.058x_3 + 0.516x_4 + 0.0077x_1x_2 - 0.016x_3x_4 - 0.044x_4^2 \quad (9)$$

Quadratic models for switchgrass pellets pretreated with *P. chrysosporium*:

$$\text{Pellet unit density} = 1219.1 + 7.19x_1 - 10.99x_2 - 59.33x_4 + 2.18x_2x_4 \quad (10)$$

$$\text{Pellet tensile strength} = -1.58 - 0.012x_1 + 0.094x_2 + 0.207x_3 - 0.034x_4 + 0.014x_1x_4 - 0.071x_2x_3 - 0.039x_4^2 \quad (11)$$

$$\text{Dimensional stability} = -16.10 + 1.11x_2 + 0.31x_3 + 0.155x_4 - 0.02x_2^2 - 0.02x_3^2 \quad (12)$$

where x_1 = fermentation temperature (°C); x_2 = fermentation time (d); x_3 = inoculum concentration (mL); x_4 = hammer mill screen size (mm)

3.4. Optimization of the Fungal Pretreatment for Fuel Pellet Production

The aim of the optimization was to determine the best pretreatment conditions at a minimum pretreatment cost while maintaining high pellet quality. This was achieved by maximizing the pellet tensile strength and unit density, while the dimensional stability was minimized. The independent variables were set in a range that was between the lower and upper limits. For the response variables, the level of importance was selected based on the degree of relevance of each of the variables to pellet transportation, storage, and handling.

Two different optimum pretreatment conditions selected from the optimization results of the responses of pellets from fungal-pretreated switchgrass for each of the fungal strains are presented in Table S3. The first optimum conditions were chosen based on the highest pellet tensile strength value, while the second optimum conditions were selected based on the shortest fermentation time. Longer fermentation time is one of the major contributors to high fungal pretreatment cost at a commercial scale [42]. Among the three fungal strains studied, PC had the shortest optimum fermentation time of 21 d with a corresponding pellet tensile strength value of 2.029 MPa, which is about 9.9% different from the highest tensile strength value obtained from the optimization result for PC-pretreated switchgrass pellets. The lowest optimum fermentation temperature of 23.3 °C was seen in TV52J-pretreated switchgrass. This implies that SSF using TV52J can be performed at room temperature, saving the cost of additional energy. However, lower temperatures require longer fermentation times than the TV52J treatment at higher temperature. The optimum fermentation time reported in this study was in a close range with the optimum fermentation time obtained for the solid-state fermentation of oat straw using the same fungal strains [38]. Although the 21 d fermentation time reported in this present study is long relative to other pretreatment methods [43,44], it represents a significant reduction in fermentation time considering the 84 d of fermentation reported by Canam et al. [22] and Kalinoski et al. [23] using TV52J and the mutant strain (m4D).

3.5. Moisture Absorption of the Switchgrass Pellets

Moisture absorption during the storage and transportation of biomass pellets affects their durability and energy value [45]. The graph of moisture content and time during moisture absorption when pellets were placed in the controlled chamber is presented in Figure 5. After the first 1 h of exposure to humid conditions (RH = 90% at 25 °C), the pellets produced from the untreated switchgrass grind showed the least hydrophobic attribute with a moisture content of 7.11%, followed by M4D- and TV52J-treated pellets with 6.6% and 5.47% moisture contents, respectively. The highest hydrophobic behavior occurred in the PC-treated pellets, with a moisture content of 4.98%. Pellets from the untreated, M4D-treated, and TV52J-treated switchgrass exhibited similar hydrophobic behavior after 5 h of exposure in humid conditions. The moisture content-time graph revealed an approximately equal equilibrium moisture content (M_e) of 10% for pellets produced from the untreated, M4D-treated, and TV52J-treated switchgrass, while the value of M_e for the PC-treated pellets was approximately 9.2%.

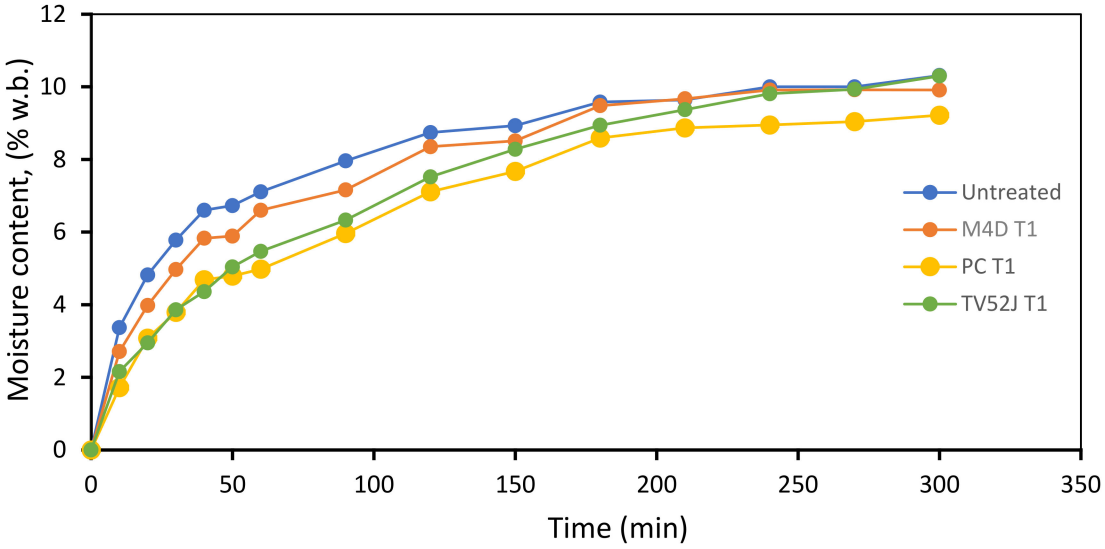

Figure 5. Moisture content with respect to time during moisture absorption of switchgrass pellets. m4D T1: *T. versicolor* m4D-treated switchgrass with the highest pellet tensile strength; PC T1: *P. chrysosporium*-treated switchgrass with the highest pellet tensile strength; TV52J T1: *T. versicolor* 52J-treated switchgrass with the highest pellet tensile strength.

3.6. Ultimate and Proximate Composition of the Switchgrass

Table S4 presents the elemental composition of the untreated and fungal-treated switchgrass grinds. The results showed that in comparison with the untreated switchgrass grind, a slight reduction in percentage carbon content and increase in oxygen content occurred in the fungal-treated samples for all three fungal strains. PC-treated switchgrass had the lowest percentage of carbon content (44.78 $\pm$ 1.41), which represents a reduction of about 7.5% as compared to the untreated switchgrass grind. In the same vein, the PC- and m4D-treated switchgrass samples had 9% more oxygen content than the untreated sample. The reduction in carbon content was slightly more in the fungal-treated samples that produced the highest pellet unit density. The difference in the percentage hydrogen, nitrogen, and sulphur content of the untreated and fungal-treated switchgrass grinds was negligible. In addition, the oxygen-carbon ratio (O/C) and hydrogen-carbon ratio (H/C) for both untreated and fungal-treated samples were approximately equal. The result of the proximate analysis portrayed in Figure S1 reveals a very small variation in the volatile matter, ash, and fixed carbon content of the fungal-treated switchgrass with respect to the control, except for the m4D-treated switchgrass, which had a 5% decrease in volatile component and a 19.8% increase in fixed carbon content. Similar results for the proximate composition of raw switchgrass were reported in previous studies [46,47].

3.7. Chemical Composition and Higher Heating Value (HHV)

The lignocellulosic composition of biomass is a major determinant of its suitability for biofuel applications. Table 4 gives the lignocellulosic composition of the untreated and fungal-treated switchgrass and the HHV of their pellets. The results showed that the percentage cellulose, hemicellulose, and lignin content ranged from 31.4 to 49.2%, from 14.9 to 24.2%, and from 23.5 to 26.8%, respectively. The hemicellulose content was mostly xylose, and the arabinose and galactose contents were negligible. The m4D-treated sample had the highest holocellulose content, with a 35.5% increase in measured cellulose content as compared to the native switchgrass. Significant degradation of the holocellulose was seen in the PC-treated sample, with a 13.5% loss in cellulose. The variation in the cellulose and hemicellulose content of the m4D- and PC-treated samples could be related to their inherent enzymes. The deficiency in cellobiose dehydrogenase in m4D reduced its ability to hydrolyze the holocellulose [22,29], and the apparent increase in cellulose content upon treatment with m4D could be due to the release of glucose from the fungal cell wall upon

acid treatment. In contrast, PC simultaneously degraded lignin and holocellulose; while apparent cellulose content decreased with PC treatment, it is likely that even more of the cellulose was actually degraded by the fungus because of the release of glucose from the fungal cell wall upon acid treatment. Although similar levels of lignin degradation were observed in all fungal strains studied, the tensile strengths of the PC- and TV52J-treated pellets were significantly higher than those of the m4D-treated samples. This indicates that besides lignin, other inherent binders such as depolymerized holocellulose may have contributed to the binding of the pellets, hence the higher pellet tensile strength recorded in the TV52J- and PC-treated samples. The impact of holocellulose on pellet strength was also reported by Frodeson et al. [48]. However, further studies on the inherent binding agents in lignocellulosic biomass and the bonding mechanism of pellets would be necessary to validate the assertion in this present study. The degradation of the holocellulose in the TV52J- and PC-treated samples was evident in the slightly significant reduction in carbon content, as seen in the elemental analysis. The HHV (18.02 $\pm$ 0.019 MJ/kg) of the untreated switchgrass obtained in this study was comparable to previous studies with this feedstock [45,47]. The HHV (17.66 $\pm$ 0.010 MJ/kg) of PC-treated switchgrass pellet was reduced by 2% relative to the untreated switchgrass pellet. The reduction in energy content is linked to the decrease in carbon content and the increase in oxygen content, which aligns with the report of Hu et al. [49]. The HHV results generally indicated that solid-state fermentation of switchgrass using M4D, TV52J, and PC had a negligible impact on the HHV of switchgrass pellets. In contrast to the findings of this study, Kalinoski et al. [23] reported a 2% increase in the HHV of TV52J-treated hardwood and miscanthus pellets.

Table 4. Lignocellulosic composition, ash content, and the higher heating value of the untreated and fungal-treated switchgrass.

Sample Treatment	Total Lignin (% Dry Weight)	Cellulose (% Dry Weight)	Hemicellulose (% Dry Weight)	Ash Content (% Dry Weight)	Higher Heating Value (MJ/kg)	Energy Loss (%)
Untreated	26.75	36.30	15.10	2.37 $\pm$ 0.24	18.03 $\pm$ 0.019	_
M4D T1	23.50	49.20	24.20	3.44 $\pm$ 0.35	17.92 $\pm$ 0.051	0.6
M4D P1	24.80	38.70	22.10	3.36 $\pm$ 0.28	17.94 $\pm$ 0.027	0.49
PC P1	24.10	42.90	23.80	3.25 $\pm$ 0.71	17.67 $\pm$ 2.010	2.01
PC T1	24.50	31.40	14.90	2.91 $\pm$ 0.83	17.71 $\pm$ 1.790	1.79
TV52J P1	24.80	39.50	23.70	3.62 $\pm$ 0.32	17.75 $\pm$ 0.032	1.57
TV52J T1	23.80	44.90	22.70	3.26 $\pm$ 0.51	17.82 $\pm$ 0.064	1.16

M4D T1 = *T. versicolor* m4D-treated switchgrass with the highest pellet tensile strength. M4D P1 = *T. versicolor* m4D-treated switchgrass with the highest pellet unit density. PC T1 = *P. chrysosporium*-treated switchgrass with the highest pellet tensile strength. PC P1 = *P. chrysosporium*-treated switchgrass with the highest pellet unit density. TV52J T1 = *T. versicolor* 52J-treated switchgrass with the highest pellet tensile strength. TV52J P1 = *T. versicolor* 52J-treated switchgrass with the highest pellet unit density.

3.8. TGA/DTG Analysis

The results of the thermogravimetric analysis of the untreated and fungal-treated switchgrass exhibited in Figure 6a,b showed a similar thermal degradation pattern for all the samples, with two DTG peaks that were indicative of the presence of hemicellulose and cellulose. However, the untreated switchgrass had higher DTG peaks than the fungal-treated switchgrass, mainly at the degradation peak for hemicellulose. This implies that the solid-state fermentation of switchgrass using the fungal strains slightly reduced its thermal stability. A similar observation was made in the TGA/DTG of hardwood and softwood pretreated with brown-rot fungus [50]. The thermal degradation of switchgrass observed in this study followed the TGA pattern for switchgrass reported in previous studies [51].

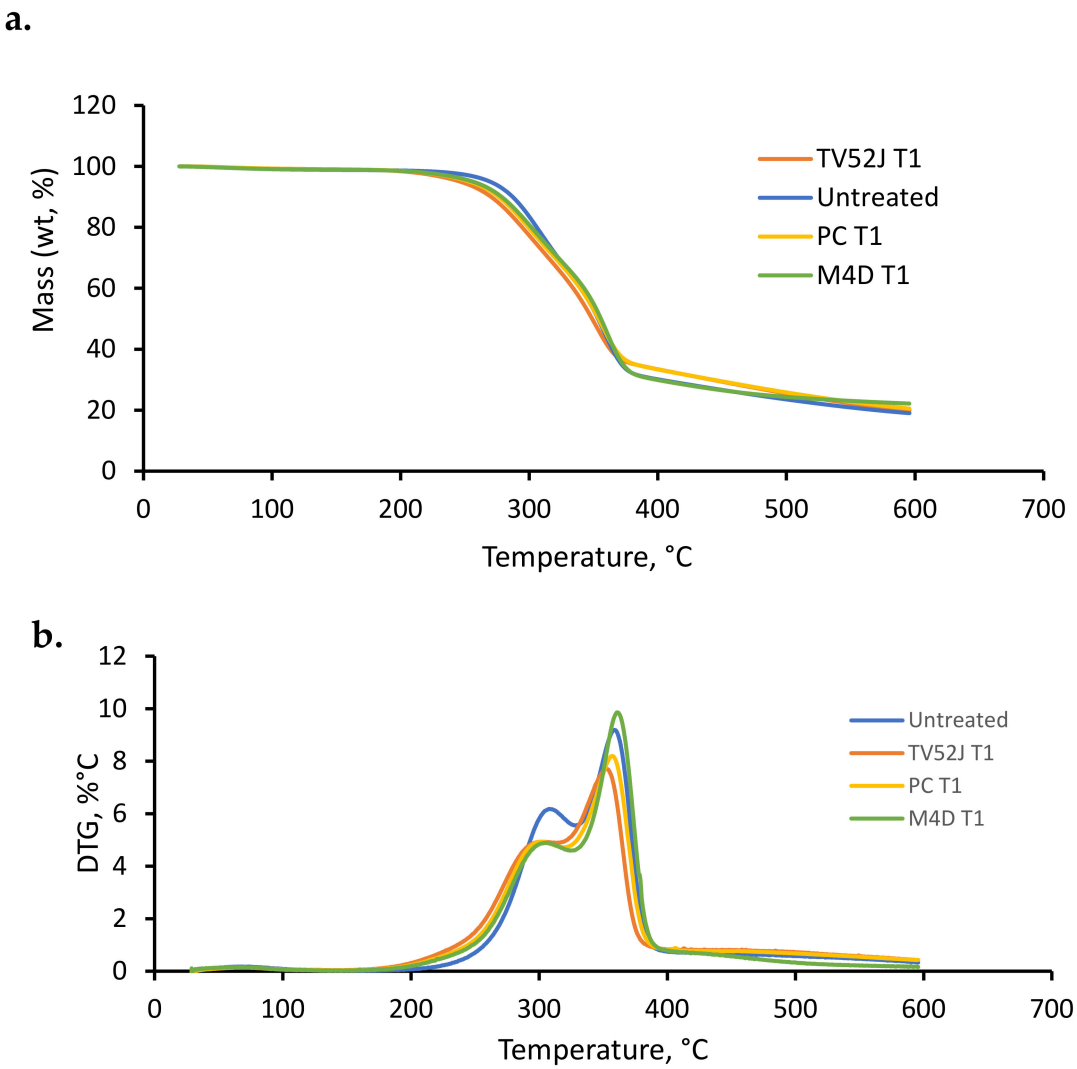

Figure 6. Effect of fungal pretreatment on the thermal degradation rate of switchgrass: (**a**) thermogravimetry curves of the untreated and fungal-treated switchgrass; (**b**) differential thermogravimetry curves of the untreated and fungal-treated switchgrass. m4D T1: *T. versicolor* m4D-treated switchgrass with the highest pellet tensile strength; PC T1: *P. chrysosporium*-treated switchgrass with the highest pellet tensile strength; TV52J T1: *T. versicolor* 52J-treated switchgrass with the highest pellet tensile strength.

3.9. Microstructure Analysis

The microstructural examination provides an understanding of the microscale impact of the fungal pretreatment of switchgrass grinds on the morphology of the pellets. The SEM micrographs of the longitudinal cross-section of the untreated and fungal-treated switchgrass pellets are shown in Figure 7a–d. Figure 7a depicts a SEM image of the pellet from the untreated switchgrass, which exhibits a rough surface with more loosely bonded particles and pore spaces. In comparison with the untreated switchgrass, the SEM images of the pellets from fungal-pretreated switchgrass (Figure 7b–d) revealed more closely bonded particles, smoother surfaces, and fewer pores. This indicates that the biodegradation of the lignocellulosic components of switchgrass during the fungal pretreatment released more of the inherent binders in the material, which was responsible for the high tensile strength witnessed in the fungal-treated switchgrass pellets relative to the untreated switchgrass.

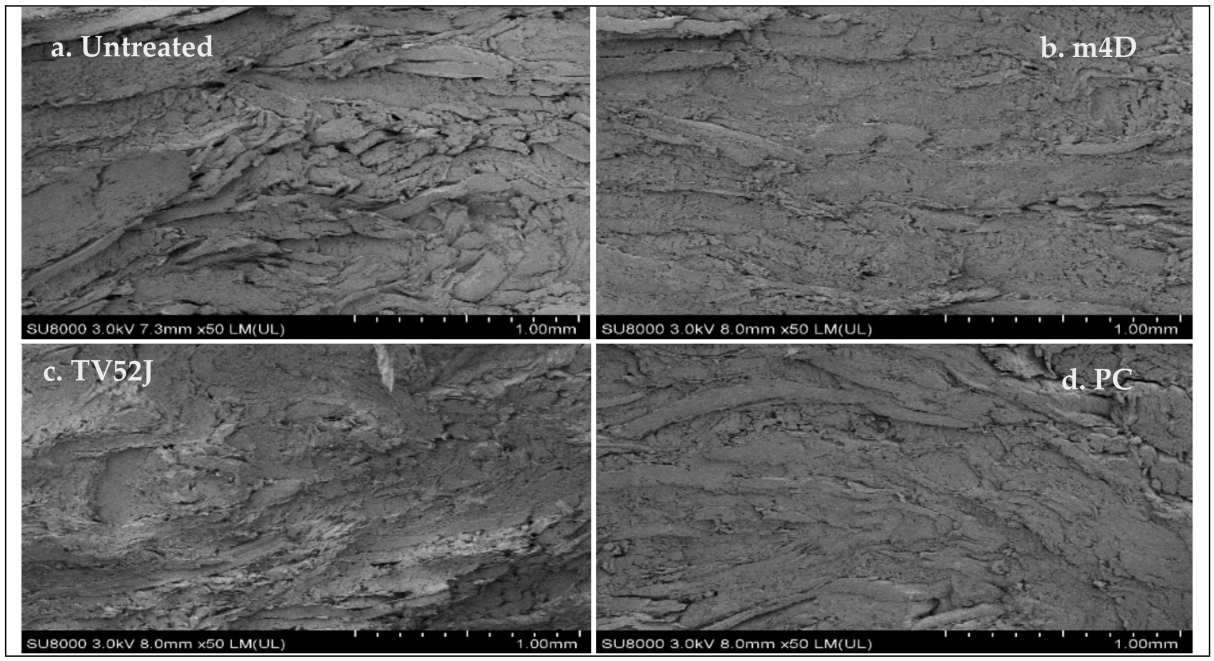

Figure 7. Scanning electron microscope images of the longitudinal cross-section of the untreated and fungal-treated switchgrass pellets: (**a**) untreated switchgrass; (**b**) *T. versicolor* m4D-treated switchgrass; (**c**) *T. versicolor* 52J-treated switchgrass; (**d**) *P. chrysosporium*-treated switchgrass.

4. Conclusions

This study showed that fermentation temperature and time were the most significant ($p < 0.05$) factors in the solid-state fermentation of switchgrass using *T. versicolor*, the mutant strain (m4D), and *P. chrysosporium* for fuel pellet production. The *p*-values of the quadratic regression models for the pellet unit density and tensile strength of all the fungal-pretreated switchgrass pellets studied showed highly significant regression models ($p < 0.01$). The wild-type fungal strains (TV52J and PC) improved the pellet quality in terms of the tensile strength compared to the mutant strain (m4D) and the untreated switchgrass pellets. Generally, the fungal strains used in this study positively influenced the quality of switchgrass pellets. However, based on the optimization and moisture absorption results, *P. chrysosporium* is preferred for the solid-state fermentation of switchgrass for the purpose of fuel pellet production. Further investigation on the inherent binders in lignocellulosic biomass and their role in pellet bonding mechanisms will be necessary to maximize the potentials of solid-state fermentation using the fungal strains as a sustainable and energy-saving pretreatment strategy for improved fuel pellet quality.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/en14227670/s1, Table S1: Actual and coded factor values of the independent variables in the fungal pretreatment; Table S2: Physical and mechanical properties of the fungal-treated switchgrass pellets; Table S3: Optimum conditions for producing switchgrass pellets pretreated by the fungal strains used in this study under solid-state fermentation; Table S4: Elemental composition of untreated and fungal-treated switchgrass; Figure S1: Proximate composition of the untreated and fungal-treated switchgrass.

Author Contributions: Conceptualization, O.O.O., L.G.T. and T.D.; methodology, O.O.O., L.G.T. and T.D.; software, O.O.O.; validation, L.G.T. and T.D.; formal analysis, O.O.O.; investigation, O.O.O.; resources, L.G.T., T.D., E.M. and D.C.; data curation, O.O.O.; writing—original draft preparation, O.O.O.; writing—review and editing O.O.O., L.G.T., T.D., E.M. and D.C.; visualization, O.O.O.; supervision, L.G.T. and T.D.; project administration, L.G.T. and T.D.; funding acquisition, L.G.T., T.D., E.M. and D.C. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gilvari, H.; de Jong, W.; Schott, D.L. Quality Parameters Relevant for Densification of Bio-Materials: Measuring Methods and Affecting Factors—A Review. *Biomass Bioenergy* **2019**, *120*, 117–134. [CrossRef]
2. Azargohar, R.; Nanda, S.; Dalai, A. Densification of Agricultural Wastes and Forest Residues: A Review on Influential Parameters and Treatments. In *Recent Advancements in Biofuels and Bioenergy Utilization*; Nanda, S., Prakash, K.S., Mohanty, P., Eds.; Springer: Singapore, 2018; pp. 27–51. [CrossRef]
3. Zamorano, M.; Popov, V.; Rodríguez, M.L.; García-Maraver, A. A Comparative Study of Quality Properties of Pelletized Agricultural and Forestry Lopping Residues. *Renew. Energy* **2011**, *36*, 3133–3140. [CrossRef]
4. Lehtikangas, P. Quality Properties of Pelletised Sawdust, Logging Residues and Bark. *Biomass Bioenergy* **2001**, *20*, 351–360. [CrossRef]
5. Picchio, R.; Latterini, F.; Venanzi, R.; Stefanoni, W.; Suardi, A.; Tocci, D.; Pari, L. Pellet Production from Woody and Non-Woody Feedstocks: A Review on Biomass Quality Evaluation. *Energies* **2020**, *13*, 2937. [CrossRef]
6. Gilbert, P.; Ryu, C.; Sharifi, V.; Swithenbank, J. Effect of Process Parameters on Pelletisation of Herbaceous Crops. *Fuel* **2009**, *88*, 1491–1497. [CrossRef]
7. Carone, M.T.; Pantaleo, A.; Pellerano, A. Influence of Process Parameters and Biomass Characteristics on the Durability of Pellets from the Pruning Residues of *Olea europaea* L. *Biomass Bioenergy* **2011**, *35*, 402–410. [CrossRef]
8. Tilay, A.; Azargohar, R.; Drisdelle, M.; Dalai, A.; Kozinski, J. Canola Meal Moisture-Resistant Fuel Pellets: Study on the Effects of Process Variables and Additives on the Pellet Quality and Compression Characteristics. *Ind. Crop. Prod.* **2015**, *63*, 337–348. [CrossRef]
9. Wongsiriamnuay, T.; Tippayawong, N. Effect of Densification Parameters on the Properties of Maize Residue Pellets. *Biosyst. Eng.* **2015**, *139*, 111–120. [CrossRef]
10. Azargohar, R.; Nanda, S.; Kang, K.; Bond, T.; Karunakaran, C.; Dalai, A.K.; Kozinski, J.A. Effects of Bio-Additives on the Physicochemical Properties and Mechanical Behavior of Canola Hull Fuel Pellets. *Renew. Energy* **2019**, *132*, 296–307. [CrossRef]
11. Onu Olughu, O.; Tabil, L.G.; Dumonceaux, T. Effect of Ultrasonic Pretreatment on the Chemical Composition and Pellet Quality of Camelina Straw. In Proceedings of the 2019 ASABE Annual International Meeting, Boston, MA, USA, 7–10 July 2019. [CrossRef]
12. Sokhansanj, S.; Mani, S.; Bi, X.; Zaini, P.; Tabil, L. Binderless Pelletization of Biomass. In Proceedings of the 2005 American Society of Agricultural and Biological Engineers (ASABE) Annual Meeting, Tampa, FL, USA, 17–20 July 2005. [CrossRef]
13. Mupondwa, E.; Li, X.; Tabil, L.; Sokhansanj, S.; Adapa, P. Status of Canada's Lignocellulosic Ethanol: Part I: Pretreatment Technologies. *Renew. Sustain. Energy Rev.* **2017**, *72*, 178–190. [CrossRef]
14. Jiang, L.; Yuan, X.; Xiao, Z.; Liang, J.; Li, H.; Cao, L.; Wang, H.; Chen, X.; Zeng, G. A Comparative Study of Biomass Pellet and Biomass-Sludge Mixed Pellet: Energy Input and Pellet Properties. *Energy Convers. Manag.* **2016**, *126*, 509–515. [CrossRef]
15. Lam, P.S.; Sokhansanj, S.; Bi, X.T.; Lim, C.J.; Larsson, S.H. Drying Characteristics and Equilibrium Moisture Content of Steam-Treated Douglas Fir (*Pseudotsuga Menziesii* L.). *Bioresour. Technol.* **2012**, *116*, 396–402. [CrossRef]
16. Zhang, P.; Deines, T.; Nottingham, D.; Pei, Z.J.; Wang, D.; Wu, X. Ultrasonic Vibration-Assisted Pelleting of Biomass: A Designed Experimental Investigation on Pellet Quality and Sugar Yield. In Proceedings of the ASME 2010 International Manufacturing Science and Engineering Conference, Erie, PA, USA, 12–15 October 2010; pp. 137–145.
17. Sarker, T.R.; Azargohar, R.; Dalai, A.K.; Venkatesh, M. Physicochemical and Fuel Characteristics of Torrefied Agricultural Residues for Sustainable Fuel Production. *Energy Fuels* **2020**, *34*, 14169–14181. [CrossRef]
18. Singh, R.; Shukla, A.; Tiwari, S.; Srivastava, M. A Review on Delignification of Lignocellulosic Biomass for Enhancement of Ethanol Production Potential. *Renew. Sustain. Energy Rev.* **2014**, *32*, 713–728. [CrossRef]
19. Laschi, A.; Marchi, E.; Gonz Alez-García, S. Environmental Performance of Wood Pellets' Production through Life Cycle Analysis. *Biosyst. Eng.* **2015**, *139*, 111–120. [CrossRef]

20. Canam, T.; Town, J.; Iroba, K.; Tabil, L.; Dumonceaux, T. Pretreatment of Lignocellulosic Biomass Using Microorganisms: Approaches, Advantages, and Limitations. In *Sustainable Degradation of Lignocellulosic Biomass—Techniques, Applications and Commercialization*; Silva, A.K.C., Da, S.S., Eds.; IntechOpen: Rijeka, Croatia, 2013; pp. 181–206. [CrossRef]
21. Wan, C.; Li, Y. Fungal Pretreatment of Lignocellulosic Biomass. *Biotechnol. Adv.* **2012**, *30*, 1447–1457. [CrossRef] [PubMed]
22. Canam, T.; Town, J.R.; Tsang, A.; McAllister, T.A.; Dumonceaux, T.J. Biological Pretreatment with a Cellobiose Dehydrogenase-Deficient Strain of Trametes Versicolor Enhances the Biofuel Potential of Canola Straw. *Bioresour. Technol.* **2011**, *102*, 10020–10027. [CrossRef]
23. Kalinoski, R.M.; Flores, H.D.; Thapa, S.; Tuegel, E.R.; Bilek, M.A.; Reyes-Mendez, E.Y.; West, M.J.; Dumonceaux, T.J.; Canam, T. Pretreatment of Hardwood and Miscanthus with Trametes Versicolor for Bioenergy Conversion and Densification Strategies. *Appl. Biochem. Biotechnol.* **2017**, *183*, 1401–1413. [CrossRef]
24. Kalyani, D.; Lee, K.M.; Kim, T.S.; Li, J.; Dhiman, S.S.; Kang, Y.C.; Lee, J.K. Microbial Consortia for Saccharification of Woody Biomass and Ethanol Fermentation. *Fuel* **2013**, *107*, 815–822. [CrossRef]
25. Guo, P.; Mochidzuki, K.; Cheng, W.; Zhou, M.; Gao, H.; Zheng, D.; Wang, X.; Cui, Z. Effects of Different Pretreatment Strategies on Corn Stalk Acidogenic Fermentation Using a Microbial Consortium. *Bioresour. Technol.* **2011**, *102*, 7526–7531. [CrossRef]
26. Rouches, E.; Herpoël-Gimbert, I.; Steyer, J.P.; Carrere, H. Improvement of Anaerobic Degradation by White-Rot Fungi Pretreatment of Lignocellulosic Biomass: A Review. *Renew. Sustain. Energy Rev.* **2016**, *59*, 179–198. [CrossRef]
27. Gao, W.; Tabil, L.G.; Liu, Q.; Zhao, R.; Zhang, M. Experimental Study on the Effect of Solid-State Fermentation on Pellet Density and Strength of Corn Stover. *J. Biobased Mater. Bioenergy* **2019**, *13*, 840–847. [CrossRef]
28. Gao, W.; Tabil, L.G.; Dumonceaux, T.; Espinel Ríos, S.; Zhao, R. Optimization of Biological Pretreatment to Enhance the Quality of Wheat Straw Pellets. *Biomass Bioenergy* **2017**, *97*, 77–89. [CrossRef]
29. Onu Olughu, O.; Tabil, L.G.; Dumonceaux, T. Ultrasonic Delignification and Microstructural Characterization of Switchgrass. *Energies* **2021**, *14*, 263. [CrossRef]
30. Dumonceaux, T.; Bartholomew, K.; Valeanu, L.; Charles, T.; Archibald, F. Cellobiose Dehydrogenase Is Essential for Wood Invasion and Nonessential for Kraft Pulp Delignification by Trametes Versicolor. *Enzym. Microb. Technol.* **2001**, *29*, 478–489. [CrossRef]
31. Schiling, C.; Wohler, M.; Yazdanpanah, F.; Bi, X.; Lau, A.; Lim, J.C.; Sokhansanj, S.; Pels, S.K. Development of a Novel Wood Pellet Durability Tester for Small Samples. In Proceedings of the 2015 World Sustainable Energy Days—Energy Efficiency & Biomass; World Sustainable Energy Days, Wels, Austria, 25–27 February 2015. [CrossRef]
32. Kashaninejad, M.; Tabil, L.G. Effect of Microwave-Chemical Pre-Treatment on Compression Characteristics of Biomass Grinds. *Biosyst. Eng.* **2011**, *108*, 36–45. [CrossRef]
33. American Society of Agricultural Engineers. *Standard Test for Moisture Content Measurement of Forages*; ANSI/ASAE S358.3; American Society of Agricultural Engineers: St. Joseph, MI, USA, 2012.
34. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. *Determination of Ash in Biomass: Laboratory Analytical Procedure (LAP)*; National Renewable Energy Laboratory: Golden, CO, USA, 2008.
35. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *NREL/TP-510-42618 Analytical Procedure—Determination of Structural Carbohydrates and Lignin in Biomass*; National Renewable Energy Laboratory: Golden, CO, USA, 2012.
36. Mani, S.; Tabil, L.G.; Sokhansanj, S. Evaluation of Compaction Equations Applied to Four Biomass Species. *Can. Biosyst. Eng. Le Genie Biosyst. Can.* **2004**, *46*, 55–61.
37. Nottingham, D.; Pei, Z.J.; Zhang, M.; Deines, T. The Effects of Pelleting Time and Ultrasonic Power during Ultrasonic Vibration-Assisted Pelleting of Switchgrass. In Proceedings of the 2010 Industrial Engineering Research Conference, Reno, NV, USA, 26–28 November 2010; pp. 1–6.
38. Gao, W.; Lei, Z.; Tabil, L.G.; Zhao, R. Biological Pretreatment by Solid-State Fermentation of Oat Straw to Enhance Physical Quality of Pellets. *J. Chem.* **2020**, *2020*, 3060475. [CrossRef]
39. Liu, S.; Wu, S.; Pang, C.; Li, W.; Dong, R. Microbial Pretreatment of Corn Stovers by Solid-State Cultivation of Phanerochaete Chrysosporium for Biogas Production. *Appl. Biochem. Biotechnol.* **2013**, *172*, 1365–1376. [CrossRef]
40. Noorfidza, H.; Muhammad, A. Effect of Particle Size on Mechanical Properties of Pellets Made from Biomass Blends. *Procedia Eng.* **2016**, *148*, 93–99. [CrossRef]
41. Reid, I.D. Solid-State Fermentations for Biological Delignification. *Enzym. Microb. Technol.* **1989**, *11*, 786–803. [CrossRef]
42. Vasco-Correa, J.; Shah, A. Techno-Economic Bottlenecks of the Fungal Pretreatment of Lignocellulosic Biomass. *Fermentation* **2019**, *5*, 30. [CrossRef]
43. Bals, B.; Rogers, C.; Jin, M.; Balan, V.; Dale, B. Evaluation of Ammonia Fibre Expansion (AFEX) Pretreatment for Enzymatic Hydrolysis of Switchgrass Harvested in Different Seasons and Locations. *Biotechnol. Biofuels* **2010**, *3*, 1. [CrossRef]
44. Hu, Z.; Wang, Y.; Wen, Z. Alkali (NaOH) Pretreatment of Switchgrass by Radio Frequency-Based Dielectric Heating. *Appl. Biochem. Biotechnol.* **2008**, *148*, 71–81. [CrossRef] [PubMed]
45. Lee, J.S.; Sokhansanj, S.; Lau, A.K.; Lim, J.; Bi, X.T. Moisture Adsorption Rate and Durability of Commercial Softwood Pellets in a Humid Environment. *Biosyst. Eng.* **2021**, *203*, 1–8. [CrossRef]
46. Sarkar, M.; Kumar, A.; Tumuluru, J.S.; Patil, K.N.; Bellmer, D.D. Gasification Performance of Switchgrass Pretreated with Torrefaction and Densification. *Appl. Energy* **2014**, *127*, 194–201. [CrossRef]

47. Via, B.K.; Adhikari, S.; Taylor, S. Modeling for Proximate Analysis and Heating Value of Torrefied Biomass with Vibration Spectroscopy. *Bioresour. Technol.* **2013**, *133*, 1–8. [CrossRef]
48. Frodeson, S.; Henriksson, G.; Berghel, J. Pelletizing Pure Biomass Substances to Investigate the Mechanical Properties and Bonding Mechanisms. *BioResources* **2018**, *13*, 1202–1222. [CrossRef]
49. Hu, Z.; Sykes, R.; Davis, M.F.; Charles Brummer, E.; Ragauskas, A.J. Chemical Profiles of Switchgrass. *Bioresour. Technol.* **2010**, *101*, 3253–3257. [CrossRef]
50. Gao, Z.; Fan, Q.; He, Z.; Wang, Z.; Wang, X.; Sun, J. Effect of Biodegradation on Thermogravimetric and Chemical Characteristics of Hardwood and Softwood by Brown-Rot Fungus. *Bioresour. Technol.* **2016**, *211*, 443–450. [CrossRef]
51. Yub Harun, N.; Muhammad, A. Torrefaction of Agricultural and Forestry Biomass Using TGA-FTIR-MS. In Proceedings of the Sixth International Exergy, Energy and Environment Symposium, Rize, Turkey, 1–4 July 2013; pp. 317–325.