Abstract: Waste management systems are overloaded with huge streams of plastic, a large part of this being originated from packaging. Additionally, the production of wheat, one of the most cultivated crops in the world, generates low-value lignocellulosic materials, which are mostly discarded. In this study, the wheat lignocellulosic byproducts straw and bran were used for the co-production of enzymes and bio-based materials with possible application as packaging via the compression molding method. The mechanical properties of the films were studied based on the effects of the removal of lignin by alkali and biological pretreatment, the growth of filamentous fungi, the size of the particles, and the enzyme recovery. Generally, the straw films were stiffer than the bran ones, but the highest Young’s modulus was obtained for the biologically pretreated bran (1074 MPa). The addition of a step to recover the fungal cellulases produced during the cultivation had no statistical effect on the mechanical properties of the films. Moreover, alkali and biological pretreatments improved the anaerobic biodegradability of the straw films. Thus, the wheat bran and straw can be used for the co-production of enzymes, materials, and biogas, potentially changing how wheat and packaging wastes are managed.

Keywords: biodegradability; bio-based material; filamentous fungi; Trametes versicolor; wheat

1. Introduction

Plastics are widely used in the modern world. They are present in a plethora of products in different applications [1]. In the European market, almost 40% of the plastic is used by the packaging industry. Usually, the plastic used in packaging is nonbiodegradable and quickly discarded, creating a huge plastic waste flow. Incorrect management of this waste ends up causing terrestrial, marine and freshwater contamination [2]. Moreover, these plastics are formed by a composite multilayer structure, which includes laminates with contaminants such as colorants, printing inks, adhesives, etc. This, as well as the presence of organic contamination in the case of food packages, can make recycling or reuse difficult [3]. A group of actions is necessary to change this scenario, including the substitution of the plastic by biodegradable materials and a better management system [1,2].

Agro-industrial residues can be used to produce biodegradable materials with the potential to replace plastic packaging in some applications [4]. These bio-based materials, however, still face limited applications because of their inherent characteristics such as hydrophilicity, thermal instability, brittleness, low melt strength, and high water vapor and oxygen permeability [5].

Wheat constitutes the crop with the largest cultivated area in the world. During the harvesting and processing of the grain, large amounts of straw and bran are produced. The straw is mostly composed of cellulose, hemicellulose, and lignin, and is partially used in the production of paper and animal
feed, but its majority is still treated as waste [6]. The bran consists mostly of carbohydrates (starch, hemicellulose, and cellulose), with low amounts of protein and lignin [7,8]. The material is used as animal feed but, because of its abundance and low nutritional value, it is sold inexpensively [8,9]. Alternatively, one can take advantage of the polymeric nature of both straw and bran and use them for the production of bio-based materials by heat compression molding, a technique involving the thermo-mechanical processing of a material [10].

It is important to notice, however, that some inherent characteristics of the lignocellulosic materials, such as the crystallinity of the cellulose and the coverage of the carbohydrates by lignin, make them resistant to chemical changes. Several pretreatment methodologies have been studied to reduce the recalcitrance of the biomass. The alkali pretreatment effectively breaks the ester bonds between lignin, hemicelluloses, and cellulose [11].

Alternatively, microorganisms can be used to pretreat the lignocellulosic material. The white-rot fungi degrade the lignin and hemicellulose and expose the cellulose [11]. The removal of the lignin and the reduction of the crystallinity of the cellulose may improve the interactions among the polymers, yielding a positive effect on the processed material. Moreover, the presence of chemically-modified lignin in polymeric materials has been reported to improve specific properties of polymeric materials [12]. The filamentous fungi, when growing on solid surfaces, form an intricate matrix of filaments known as mycelium [13]. When using lignocellulosic materials, the natural polymers present in the substrate (cellulose, hemicelluloses, starch, etc.) are degraded by the action of enzymes released by the fungi, and converted into biomass, containing different polymers (protein, chitin, β-glucans, etc.) [13,14]. Growing filamentous fungi in lignocellulosic residues may improve the final bio-based material because of the presence of these polymers and the entanglement of the mycelium in the material structure.

In this paper, the production of bio-based materials from wheat bran and straw was studied. The effects of an alkali pretreatment, the growth of the white-rot fungus Trametes versicolor, and the cultivation of the filamentous fungi Neurospora intermedia and Aspergillus oryzae on the mechanical characteristics of the materials and their biodegradability were investigated. Lastly, the recovery of the enzymes produced by the fungi during solid-state fermentation was investigated. A summary of the steps involved in this study are presented in Figure 1. The utilization of the wheat byproducts for the production of material, enzyme, and fuel (biogas) was shown through a bioprocess route.

![Flowchart showing the stages studied for the production of wheat bio-based materials.](image-url)
2. Materials and Methods

2.1. Material

Wheat straw was kindly provided by Lantmännen Agroetanol (Norrköping, Sweden) and the wheat bran was purchased locally from Granngården (Borås, Sweden). Wheat straw was milled to a particle size of less than 1 mm using a cutting mill (Retsch SM 100, Haan, Germany). The moisture content of wheat straw and wheat bran was measured in triplicate by the weight difference before and after drying at 105 °C until constant weight.

2.2. Microorganisms

Strains of Neurospora intermedia (CBS 131.92), Aspergillus oryzae var. oryzae (CBS 819.72), and Trametes versicolor (CBS 114372) used in this study were obtained from the Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands). N. intermedia and A. oryzae were grown in Potato Dextrose Agar (PDA) plates, containing potato extract (4 g/L), glucose (20 g/L), and agar (15 g/L) at 30 °C until the formation of spores (3–5 days). T. versicolor was grown in the same PDA plates sealed with Parafilm® for eight days at room temperature. The cultured plates were stored at 5 °C for a maximum period of 30 days.

2.3. Material Processing

2.3.1. Biological Pretreatment

For the biological pretreatment, an amount of 4 g of sterile wheat bran or straw was added to a sterile Petri dish (diameter of 90 mm) and wetted with 20 mL of potassium phosphate buffer (0.1 M, pH 5.5). The sterilization of the solid materials was carried out in autoclave (Systec VX-95, Linden, Germany) in three cycles of 10 min at 121 °C. T. versicolor was inoculated by transplanting three square agar plugs of 1 cm² from the stock PDA plates to the new plates. The plates were sealed with Parafilm® and kept at room temperature for seven (bran) or 14 days (straw). At the 5th or 7th day (for bran and straw, respectively) the material was mixed for T. versicolor to grow evenly on the material. Five plates were prepared in replicate for each material. Each yielding one sample for compression molding (see Section 2.3.4).

2.3.2. Alkali Pretreatment

The alkali pretreatment of the wheat straw was carried out using a 1% (w/v) NaOH solution mixed with the lignocellulosic material at a proportion of 5 mL NaOH/g straw. Batches containing 400 g of each material were prepared. The obtained slurry was then autoclaved for 30 min at 121 °C (Systec VX-95, Linden, Germany). Starch retrogradation during pretreatment of wheat bran was avoided by enzymatic treatment. Thus, bran was mixed with distilled water at 20.83% (w/v) ratio and α-amylase (Fermgen, Genencor) was added at 0.4 mg per gram of substrate. The mixture was slowly heated up to 70 °C in a water bath and kept for 2 h. Thereafter, a 25% NaOH solution was added to bring the final mixture to the same conditions as those of wheat straw (i.e., 1% w/v NaOH and 20% w/v solids). Lastly, the material was autoclaved at 121 °C for 30 min.

2.3.3. Fungal Cultivation

The cultivation of the filamentous fungi using the raw wheat byproducts was carried out in Petri dishes (diameter of 90 mm) containing 4 g of the sterile material mixed with 20 mL of potassium phosphate buffer (0.1 M, pH 5.5). The sterilization of the material and solutions followed the same procedure previously described. Spore suspensions of N. intermedia and A. oryzae were obtained by pouring 12 mL of sterile water on the sporulated plates and gently stirring it with an L-shaped sterile Drigalski spatula. A volume of 2 mL of this suspension was transferred to the new Petri dishes containing the lignocellulosic material (i.e., straw or bran). The material was mixed with sterile
spreaders and incubated in a climate chamber (Memmert, Schwabach, Germany) at 35 °C, 90% relative humidity, for six days. On the 4th day, when spores started appearing on the surface of the material, the plates were opened near a flame to keep the sterile condition, and the material was mixed again.

When growing the fungus in the alkali-pretreated material, 24 g of the pretreated slurry were added to a Petri dish together with some orthophosphoric acid (85%, Scharlau, Spain) to bring the pH to 5.5. The amount of acid was determined by mixing 5 g of the slurry with 100 mL of ultrapure water and titrating orthophosphoric acid until the aimed pH (160 µL per plate for straw and 120 µL for bran). Thereafter, 2 mL of a spore suspension was added to the plates and the cultivations were carried out following the same procedure described above. All cultivations were performed in five replicates.

2.3.4. Compression Molding

The samples were pressed following three methods: i) Direct pressing, ii) milling and pressing, and iii) extraction of enzymes, milling, and pressing. For the first method, after the pretreatment and/or cultivation, the material was dried overnight at 70 °C. The dry material was then pressed at 120 °C and 150 kN (3.75 MPa) for 10 min in a Rondol 20 Ton molding press (Rondol Technologies Ltd., Strasbourg, France). For the second method, the dried samples were milled to a powder passing a 0.2 mm screen using a variable speed rotor mill (Pulverisette 14, Fritsch, Idar-Oberstein, Germany). The powder was mixed with glycerol at a 7:3 weight ratio, kept overnight at 5 °C, and then pressed at the same conditions described before. Lastly, for the third method, the cultivated samples were washed to extract the enzymes. The wet content of a Petri dish was mixed with 100 mL of phosphate buffer (0.1 M, pH 5.5) and agitated for 1 h. Finally, the material was sieved using a kitchen sieve (1 mm² pore size). The liquid was used to determine the enzymatic activity (see Section 2.5.3) and the solids were dried, milled, mixed with glycerol, and pressed respecting the same conditions previously described.

2.4. Anaerobic Digestion

The biodegradability of the produced new materials was investigated through batch anaerobic digestion according to Hansen et al. [15]. The bacterial inoculum was kindly provided by a local thermophilic biogas plant (Borås Energi and Miljö AB, Borås, Sweden). The material and inoculum were mixed with water to a final working weight of 50 g in 120 mL glass bottles. The ratio of substrate to the inoculum was kept at 1:2 based on their content of volatile solids (VS). The glass bottles were sealed with a rubber septum and a metallic cover (Apodan Nordic, Copenhagen, Denmark) and flushed with an anaerobic gas mixture containing 80% N₂ and 20% CO₂ for 3 min. The bottles were then incubated at 55 °C for 30 days. A sample set without added substrate was used as control and all samples were carried out in triplicate. The accumulation of methane during the anaerobic digestion was monitored through sampling from the bottle headspace using a 0.25 mL pressure-tight syringe (VICI, Precision Sampling Inc., Baton Rouge, LA, USA) followed by gas chromatography analysis.

2.5. Analytical Methods

2.5.1. Biomass Characterization

Structural carbohydrates were analyzed according to the NREL methodology [16]. An HPLC system (Alliance 2695, Waters, Milford, MA, USA) equipped with a UV detector (Waters 2487), operating at 210 nm wavelength, and a refractive index (RI) detector (Waters 2414) was used. Two columns were used. A hydrogen-ion based ion-exchange column (Aminex HPX-87H, Bio-Rad, Hercules, CA, USA), running at 60 °C and using 0.6 mL/min of 5 mM H₂SO₄ as mobile phase, was used to detect acetic acid, lactic acid, furfural, and 5-(hydroxymethyl)furfural (HMF). A lead (II)-based column (Aminex HPX-87P, Bio-Rad), running at 85 °C and 0.6 mL/min of ultrapure water, was used for analyses of glucose, xylose, cellobiose, maltose, mannose, and arabinose.

The determination of α-glucans was made using the total starch assay kit (code K-TSHK) from Megazyme (Ireland).
The nitrogen content of the samples was determined using the Kjeldahl method previously described by Ferreira et al. (2015) [17], and the results are presented as total Kjeldahl nitrogen (TKN). The Kjeldahl tablets from Merck (Germany) and the antifoam tablets from Thompson and Capper (United Kingdom) were used.

The amount of volatile solids (VS) present in the samples used for anaerobic digestion tests was quantified according to Method 1684 from the US Environmental Protection Agency [18]. All analyses were performed in triplicates.

2.5.2. Methane Analysis

Gas chromatographic analysis of the methane content in the headspace of bottles used for anaerobic biodegradability tests was carried out according to Kurniawan et al. (2018) [19].

2.5.3. Enzymatic Activity

The cellulase activity in the enzymatic extracts was determined using filter paper as a substrate (FPase) and following the method described by Ghose (1987) [20]. The determination of sugars released in the enzymatic hydrolysis was performed using the DNS method. One unit of FPase (1 FPU) represents the amount of enzyme responsible for producing 1 µmol of glucose equivalent per minute. Enzymatic activity was analyzed in duplicate for each of the five replicates prepared in fungal cultivation.

2.5.4. Tensile Analyses

The films were laser cut (GCC LaserPro Spirit GLS, Taiwan; settings: speed 10%, power 100%, PPI 1524) into a dog-bone shape according to ISO 32-7. One specimen was removed from each film and subjected to a tensile analysis using an Elastocon H10KT tensile testing machine (Elastocon AB, Bråmhult, Sweden). The tensile strength ($\sigma_b$), the elongation at break ($\varepsilon_b$), and the Young’s modulus (Y) were obtained using a load cell of 100 N (Tinius Olsen FBB-100N), gauge length of 20 mm, and width of the specimen of 4 mm. The elongation at break was determined using the variation of the distance between the grips. The data was processed using the QMat 5.41a-Dongle 4631 software processor. The thickness of the films was measured using a thickness gauge Elastocon EV 01F (Bråmhult, Sweden).

2.6. Statistical Analyses

Statistical analyses of the data obtained in this study were conducted in Minitab© (version 17.1.0). The analysis of variance (ANOVA) used general linear models with a 95% confidence interval. The values in the graphs are presented as the averages with one error bar up and one down, each of them representing one standard deviation. In the tables, the results are presented as the average ± one standard deviation.

3. Results and Discussion

3.1. Characterization of the Wheat Residues

The wheat bran, straw, and the washed solids recovered after the pretreatment of these materials were characterized in terms of carbohydrates, lignin, solids, ashes, and nitrogen. Table 1 summarizes the results of the characterization. Approximately half of the total glucans present in bran are formed by $\alpha$-glucans (15.8% of 30.7%, dry weight). The material is rich in xylans (23.2%) and contains significant amounts of arabinans (9.9%) and acid insoluble lignin (14.1%). Arabinoxylans are the main structural component of wheat bran. Among the nonstarch carbohydrates, 69% are arabinoxylans and 31% are other forms of glucans ($\beta$-glucans, including cellulose). The results are in agreement with Yin et al. [21] who determined the arabinoxylans to represent 70% of the nonstarch carbohydrates and the glucans represent the other 30%. Considering a factor of 5.37 for the conversion of total nitrogen to protein (determined for whole-grain wheat flour) [22], wheat bran consists of 14.5% of proteins.
Table 1. Characterization of the lignocellulosic materials before and after the alkali pretreatment (% w/w, dry matter).

|                          | Bran Pretreated Bran | Straw Pretreated Straw |
|--------------------------|----------------------|------------------------|
| Total glucans            | 30.7 ± 0.8           | 25.4 ± 1.1             | 38.5 ± 1.1 | 50.9 ± 1.8 |
| α-glucans                | 15.8 ±0.2            |                        | 0.8 ± 0.3 |           |
| Xylans                   | 23.2 ± 0.9           | 21.3 ± 1.5             | 25.9 ± 0.3 | 26.8 ± 0.8 |
| Arabinans                | 9.9 ± 0.1            | 10.0 ± 0.3             | 3.7 ± 1.0 | 2.9 ± 0.3 |
| Acid insoluble lignin    | 14.1 ±1.2            | 3.3 ± 1.7              | 22.8 ± 1.3 | 1.6 ± 0.9 |
| Moisture<sup>1</sup>     | 10.5 ±0.1            |                        | 8.5 ± 0.1 |           |
| Ashes                    | 5.8 ± 0.1            |                        | 9.8 ± 0.1 |           |
| Total Kjeldahl nitrogen  | 2.7 ± 0.1            |                        | 0.6 ± 0.0 |           |

<sup>1</sup> Wet basis.  <sup>2</sup> Values not measured.

Wheat straw contained more glucans (38.5%), with only 2% of this being α-glucans. The amount of xylans (25.9%) was superior to that of bran, but the arabinans were less present in the straw (3.7%). Straw is very poor in proteins (3.2%) and 9.8% of it is formed by ashes. Qi et al. [23] reported 37.2% of glucan, 28.8% of xylan, and 18.9% of acid-insoluble lignin for the wheat straw composition. The results are similar to those obtained in this study. HMF and furfural were not detected in the pretreated samples.

3.2. Fungal Cultivation

For *N. intermedia* and *A. oryzae*, it took four days until fungal growth was visible on the surface of the material. After that, the material was mixed again and left for two more days for further growth. *T. versicolor* took five days when growing on bran and seven days when growing on straw, to completely grow on the surface of the material. Afterwards, the material was mixed and the cultivation was carried out for two more days for bran or seven more days for straw.

3.3. Mechanical Analyses

Pressing of the raw or fermented byproducts yielded bad quality materials, whose edges were not well aggregated, as shown in Supplementary Figures S1 and S2. Therefore, experiments were also conducted with the material milled to a powder (<0.2 mm) and mixed with glycerol, which acted as the plasticizer. Results of the tensile strength (σ<sub>b</sub>, MPa), the elongation at break (ε<sub>b</sub>, %), the thickness (mm), and the Young’s modulus (Y, MPa) of the films are presented in Figure 2 for wheat bran and Figure 3 for wheat straw. The maximum tensile strength obtained was 7.2 MPa for the bran film (raw, not milled). Generally, although the edges of the films were better aggregated when the bran was milled, the tensile strength for these films decreased. On the other hand, for the films made with straw, the milling of the material improved the tensile strength—the highest one being observed for the raw straw cultivated with *A. oryzae* and milled (3.5 MPa). Comparatively, high-density polyethylene [24] and low-density polyethylene [25] have been reported to have tensile strength around 21 and 11 MPa, respectively. All the films presented elongation at break below 3%, except for the bran pretreated with *T. versicolor* and milled (3.7%).
Figure 2. (a) Tensile strength (MPa), (b) elongation at break (%), (c) thickness (mm), and (d) Young’s modulus (MPa) of the films made with wheat bran. Individual standard deviations were used to calculate the intervals. Number of replicates = 5; confidence interval for the means of 95%. Ni, Ao, and Tv stands for *N. intermedia*, *A. oryzae*, and *T. versicolor*, respectively.
Figure 3. (a) Tensile strength (MPa), (b) elongation at break (%), (c) thickness (mm), and (d) Young’s modulus (MPa) of the films made with wheat straw. Individual standard deviations were used to calculate the intervals. Number of replicates = 5; confidence interval for the means of 95%. Ni, Ao, and Tv stands for N. intermedia, A. oryzae, and T. versicolor, respectively.
Using rice straw, Bilo et al. (2018) have produced bioplastics via the solution casting method with trifluoroacetic acid (TFA) as solvent. The yielded films (approx. 0.3 mm thick) had tensile strength of 43 MPa, elongation at break of 6.1%, and Young’s modulus of 45 MPa [1]. Although less strong, the films produced in this study using wheat straw and bran via compression molding have been determined to be stiffer with a maximum Young’s modulus of 668 MPa for the straw film (A. oryzae, milled) and 1074 MPa for the bran film (T. versicolor). It is noteworthy that the use of the solution casting method includes the evaporation and recovery of this solvent, which are time and energy-consuming operations that are avoided in the compression molding technique [26]. Additionally, the application and uncertain emission of TFA in the environment raises continuous concerns [27].

The high Young’s modulus for the biomaterial obtained from biologically pretreated bran (i.e., after cultivation of T. versicolor) is probably due to the uniform and consistent growth of the microorganism on the whole surface of the material, producing a fungal layer, which effectively changed the mechanical properties of the pressed sheet. When the samples were milled before pressing, the growth of the fungus caused no effect on the modulus.

The cultivation of T. versicolor was considered in this study as a biological pretreatment of the lignocellulosic biomass, as an alternative to physicochemical alkali pretreatment. T. versicolor is a white-rot fungus, belonging to the Basidiomycota division. It delignifies the biomass using a complex ligninolytic system, including the production and excretion of ligninolytic enzymes such as lignin peroxidase, manganese peroxidase, and laccase [28].

Milling was the only factor that influenced the tensile strength (Table 2). The type of material (bran or straw) was the most important factor influencing the elongation at break, thickness, and Young’s modulus. The other factors affecting the thickness and the Young’s modulus were the alkali pretreatment and the milling. The T. versicolor pretreatment and the washing had statistical significance on the elongation at break. The cultivation of filamentous fungi (N. intermedia or A. oryzae) caused no statistical effect on any of the analyzed responses.

### Table 2. Coefficients of the general linear models.

| Term          | Tensile Strength Coef | p-Value | Elongation Coef | p-Value | Thickness Coef | p-Value | Young’s Modulus Coef | p-Value |
|---------------|-----------------------|---------|----------------|---------|----------------|---------|----------------------|---------|
| Constant      | 2.401                 | 0.000   | 0.769          | 0.000   | 0.7781         | 0.000   | 217.2                | 0.000   |
| Material      | Bran                  | 0.239   | 0.159          | 0.5826  | −0.2067        | 0.000   | −130.3               | 0.000   |
| Pretreatment  | Alkali                | 0.309   | 0.257          | 0.194   | 0.087          | −0.0715 | 0.006                | −100.2  | 0.001               |
|               | No                    | 0.021   | 0.939          | −0.328  | 0.004          | 0.0009  | 0.971                | −45.6   | 0.122               |
| Microorganism | A. oryzae             | −0.216  | 0.415          | −0.115  | 0.295          | 0.0133  | 0.595                | −17.1   | 0.553               |
|               | N. intermedia         | −0.199  | 0.453          | 0.034   | 0.754          | 0.0199  | 0.426                | −1.3    | 0.963               |
| Mill          | No                    | 0.539   | 0.003          | −0.0108 | 0.882          | 0.0868  | 0.000                | −77.3   | 0.000               |
| Wash          | No                    | −0.462  | 0.253          | 0.457   | 0.007          | −0.0458 | 0.231                | 56.2    | 0.203               |

The alkali pretreatment acts on the lignocellulosic structure by modifying the cellulose and by partially solubilizing the hemicelloses and lignin [29]. The addition of alkali lignin in soy protein isolate plastics made by compression molding has been reported to increase the tensile strength and the Young’s modulus. Moreover, the hydrophobicity of the lignin is suggested to cause a decrease in the amount of water absorbed by the films [30]. In this study, the solubilization of the lignin present in the material itself caused a reduction in the Young’s modulus and an increase in the elongation at break of the bio-based films. No significant effect on the tensile strength was identified.
3.4. Compositional Characterization and Enzymatic Activity

The presence of proteins in the bio-based materials is interesting because of the amphiphilic nature of these polymers, i.e., the presence of hydrophilic and lipophilic regions that result in improved interaction with different types of molecules [31]. Therefore, some promising conditions were selected among the produced films to determine the effect of the pretreatment, fungal cultivation, and enzyme extraction on the nitrogen content. According to the results obtained for the straw biologically pretreated with *T. versicolor*, washing and milling caused no effect on the nitrogen content of the samples (see Table 3), considering that milled samples contain 30% of added glycerol. It is expected, however, that the nitrogen compounds have been modified by the fungus, with higher amounts of proteins in the cultivated samples. Similarly, the alkali pretreatment and the cultivation of the filamentous fungi did not significantly change the nitrogen content of the samples.

| Material | Pretreatment | Microorganism | Mill | Washing | TKN\(^1\) (% w/w) |
|----------|--------------|---------------|------|---------|---------------------|
| Bran     | *T. versicolor* | No            | No   | No      | 2.8 ± 0.1           |
|          |              | Yes           | Yes  |         | 1.8 ± 0.0           |
| Straw    | Alkali       | *N. intermedia* | No   | No      | 0.6 ± 0.0           |
|          |              | *A. oryzae*   | No   | No      | 0.6 ± 0.0           |
|          | *T. versicolor* | No            | Yes  | No      | 0.4 ± 0.0           |
|          |              |               | Yes  | Yes     | 0.4 ± 0.0           |

\(^1\) Total Kjeldahl nitrogen.

The cultivated samples were also analyzed regarding the cellulase activity of the washing solution. The results are presented in Table 4. The use of alkali pretreated straw with the ascomycetes *N. intermedia* or *A. oryzae* has not yielded statistically different results of FPase (\(p = 0.27\)). Similarly, *T. versicolor* yielded the same amount of FPase when growing on both wheat bran and straw (\(p = 0.24\)). On the other hand, the enzymatic activities of the *T. versicolor* and the ascomycetes were different (\(p ≤ 0.001\)).

| Material | Pretreatment | Microorganism | FPU/g Substrate |
|----------|--------------|---------------|-----------------|
| Bran     | *T. versicolor* | No            | 17.0 ± 7.9      |
|          |              | *N. intermedia* | 34.1 ± 8.4     |
| Straw    | Alkali       | *A. oryzae*   | 44.4 ± 12.4     |
|          | *T. versicolor* | No            | 6.3 ± 3.0       |

Using wheat straw and *Aspergillus tubingensis* JP-1, Pandya et al. [32] obtained an FPase activity of 0.669 FPU/g. For wheat bran, the activity was 0.024 FPU/g. White-rot basidiomycetes have been tested for the production of cellulase using wheat straw [33]. The FPase produced by *Funaalia trogii*, *Lentinus edodes*, *Pleurotus dryinus*, and *Pleurotus tuberregium* were 18, 26, 18, and 24 FPU/g of straw, respectively. Nitrogen supplementation by the addition of NH\(_4\)NO\(_3\) increased the enzymatic activity to 46 and 30 FPU/g of the *P. dryinus* and *P. tuberregium*, respectively [33]. In the present study, with no supplementation of nitrogen, the basidiomycete *T. versicolor* produced 6.3 FPU/g of straw and 17.0 FPU/g of bran. Although it contains less cellulose, the physical and chemical characteristics of the wheat bran, favored the secretion of cellulases, as well as the faster growth on the substrates, compared to wheat straw. Bran has been reported to contain sufficient nutrients for microbial growth and, even when humid, it remains loose and well-aerated in mass [34]. Similarly, the FPase activities reported in the literature [35] when growing *Aspergillus niger* in wheat bran and straw were 16.8 and 2.8 FPU/g,
respectively. When the materials were alkali pretreated, the FPase obtained were 18.0 and 30.6 for bran and straw, respectively.

The extraction of enzymes did not change the mechanical characteristics of the material and, therefore, cellulolytic enzymes can be recovered as a valuable co-product in the production process of bio-based materials from wheat byproducts.

3.5. Biodegradability by Anaerobic Digestion

The amount of methane produced during the anaerobic digestion of the films is presented in Figure 4. For the bran, the raw material yielded a faster production of methane compared to the films made of bran biologically pretreated, washed, and milled ($p = 0.042$). For the films made with fermented bran, a plateau was reached on the 25th day of the anaerobic digestion (maximum values of $415.5 \pm 22.6$ and $449.2 \pm 27.2$ NmL CH$_4$/gVS were reached at the 30th day for the _N. intermedia_ and the _T. versicolor_ fermented film, respectively), while for the raw bran film, on the 18th day, a decrease in the rate of the production of methane was observed (maximum value of $414.4 \pm 12.2$ NmL CH$_4$/gVS obtained on the 18th day). The films made with _N. intermedia_ cultivated, milled bran did not yield a statistically different methane production at the 18th day.

For the straw, the films made with the raw material degraded slower than the alkali-pretreated, _A. oryzae_-cultivated, milled straw during the first 18 days of anaerobic digestion ($p = 0.018$). According to Table 1, the amount of carbohydrates present in the straw biomaterial after the alkali pretreatment was increased from 68.1% to 80.6% (dry weight) and the acid insoluble lignin was reduced from 22.8% to 1.6%. This may explain the faster biodegradability of the pretreated material over the raw straw.

After 30 days of anaerobic digestion, an accumulated volume of 289 NmL CH$_4$/gVS was produced from the raw straw. Comparatively, the films made with the _T. versicolor_ cultivated, washed, milled straw reached a plateau about the 18th day, with a cumulative methane production of 277 NmL CH$_4$/gVS, which is not statistically different from the raw straw ($p = 0.066$). Comparatively, the
biological pretreatment with *T. versicolor* decreased the biodegradability rate of the bran films but increased the rate for the straw films. Patinvoh et al. (2017) highlight that, although the solubilization of the lignin achieved by white-rot fungi increases the digestibility of the material, losses of cellulose and hemicellulose also occur [36].

The alkali pretreatment may be pointed as responsible for the increase in the biodegradability of the straw films. Similarly, an alkali pretreatment of wheat straw more than doubled the production of methane compared to the production from raw straw [37]. In their work with alkali-pretreated rice straw, Elsayed et al. (2018) demonstrated that the enzymatic hydrolysis combined with fermentation (using cellulases and *Saccharomyces cerevisiae*, respectively) improved the biogas production from 286.9 to 372.4 mL/gVS [38]. The authors suggest that the presence of short molecules such as the ethanol produced by the yeast and the xylose released during the hydrolysis may play a role in the higher biogas yield. In the present study, pretreatment and fermentation were used to act on the structure of the biomass, modifying the recalcitrance. The term recalcitrance refers to the resistance to microbial and enzymatic digestion [39]. Accordingly, the treatments given to wheat straw succeeded in yielding a less recalcitrant material. On the other hand, the recalcitrance of the bran was increased possibly because of the digestion of the less recalcitrant parts of the material by the microorganisms.

Therefore, among the conditions tested, no improvement in the biodegradability of the bran was achieved. On the other hand, the combination of the alkali pretreatment, with *A. oryzae* cultivation, and size reduction effectively decreased the time for anaerobic digestibility of the straw films.

4. Conclusions

In this study, the agro-industrial residues wheat bran and straw have been used for the co-production of bio-based materials and enzymes. The cultivation of filamentous fungi in the lignocellulosic material via solid-state fermentation did not change the mechanical properties of the yielded films. The solubilization of the lignin using an alkali pretreatment significantly reduced the Young’s modulus of the films, and the growth of the basidiomycete *T. versicolor* increased the elongation at break. Lastly, the addition of a step to recover the enzymes produced by the fungi had no statistical effect on the characteristics of films. The method can potentially improve the management of the residues generated during the wheat production and processing. Moreover, the produced bio-based materials may be suitable for packaging, improving the plastic waste management system.

**Supplementary Materials:** The following are available online at [http://www.mdpi.com/1996-1073/13/6/1300/s1](http://www.mdpi.com/1996-1073/13/6/1300/s1), Figure S1. Wheat bran (a and c) and straw (b and d) cultivated with *Aspergillus oryzae* (a and b) and *Neurospora intermedia* (c and d); Figure S2. Alkali-pretreated wheat straw after fermentation by *Aspergillus oryzae* and pressing; Figure S3. Wheat straw after fermentation by *Aspergillus oryzae* and pressing.

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