Enzyme-treated Wheat Straw-based PVOH Bio-composites: Development and Characterization

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Valorization of lignocellulosic waste residues in the development of potential biodegradable composites has been of recent research interest. Recent research has shown that wheat straw can be used as a reinforcement material for the synthesis of novel polyvinyl alcohol (PVOH)-based composites. However, certain pretreatment methodology needs to be used for the selective removal of the lignin component. The de-lignification of native wheat straw was performed using an in-house isolated ligninolytic consortium. The bio-composites were developed using the de-lignified wheat straw along with PVOH as the matrix phase and glycerol as a plasticizer *via* a compression molding technique. In this study, a structural analysis by Fourier transform infrared spectroscopy (FT-IR) showed that the enzymatic treatment led to noticeable changes in the chemical structure of the materials used. A dynamic mechanical analysis (DMA) of the composites revealed an increase in the tensile strength of the sample from 46.1 MPa ± 0.1 MPa to 53.0 MPa ± 0.9 MPa, upon the addition of the plasticizer. Also, there was a noticeable increase in the tensile modulus of composites from 2,130 MPa to 4,520 MPa, respectively. Topographical features of the newly synthesized PVOH-based bio-composites were observed using scanning electron microscopy.

Keywords: Ligninolytic enzymes; Biofibres; Bio-composites; Delignification; Compression molding

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INTRODUCTION

Owing to increasing environmental concerns and legislation by government authorities coupled with the demands of industrialists, the use of the synthetic polymers and structural composites is considered critical (Iqbal *et al.* 2013). This concern has led to the development of sustainable, industrially useful, ecologically green bio-composite materials (Awal *et al.* 2015; Bai *et al.* 2015; Vaisanen *et al.* 2016). These biocomposites are contributing more efficiently and cost effectively towards the environmental constraints, such as recycling of the products (Karsli *et al.* 2013; Khanam and Almamdeed 2014). Bio-composites contain two major components; one of the components is based on natural fibres, which serve as a reinforcement phase (Horrocks 2011). This is due to its environmentally friendly properties, such as natural availability, biodegradability, suitable physical and mechanical strengths, low cost, high specific stiffness, and low density (Mantia and Morreale 2011). Because of these highly remarkable features, biofibres have replaced several synthetic fibre composites and have occupied a prominent position in marine, automotive, aeroscope, and construction industries (Koronis *et al.* 2013). Moreover, the use of biomass provides a viable solution to the waste disposal, leading to a reduction in environmental pollution, and thus providing an economical way for the development of useful materials (Fink *et al.* 2010; Anandjiwala *et al.* 2013).

Besides these promising and remarkable properties of bio-fibres, several problems of compatibility between the fibres and the matrix phase occur due to a weak interface (Kalia *et al.* 2011). This compatibility issue is usually surmounted by the chemical modification of the filler surface, incorporation of a coupling agent, de-lignification, bleaching, or chlorination (Supri *et al.* 2013; Zaaba *et al.* 2016). This weak interface leads to poor stress transfer between the reinforcement and the matrix, which is mainly dependent on the strong degree of bonding between the two phases (Kalia *et al.* 2009). Therefore, to improve the interface between the matrix and the fibre it is necessary to increase the hydrophobicity of the cellulose fibres. The addition of natural fibers in a polymer matrix may increase or decrease its melting point depending on several factors, such as the nature of the natural fibers and interface. The pretreatment can modify the fibre surface, and such surface functionalization increases the surface roughness and reduces the moisture absorption process (Kalia *et al.* 2009; 2011). The main reason for the surface treatment is to enhance the fibre/matrix interfacial bonding and to transfer the stress in-between the components (Hidayat and Tachibana 2012). Regarding the development of biocomposites, the biological pretreatment of fibres with enzymes can improve the surface morphology of cellulosic fibres (Wu *et al.* 2011). For biological treatments, the use of various fungi shows unique characteristics when the de-lignification of the lignocellulosic biomass produces residues. The white rot fungi (WRF) are the most efficient degraders of lignin due to their ligninolytic enzymes, such as lignin peroxidase (LiP), manganese peroxidase (MnP), laccase, and H_2O_2-producing oxidases (Iqbal *et al.* 2011). These ligninolytic enzymes have low substrate specificity, non-stereo selectivity, and strong oxidative abilities (Iqbal *et al.* 2011; Asgher *et al.* 2013). Among WRF, *Ganoderma lucidum*, *Trametes versicolor,* and *Pleurotus ostreatus* are efficient producers of ligninolytic enzymes (Xavier *et al.* 2007; Batool *et al*. 2013).

Many materials exhibit excellent mechanical properties in their fibrous forms. However, their properties can be enhanced *via* binding them to a suitable matrix (Nitin and Singh 2013). The function of the matrix is to isolate the fibres from each other to prevent abrasion, minimize the formation of surface flaws, and act as a bridge to hold the fibres in place (Prajapati and Qureshi 2015). Among the polymers, polyvinyl alcohol (PVOH) is widely used as a matrix in the processing of composites. It exhibits remarkable adhesion, water soluble, film forming and emulsification properties and therefore had potential application as an adhesive agent in the making of films (Wang *et al*. 2011; Asgher *et al*. 2017). Coupling agents are the chemicals that act as an interphase to create a chemical linkage between the matrix and the reinforcement (Xie *et al*. 2010). Among different coupling agents, maleic anhydride is a compatibilizer widely used as it leads to an increase in the compatibility between the matrix and fibre (Pickering *et al*. 2016; Asgher *et al*. 2017). Plasticizer also adds prominent features to the composites as it increases the flexibility and tends to reduce the glass transition (T_g) temperature of the matrix (Razavi *et al*. 2015). Glycerol is a highly hygroscopic plasticizer and prevents the brittleness of the films when added in the reaction mixtures (Karbowiak *et al.* 2006; Ma *et al.* 2009).

The processing of thermoplastic-based composites can be performed *via* a variety of techniques (Rao *et al.* 2015). Among the various techniques of extrusion, sheet thermoforming, rotational, and compression moldings, extrusion coverings are involved in the continuous manufacturing of the composite products with a uniform cross-sectional area. Compression molding is considered as the most efficient process for the manufacturing of thin, strong, stiff, and lightweight composites (Park *et al.* 2001). The extrusion and the injection molding processes sometimes lead to the damaging of the fibres due to the rotational screw (Carneiro and Maia 2000). In comparison to injection molding, the compression molding technique does not pose any damage to the fibres and therefore preserves the isotropic behavior of composites. Compression molding process is widely used in the making of high strength, complex parts (Howell and Fukumoto 2014) and in the manufacturing of high volume objects (Asgher *et al*. 2017). It possesses a short cycle time and results in a higher production rate (Woehl *et al.* 2010).

Bio-composites are used in a variety of applications from the production of advanced spacecraft to sporting goods to joints implants. Also, they are widely used in the construction, food, electronic, and biomedical industries. The use of bio-composites in the packaging materials of food has been extensively improved and is expected to grow in the next decade, reducing environmental impacts (Soulestin *et al.* 2011). In the construction industries, bio-composites are potentially used in the scaffolding, decking, railing, and framework, informing walls, flooring, fencing, ceiling panels, and decorative panels (Roy *et al.* 2014). The present study was conducted to develop bio-fibre based PVOH bio-composites with enhanced mechanical properties. However, the effect of the biological treatment on the structural, mechanical, and water absorption properties and the morphology of the bio-composites were studied using different mechanical and imaging techniques.

EXPERIMENTAL

Materials

All of the chemicals used in this study were of analytical grade and purchased from Merck (Dramstadt, Germany) and Sigma-Aldrich (Missouri, USA. The lignocellulosic substrates (wheat straw and corncobs) were obtained from the students' research farms, (Faisalabad, Pakistan) and sliced into small pieces, oven-dried, ground to 40-mm mesh size, and stored in air tight plastic jars to avoid moisture. Three white rot fungal strains *Ganoderma lucidum* IBL-05, *Trametes versicolor* IBL-04, and *Pleorotus ostreatus* IBL-02 used for the production of ligninolytic enzymes were obtained from the Industrial Biotechnology Laboratory, Department of Biochemistry, University of Agriculture Faisalabad, Pakistan.

Ligninolytic enzymes production and activity determination

The three ligninolytic enzymes (LiP, MnP, and laccase) were produced from *G. lucidum* IBL-05, *T. versicolor* IBL-04, and *P. ostreatus* IBL-02 using freshly prepared fungal inoculums as reported earlier (Ahmad *et al.* 2016). The enzymes LiP and MnP were produced using wheat straw moistened with Kirk's basal medium (60% w/w moisture) containing an additional 2 mL of glycerol as a carbon source, 2 g urea as a nitrogen source, 1 mM oxalates (1 mL) as a mediator, and 1 mM $CuSO_4$ (1 mL) as a metal ion activator

(Ahmad *et al.* 2016). Laccase was produced using corncobs as a fermentative substrate additionally supplemented with 1 mM 2, 2'-azino-bis (3-ethylbenzothiazoline- 6-sulfonic acid (ABTS, 1 mL) as a laccase-mediator. All of the flasks were autoclaved and inoculated with 5 mL of freshly prepared respective inoculums under sterilized conditions. The inoculated flasks were set at a pH of 4.5 and allowed to ferment at 30 °C (Asgher *et al.* 2013). After the stipulated fermentation (120 h), the enzymes were isolated by filtration and centrifugation of the filtrate at 3000 *g* and used for activity calculation purposes.

The LiP activity was measured *via* the Tien and Kirk method (1988), following the H_2O_2-dependent oxidation of veratryl alcohol to veratraldehyde at 25 °C. The absorbance of the reaction mixture was monitored at 310 nm (ε_{310} = 9300). The MnP was assayed at 25 °C by the H_2O_2-dependent oxidation of manganic-malonate complex, and its optical density (OD) was recorded at 270 nm (ε_{270} = 11,570). The laccase activity was determined by monitoring the oxidation of ABTS. The oxidation was followed at 25 °C and its OD was recorded at 420 nm (ε_{420} = 36,000).

Delignification of lignocellulosic material

Prior to the development of the biocomposites, the wheat-straw bio-fibre was subjected to enzymatic modification. The three ligninolytic enzymes were used as a mixture for the delignification, with each enzyme having 200 U/mL in the consortium. Each flask containing 15 g of the substrate was made up to 100 mL with 0.5 mM of a sodium malonate buffer (pH 4.5). The treated substrate was then analyzed to determine the percentage of lignin and cellulose contents as described (Ahmad *et al.* 2016).

Development of bio-composites

A compression mold technique was used to develop various composites using enzyme-treated de-lignified wheat straw (DWS) and the matrix polymer polyvinyl alcohol (PVOH) with and without glycerol as a plasticizer agent. Briefly, the DWS was initially blended with PVOH at 700 rpm, at 35 °C using distilled water (as required) for 15 min. Next, maleic anhydride (as a compatibilizer) and glycerol (as a plasticizer) were added based on 25% and 50% of the total weight of the composite, respectively. The blended mixtures were then molded using a hot press in a pre-heated metal mold (150 mm in diameter and 1 mm in thickness) at 60 °C for 20 min. The pressure applied ranged from 3 MPa to 4 MPa depending on the loading of the reinforcing material. All of the specimens were then post cured at 50 °C for 12 h (Asgher *et al.* 2017). The newly developed bio-composites were then removed from their respective molds and labeled as PVOH-DWS (prepared using just the matrix polymer and the reinforcement) and PVOH-DWS-PL (prepared using the matrix polymer and glycerol). The prepared bio-composites were then characterized using different analytical and imaging techniques as described below.

Methods

Morphological investigations by SEM

The surface morphologies and the microstructural characteristics of the newly developed bio-composites, *i.e.*, PVOH-DWS and PVOH-DWS-PL samples, along with the individual counterparts were studied in ultra-high vacuum mode at an accelerating voltage of 5 kV (Quanta 250, FEG-SEM; FEI, Hillsboro, Oregon, USA). The test composites were placed on 8-mm diameter aluminum stubs using adhesive tape before record images. High definition images (HDI) were then recorded to study the surface morphologies of each sample. An operating pressure of 700 bar and a deposition current of 20 mA for 2 min was used for analysis as previously described (Iqbal 2015).

Fourier transform infrared spectroscopy

The structural elements of the newly developed bio-composites, *i.e.*, PVOH-DWS and PVOH-DWS-PL, along with the individual counterparts were studied using a Perkin-Elmer system 2000 FT-IR spectrophotometer (Waltham, Massachusetts, United States). The test material was directly placed onto the diamond crystal, and the infrared absorption spectra were recorded from the wavenumber region of 4000 cm^{-1} to 800 cm^{-1}. All of the spectra were collected with 64 scans and 2 cm^{-1} resolutions and assigned peak numbers.

Tensile test on biocomposites

The mechanical analyses were performed using DMA in tensile mode (Universal Test Machine, AMETEK Lloyd Instruments Ltd., West Sussex, UK). Before loading on a crosshead, the test samples were cut into a rectangular shape with 8 mm × 4 mm × 0.25 mm dimensions. The load was set within the range of 1 mN to 6000 mN, and the crosshead speed was set at a constant tensile rate of 200 mN/min^{-1}. All of the tests were conducted at ambient temperature, and an average value of the three repeated tests was taken for each treatment (Lou *et al*. 2007; Asgher *et al*. 2017).

Water uptake test

The water uptake test was performed for all of the composites developed from compression molding. The samples were placed in the water bath for a specific time interval of 30 min to 120 min. The weight gain by the composites was recorded after the specific time intervals on an analytical balance. Before the weight measurement, the surface water was removed from the samples by blotting carefully. The water uptake level of the composite was expressed as a percentage of weight gain. The percentage of water uptake was calculated using Eq. (1). The data reported was the average of three replicates,

$$Water\ uptake\ (\%) = \frac{Wa-Wo}{Wo} \times 100 \tag{1}$$

where *W*o is the initial dry weight of the composites and *W*a is the weight of the composites after exposure to water (Asgher *et al*. 2017).

Statistical analysis

All of the experiments were conducted in triplicate and presented as mean ± standard error (SE). The means and standard errors of means (mean ± SE) were computed for each treatment, and the SE values have been displayed as Y-error bars in figures.

RESULTS AND DISCUSSION

Production of Ligninolytic Enzymes

Three fungal strains, *i.e., G. lucidum* IBL-04, *T. versicolor* IBL-03, and *P. ostreatus* IBL-02, were used for the production of ligninolytic enzymes in solid state fermentation (SSF). The enzyme extracts obtained were assayed and revealed the enzymatic and specific activities to be 1277.91 U/mL and 493.3 U/mg for LiP, 1436.32 U/mL and 460.21 U/mg for MnP, and 911.11 U/mL and 219 U/mg for laccase, respectively. Previously, Oliveira *et al.* (2016) used two WRF strains, *i.e.*, *Ganoderma lucidum* and *P. ostreatus*, for the production of LiP and observed the enzyme activity of 9419.35 IU/mL.

Delignification of Wheat Straw

Wheat straw was enzymatically pre-treated with a mixture of enzyme consortiums that contained various ligninolytic enzymes. The same ratio was used for the three ligninolytic enzymes by keeping their international activity units the same (200 IU/mL). As reported in our previous paper (Asgher *et al*. 2017), in response to the enzymatic pretreatment, the lignin contents of wheat straw were reduced from 24.6% to 10.20%, which led to a maximum de-lignification of approximately 58.5% when the consortium (LiP, MnP, and Laccase) ratio (1:2:2) was used at 25 °C (Table 1). The cellulosic content of 24% in the native straw increased to 76.5% after pretreatment. Previously, Knezevic *et al*. (2013) reported a reduction of 34.1% in the lignin content of wheat straw after incubation for 14 days with *Dichomitus squalens.* Compared with this data, *T. versicolor* BEOFB 320 showed a higher de-lignification of approximately 20.9% for 14 days of wheat straw fermentation, while the levels of the analyzed ligninolytic enzyme activities were similar or even lower. However, a 24 h treatment for the straw at a ratio of 2:5 (v/w) showed a marked reduction in these cell wall components, and in the *in vitro* digestibility (Sridhar *et al.* 2014). Chang *et al.* (2012) screened different fungi for the selective de-lignification of rice straw. They found that traditional white-rot fungus *Phanerochaete chrysosporium* yielded 28.3% lignin degradation and 28.4% holo-cellulose degradation.

Table 1. Percent of Lignin and Cellulose Content before and after the Enzymatic Treatment of Wheat Straw (WS) With a Mixture of Ligninolytic Enzyme Consortium at 25 °C (Ahmad *et al.* 2016)

Enzyme combination	Ratio	Lignin (%)		Delignification (%)	Cellulose (%)	
		Before treatment	After treatment		Before treatment	After treatment
LiP:MnP:Lacc	1:2:2	24.60±0.13	10.20±0.41	58.50±1.05	24.00±0.36	76.54±0.27

The morphological analysis of the native and de-lignified wheat straw was performed *via* scanning electron microscopy and is presented in Fig. 1. It revealed a rigid and ordered structure in the native straw that exposed the presence of a lignin polymer on the surface of the cell with interconnected hemicellulose and cellulose. The de-lignified substrate obtained after the enzymatic pretreatment presented a distorted image with a change in the internal structure of the cell wall, which indicated the removal of lignin and a concomitant increase in the porosity and surface area of the substrate (Ruiz *et al.* 2011).

Characterization of Bio-composites

Fourier-transform infrared spectroscopy (FT-IR)

The structural elements of the de-lignified wheat straw (DWS), polyvinyl alcohol (PVOH), and composites developed from the PVOH-DWS and PVOH-DWS-PL samples were characterized using FT-IR spectroscopy to confirm the structural changes in the bio-composites synthesized from them (Fig. 2).

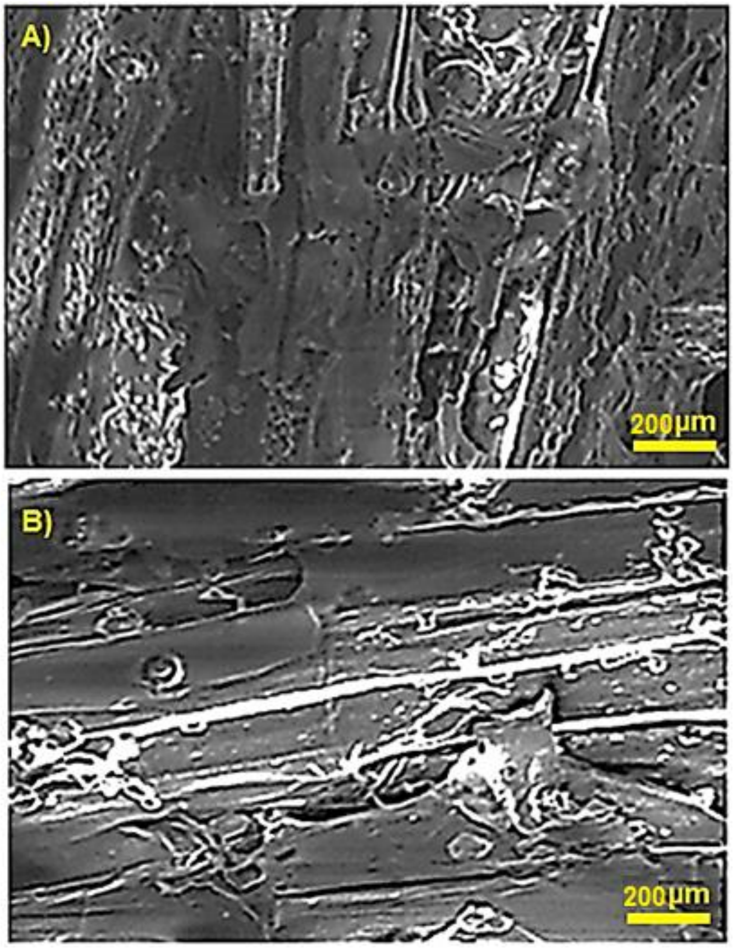

Fig. 1. SEM images of native (A) and de-lignified wheat straw (B)

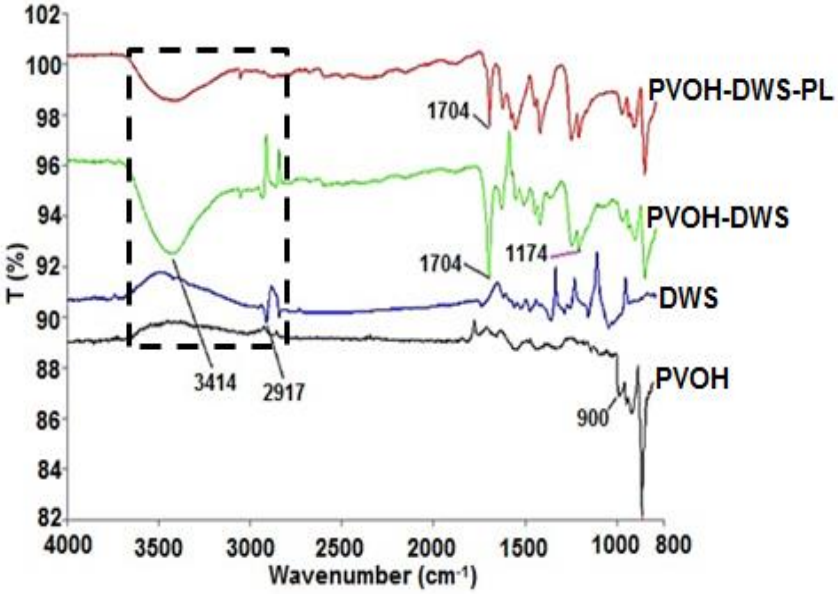

Fig. 2. FTIR spectra of the pure PVOH, pure DWS, PVOH-DWS, and PVOH-DWS-PL-based biocomposites

The characteristic band at 900 cm^{-1} in the case of pure PVOH, but not in the de-lignified substrate (DWS), was assigned to vinyl polymers due to CH_2 deformation vibrations (Długa and Kaczmarek 2014). In the case of PVOH, and the composite PVOH-DWS, the bands in the region between the 4000 cm^{-1} to 3000 cm^{-1} represented the O-H stretching vibration.

The bands in the 3070 to 2860 cm^{-1} region were assigned to the –CH, –CH_2 and –CH_3 vibrations (Iqbal *et al.* 2015a,b). The maximum of the O-H stretching bands of cellulose was situated at 3414 cm^{-1} in the case of the PVOH-DWS-based composite (Marechal and Chanzy 2000). The peaks in pure PVOH around 900 cm^{-1} were attributed to the residual acetate groups (Jayasekara *et al.* 2004).

Scanning electron microscopy (SEM)

A scanning electron microscopy analysis was performed on the individual polymer, *i.e.*, PVOH and DWS, and their corresponding biocomposites, *i.e.*, PVOH-DWS and PVOH-DWS-PL, samples to evaluate their surface morphologies. It was observed that the surface morphology of the polymer (PVOH) appeared to be smooth without any holes in the surface, as shown in Fig. 3. This was in agreement with the findings of Długa and Kaczmarek (2014) who also observed the similar flat, homogenous surface of PVOH without any defects.

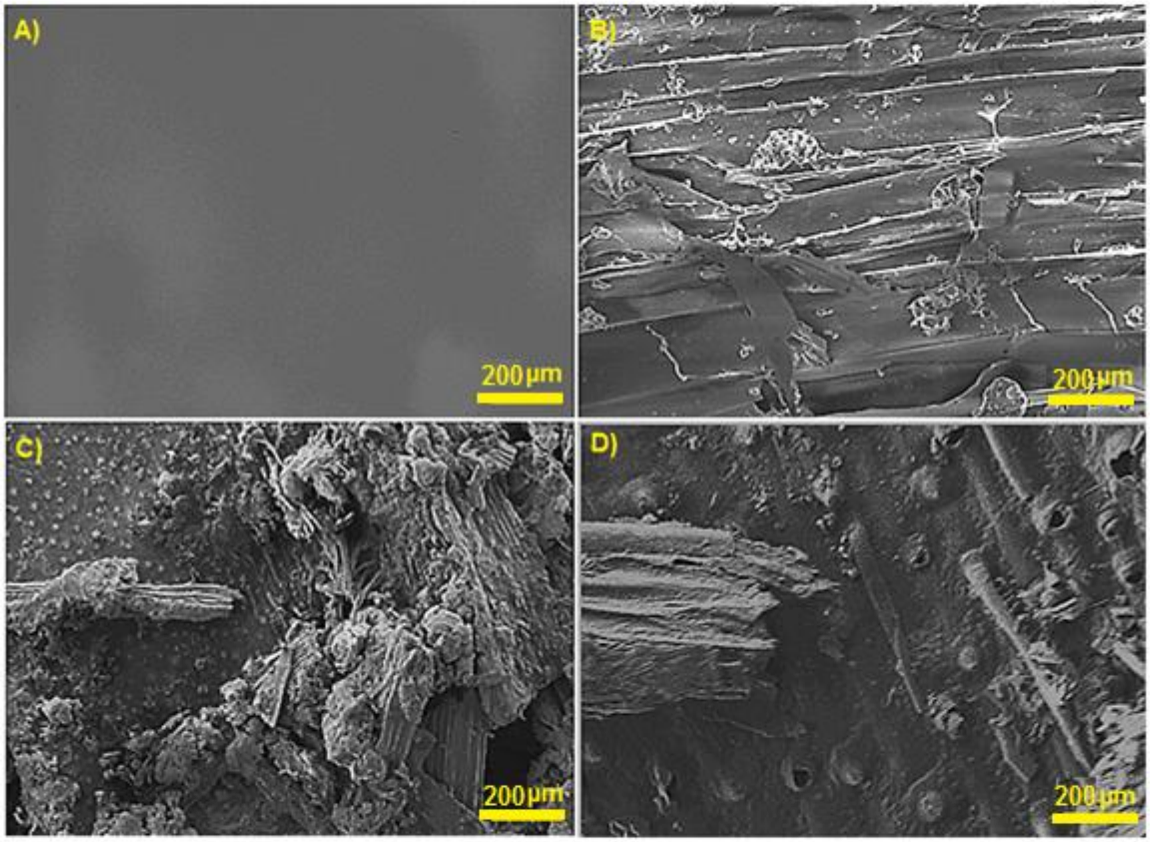

Fig. 3. Scanning electron microscopy of pure PVOH (A), DWS (B), PVOH-DWS (C), and PVOH-DWS-PL (D) samples

The enzymatically-treated wheat straw, when viewed through a SEM, showed that the lignin linkages and other artificial impurities that produce rugged, rough, and broken surfaces were removed after the enzymatic pretreatment. The fragments were completely different from the initial connected structure of native straw, thus an increased external surface area and porosity of the substrate (Qi *et al.* 2009).

The authors' results were in close agreement with those of Zhu *et al.* (2015), who observed the rough surface of the biomass samples after a NaOH pretreatment that exposed more cellulose fibres and interpreted the complete removal of hemicellulose and lignin. The composite (PVOH-DWS), revealed an uneven dispersion of the fibres in the matrix and thus presented as ridges and holes in the surface. However, on top of the fractured surface of the composites, large aggregates had appeared, which may have affected the mechanical properties of the composites (Chen *et al*. 2011). The introduction of the glycerol as a plasticizer led to the homogenous distribution of the fibre into the PVOH matrix. This provided the smooth composite surface and filled the ridges and pores, which interpreted the better interfacial adhesion between the components (Haafiz *et al.* 2013). This reduction in ridges was due to the plasticizing effect of glycerol that acted as a cross-linker, facilitated the interfacial adhesion, and improved the mechanical properties of the composites (Yao *et al.* 2013).

Dynamic mechanical analysis (DMA)

The mechanical properties of the composites prepared from the de-lignified wheat straw (DWS) as a reinforcement material and PVOH as a matrix, along with the use of glycerol as a plasticizer are presented in Table 2. The enzymatically-treated WS-based bio-composites had a pronounced effect on the tensile modulus of the composites. A few factors that could have affected the tensile modulus of the treated bio-composites were the aspect ratio and the degree of molecular orientation (Zaaba *et al.* 2016).

As presented in Table 2, the mechanical properties of the individual component, *i.e.*, pure PVOH, possessed the tensile strength of 25.98 MPa ± 0.98 MPa, a tensile modulus of 1472 MPa ± 0.28 MPa, and the elongation at break of 110.58% ± 1.12%. The other counter current part, de-lignified wheat straw (DWS), was too fragile to be measured. However, the composites prepared from DWS-PVOH showed the improved mechanical properties that exhibited the tensile strength of 46.12 MPa ± 0.13 MPa, a tensile modulus of 2,134 ± 0.76 MPa, and the elongation at break of 11.32 ± 0.47%. The present results could be attributed to the fact that the de-lignification of the straw fibers improved the hidden cellulose network of the composites imparting good mechanical properties for the composites prepared (Sardhashti 2009). However, the composites that imparted the glycerol effect in it showed the best tensile strength of 52.98 MPa ± 0.93 MPa, a tensile modulus of 4,521 MPa ± 0.24 MPa, and the minimum elongation at break of 4.63% ± 0.65%.

Beyond an optimum range, the increase in the concentration of plasticizer led to remarkable results in the mechanical strength of the composites (Liu *et al*. 2014). The use of a plasticizer resulted in the conformational changes, *via* altered interactions between the host polymers to the reinforcement fibre (Kfoury *et al.* 2013), and thus contributed to increased mobility.

Table 2. DMA-mechanical Properties of the Individual Polymers, *i.e.,* PVOH and DWS and their Bio-composites, *i.e.,* PVOH-DWS, and PVOH-DWS-PL

No.	Sample ID	Tensile Strength (MPa)	Tensile Modulus (MPa)	Elongation at Break (%)
1	PVOH	25.98 ± 0.98	1,472 ± 0.28	110.58 ± 1.12
2	DWS	N.D*	N.D*	N.D*
3	PVOH-DWS	46.12 ± 0.13	2,134 ± 0.76	11.32 ± 0.47
4	PVOH-DWS-PL	52.98 ± 0.93	4,521 ± 0.24	4.63 ± 0.65

*N.D: not determined

Water uptake test

Biocomposites developed from the DWS along with PVOH, with and without the incorporation of plasticizer, were analyzed *via* a water absorption test, and the results are presented in Fig 4.

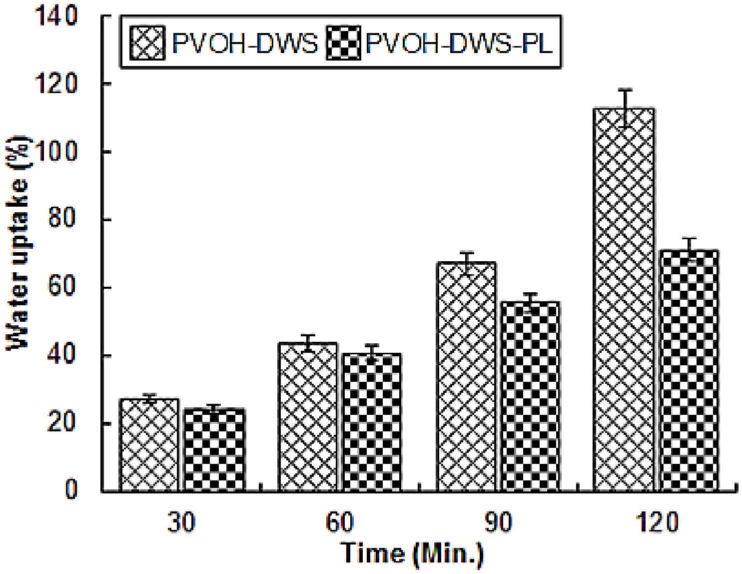

Fig. 4. Water uptake measurements of PVOH-DWS and PVOH-DWS-PL based bio-composites

When the biocomposites developed from DWS-PVOH were placed in water for 30 min, they showed a water absorption rate of 26.87%, which was further increased by immersing the sample for a longer duration. The composite showed the maximum water absorption of 112% when it was immersed for 120 min. In comparison, the sample prepared from de-lignified WS and PVOH, along with the plasticizer (glycerol) presented a moisture content of 71% when it was immersed for 120 min. The enzymatic pre-treatment used on the straw fibers led to the degradation of lignin content and therefore exposed the cellulose surface (Isa *et al.* 2014). This effect improved the water absorption behavior of the composites by providing additional interlocking mechanical sites and led to increased interfacial bonding (Mishra *et al.* 2003). The decrease of the moisture uptake of the samples may have been due to the decrease in the content of hydroxyl groups and the presence of microvoids in the composites (Ishak *et al.* 2013). The presence of hydroxyl groups could have led to the inter- and intra-chain hydrogen bonding in the fibres and the polymer. This would eventually have led to the decrease in the fibre agglomeration and an improvement in the dispersion of the fibres within the matrix, thus reduced micro-voids (Laxmeshwar *et al.* 2012; Ali *et al.* 2015) present in the composites.

CONCLUSIONS

1. The newly developed bio-composites with the matrix polymer polyvinyl alcohol and delignified wheat straw (DWS), with or without glycerol as a plasticizer (PL), *i.e.* PVOH-DWS, and PVOH-DWS-PL, showed improved topographical and mechanical properties that individual DWS failed to demonstrate on its own.
2. The morphological analysis revealed a more fractured and rough surface of the bio-composites after the enzymatic treatment of the reinforcing phase.
3. The technology developed enables to revalorize the lignocellulose-based materials to develop products with new/improved characteristics.

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