



Development of biocomposites based on bacterial cellulose reinforced delignified rice husk-PVA plasticized with glycerol

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Abstract

Eco-friendly materials production from industrial wastes and renewable raw materials is being promoted for green construction applications. Therefore, considerable value can be added by combining green chemistry with microbial biotechnology to construct improved and far better, biologically stable, and compatible materials. For this purpose, herein, *Schizophyllum commune* IBL-06 was exploited for the production of ligninolytic enzymes (MnP, LiP, and Lac). This in-house extracted enzymatic consortium was used for the delignification of pristine rice husk. The enhanced level of delignification (40.35%) was achieved with maximum cellulose exposure (from 32 to 72%). The delignified rice husk was further reinforced with bacterial cellulose from *Acetobacter xylinum*. Subsequently, biocomposites were prepared from both pristine and bacterially treated rice husk using compression molding technique. Glycerol/maleic anhydride combination was used as a plasticizer/compatibilizer and chitosan (10 wt.%) as a filler for newly developed biocomposite specimens. The newly synthesized biocomposite specimens were characterized using different imaging and analytical techniques including scanning electron microscopy (SEM) and Fourier transform infrared (FT-IR) spectroscopy. The characterization studies of newly developed biocomposites revealed significant improvement in morphological and mechanical properties of biocomposites. In addition, moderate increase in water uptake capacity was also observed due to the presence of hygroscopic filler among polymer matrix. In conclusion, the improved characteristics of newly developed biocomposites based on bacterial cellulose-reinforced delignified rice husk-PVA using chitosan filler suggest high throughput of enzymatic treatment for several industrial and biotechnological sectors.

Keywords Biofibers · Ligninolytic enzymes · Delignification · *Schizophyllum commune* · Biocomposites · Polyvinyl alcohol

Introduction

During past few years, the growing concern related to fossil-based functional materials has directed towards biobased polymer composites production for various applications [1–3]. Microbial polymers such as extracellular polymeric substances and agro-industrial based lignocellulosic raw materials possess a huge potential to replace petrochemically originated materials for wide range of applications in different sectors such as pharmaceutical, food, automotive, construction, biomedical, packaging and cosmaceutical industries [2, 4–6]. However, the non-homogeneous nature of biofibers with a plenty of lignin remains a problem while

dealing with lignocellulosic biomaterials. The improved compatibility i.e., uniform fiber-distribution and interfacial adhesion among the matrix backbone and the biofibers can be achieved by fibrous modifications [7]. Biofiber modifications can be achieved through different types of treatments including alkaline, bleaching, dewaxing, cyanoethylation, enzymatic and the grafting etc.[8]. In this study, ligninolytic enzymes were produced from *Schizophyllum commune* IBL-06 and the delignification of rice husk was carried out to reduce the lignin content. The delignified natural fibers were further modified using bacterial cellulosic (BC) reinforcement from *Acetobacter xylinum* to increase the coupling between biofiber and the polymer matrix. Several researches have reported the excellent compatibility of biopolymer composites fabricated with bacterial cellulose [9, 10]. Except compatibility, several other remarkable characteristics of BC have been reported such as water absorption,

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mold-ability, porosity and biodegradability for both *in-vitro* and *in-vivo* applications [11–13].

Polyvinyl alcohol (PVA) is a synthetic polymer commonly being applied as a matrix in composite manufacturing. It is being used in composite film formation as an adhesive agent because of its exceptional physicochemical characteristics such as emulsification, adhesion, aqueous solubility and film forming characteristics [14]. High structural stability of PVA/cellulose-based bio and nanocomposite have been reported for various applications in textile, packaging, heavy metal removal, and biomedical sectors [15–17]. Maleic anhydride is an organic compound often used in composites materials as a coupling agent as it enhances the compatibility among biofibers and the polymer matrix [18]. Additionally, the plasticizers have also been used in the composite development to reduce the glass transition temperature and increase the flexibility of biocomposites [19, 20]. Low molecular weight organic compounds are often used as plasticizers viz. sorbitol, glycerol and polyethylene glycol. However, glycerol due to its hygroscopic nature is considered as the favorable one for biocomposite development [21, 22].

Chitosan is a biopolymer with high compatibility and strong antimicrobial effects [23]. Highly crystalline structure and the presence of H-bonds among the polymer molecules make chitosan an excellent agent for oxygen-barrier applications [24]. So, it would be a promising way to use chitosan as a filler for PVA-based biopolymer composites for improved compatibility and oxygen barrier properties. Chitosan blends and composites have been successfully used for the development of packaging material with improved food security, ultimately leading to increased shelf-life [25, 26]. Variety of different techniques have been adapted for the development of biocomposites. Among other techniques, the compression molding poses less or no damaging effect to the fibers with potential to preserve the isotropic characteristics of biocomposites [27].

This research was designed to investigate the characteristics of biocomposites based on microbial cellulose-reinforced delignified rice husk-PVA. The effect of plasticizer/compatibilizer among polymer matrix was determined by the addition of glycerol/maleic anhydride. Finally, the filler effect of chitosan was determined in both pristine and microbial cellulose-reinforced delignified biofibers. All newly developed biocomposites were studied for their mechanical and barrier characteristic using modern techniques including SEM and FTIR. In addition, the water absorption and tensile testing was also performed using variety of different techniques to analyze the novel characteristics of biocomposites.

Materials and methods

Microbial strains, substrate and chemicals

White-rot fungus (*Schizophyllum commune* IBL-06) was obtained from Industrial Biotechnology Laboratory of University of Agriculture, Faisalabad, Pakistan and used for the production of ligninolytic enzymes (MnP, LiP and Lac). The bacterial spp. (*Acetobacter xylinum*) was obtained from Department of Mycology & Plant Pathology, University of Punjab, Pakistan and exploited for the development of microbial cellulosic fibers. Different biomaterials from agricultural-based waste (sugarcane bagasse and rice husk) were collected from students' research farm, UAF, Pakistan. Biomaterials were dried in convection oven (LabTech LDO-150 N) at 60 °C for 48 h and pulverized to 40 mm mesh size. Dried biomaterials were preserved in polythene zipper bags to prevent moisture uptake. All the chemicals used were purchased from local distributors of Merck KGaA, USA and Sigma Aldrich, Germany.

Development of ligninolytic consortium from *Schizophyllum commune* IBL-06

The inoculum of *Schizophyllum commune* IBL-06 was prepared in Kirk's basal medium of pH 4.5 [28]. The media was sterilized by autoclaving at 121 °C for 15 min. After autoclaving, loopful of fungal culture was added in the flasks containing basal media and incubated at 30 °C for 5–6 days.

Solid state fermentation (SSF) was carried out for the production of ligninolytic enzymes using sugarcane bagasse as a substrate. Flasks containing SSF media (15 g dried substrate moistened with 7 mL of Kirk's basal medium) were autoclaved, pipetted with 5 mL of *S. commune* inoculum, and incubated at 30 °C for 5 days in still culture incubator (LabTech LDO-150 N). After five days, ligninolytic enzymes were extracted by the addition of 100 mL of 0.5 mM sodium malonate buffer (pH 4.5) and placed in incubator shaker for 30 min at 150 rpm [29]. The biomass filtrate was centrifuged at 4 °C and 3000 rpm for 5 min. The supernatant was collected and assayed for ligninolytic enzymes using standard protocol [29] and used for delignification of rice husk.

Ligninolytic enzyme assays

Enzyme assays were performed using spectrophotometric method (Dynamica, HALO DB-20S) as reported earlier [30, 31]. Briefly, the MnP activity assay was performed at 270 nm ($\epsilon_{270} = 11,570$) by hydrogen peroxide-dependent oxidation of manganic-malonate. The LiP activity was assayed at 310 nm ($\epsilon_{310} = 9300$) by hydrogen peroxide-dependent

conversion of veratryl alcohol to veratraldehyde. The laccase activity assay was performed at 420 nm ($\epsilon_{420} = 36,000$) by determining the oxidation of ABTS. Quartz cuvettes were used for ligninolytic enzyme assay having an exterior height, width and length (H × W × L) dimensions of 45 × 12.5 × 3.5 mm with 1 mm path length.

Enzymatic delignification of rice husk

The delignification of native rice husk (NRH) was performed using mixture of ligninolytic enzyme (MnP, LiP and lac). The total volume of the flasks was adjusted to 100 mL with sodium malonate buffer (0.5 mM, pH 4.5). Percentage delignification was determined from both treated and untreated substrates as reported previously [32]. Briefly, the samples were taken in glass test tubes and mixed with 25% acetyl bromide. The tubes were stoppered and kept in water bath for 30 min at 70 ± 5 °C temperature. The samples were transferred to volumetric flask of 250 mL capacity, containing $\text{CH}_3\text{COOH}_{(g)}$ (25 mL) and 2 M-NaOH (10 mL) supplemented with 1 mL of hydroxylammonium-chloride (7.5 M). The readings were taken at 280 nm using UV/Vis spectrophotometer.

Cellulose content of the samples was determined by the method of Updegraff, [33] with modifications. Briefly, the samples were homogenized in distilled water using a waring blender with 2% (w/v) of biomass concentration. The pellets were obtained by centrifugation at 3000 rpm and added with 3 mL of acetic/nitric reagent. The mixture was incubated in boiling water bath for 30 min followed by centrifugation at 5000 rpm for 5 min. Pellets were collected and added with 10 mL of 67% H_2SO_4 and incubated for 1 h. The total volume of the solution was adjusted to 1L with distilled water. The hydrolysate 1 mL was added with 4 mL of distilled water and placed in ice. 4 mL of chilled anthrone reagent was gently mixed and incubated for at least 15 min. The readings were taken at 620 nm using VU/Vis spectrophotometer.

Bacterial treatment of delignified rice husk

The delignified rice husk was modified by treating with bacterial cellulose from *Acetobacter xylinum*. For this purpose, bacterial suspension was prepared using Hestrin-Schramm (HS) medium with g/L composition of peptone (5.0), yeast extract (5.0), Na_2HSO_4 (1.1), citric acid (1.15) and glucose (20). Media was adjusted to pH 5.5 and autoclaved at 121 °C for 15 min. After sterilization, media was inoculated with a loopful of bacterial culture and placed in incubator shaker at 30 °C for 3 days.

The delignified rice husk was placed in 250 mL Erlenmeyer flasks in the presence of HS culture media. The media was sterilized and inoculated with 5 mL of *Acetobacter*

xylinum culture broth. The media was placed in incubator shaker at 130 rpm and 30 °C for 7 days [34]. After 7 days, the treated biofibers were recovered by washing with NaOH (0.1 N) at 80 °C in order to remove soluble polysaccharides, media components and microorganisms [35]. The harvested biofibers were designated as bacterially treated rice husk (BTRH) which were dried in oven at 50 °C for 2–3 days and used for the development of biocomposites.

Development of biocomposites

The biocomposites were developed using pristine (NRH), bacterially treated rice husk (BTRH) and PVA with and without chitosan as a filler and glycerol/maleic anhydride as plasticizer/compatibilizer. Briefly, the PVA was gently mixed with biofibers (ratio 1:1) at 35 °C and 700 rpm for 15 min using dH_2O (as required). The resulting mixture was processed for biocomposite development. In next step, the thick composite biomass was further compatibilized/ plasticized with maleic anhydride and glycerol with 25% and 50% concentration of total composite biomass respectively. To analyze the filler effect of chitosan, a 2% (w/v) solution was prepared in 1% acetic acid solution in water and the thick solution (50 mL) was blended with composite biomass at 700 rev/min for 15 min. The composite mixture was then molded using pre-heated plates (150 mm diameter; 2 mm thick) at 80 °C for 15 min. The pressure (3–4 MPa) was applied depending upon reinforcement biomass. All the newly developed biocomposites were then placed at 50 °C for 12 h [36]. The constituent composition of all newly developed biocomposites have shown in Table 1.

The resulting biocomposites were designated as PVA-NRH (synthesized using PVA and pristine rice husk), PVA-BTRH (synthesized using PVA with delignified and BC reinforced rice husk), PVA-NRH-GLY (synthesized using PVA with pristine rice husk using plasticizer/compatibilizer), PVA-BTRH-GLY (synthesized using PVA with bacterially treated rice husk using plasticizer/compatibilizer), PVA-NRH-GLY-CHT (synthesized using PVA with pristine rice husk further supplemented with plasticizers and chitosan filler), PVA-BTRH-GLY-CHT (synthesized using PVA with bacterially treated rice husk further supplemented with filler and plasticizers). Figure 1 represents the digital image of newly synthesized composites.

Characterization of biocomposites

Structural composition – FTIR

The structural components of test composites were examined by Fourier transform infrared spectrometer (Bruker Alpha FT-IR, Germany). Composites were placed directly

Table 1 Constituent composition of newly developed biocomposite specimens

Sample ID	PVA ^a (g)	NRH ^b (g)	BTRH ^c (g)	MA ^d (g)	GLY ^e (mL)	2% CHT ^f (mL)
PVA/NRH	5	5	-	-	-	-
PVA/NRH-GLY	5	5	-	2.5	5	-
PVA/NRH-GLY-CHT	5	5	-	2.5	5	50
PVA/BTRH	5	-	5	-	-	-
PVA/BTRH-GLY	5	-	5	2.5	5	-
PVA/BTRH-GLY-CHT	5	-	5	2.5	5	50

^a Poly (vinyl alcohol); ^b Native rice husk (untreated); ^c Bacterially treated de-lignified rice husk; ^d Maleic anhydride; ^e Glycerol (plasticizer); ^f Chitosan (2%, w/v) dissolved in 1% (v/v) acetic acid solution in dH₂O

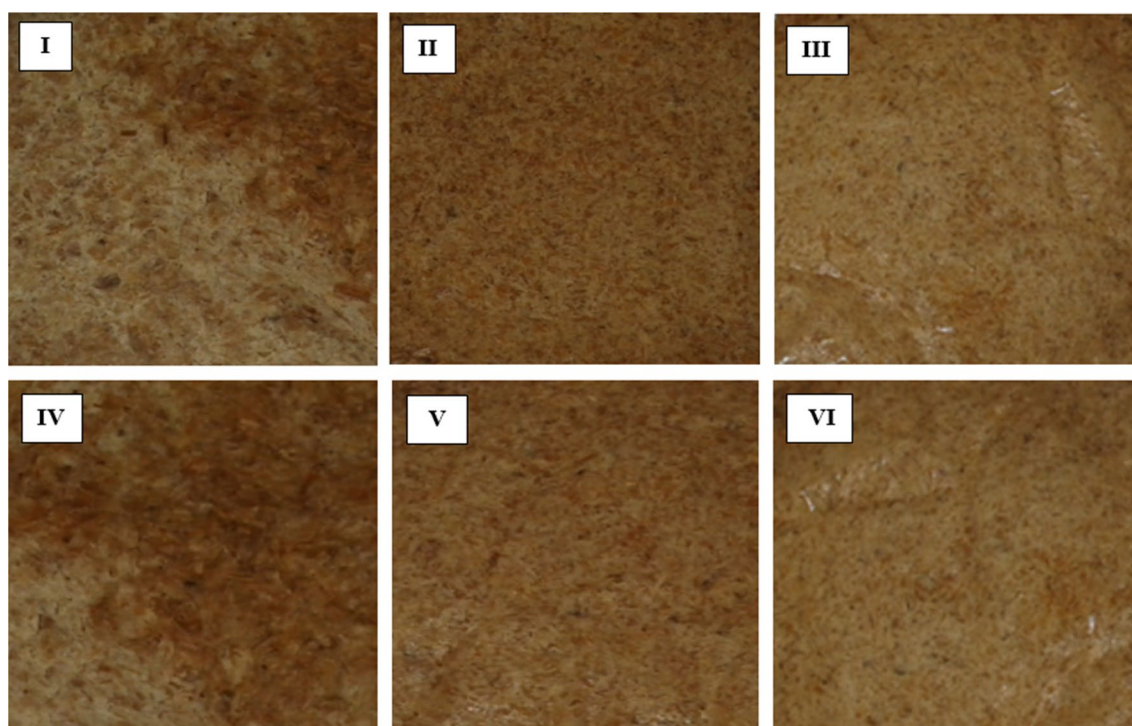


Fig. 1 Digital images of biocomposite specimens (I) PVA-NRH; (II) PVA-NRH-GLY; (III) PVA-NRH-GLY-CHT; (IV) PVA-BTRH; (V) PVA-BTRH-GLY; (VI) PVA-BTRH-GLY-CHT

under the infrared beam and the spectrum was recorded from 4000–500 cm⁻¹ and given peak numbers.

Surface morphologies – SEM

All the newly developed biopolymer composites were analyzed for their surface morphologies in ultra-high vacuum model of scanning electron microscope (JSM 6409A, JEOL, Japan) at an accelerating voltage about 10 kV. The biocomposites were gold coated using sputter coater machine (Emitech-K550) and high-quality images were obtained. The deposition current of 20 mA and pressure of 7×10^2 bar was used for analysis [35].

Mechanical analysis of biocomposites

Mechanical characteristics of biocomposites, i.e., tensile strength, elongation at break and Young's modulus were determined using Universal Testing Machine (UTM: AMETEK Lloyd Instruments Ltd.). Before mechanical measurements, the composite specimens were cut into 80 mm × 20 mm × 2 mm dimensions and were placed at 40 mm distance according to the ASTM-D790 standard. The crosshead speed of 2 mm/min and the load range of 1–6000 mN was adjusted. All measurements were taken in the form of triplicate and the results were reported as average of three repeated experiments.

Water absorption capacity

The water absorption analysis of all the newly developed biocomposites was determined. The composite specimens were weighted and immersed in distilled water. The weight gain was recorded after 30, 60, 90- and 120-min. Water absorption capacity was expressed as the percentage weight gain of biocomposite specimens. All experiments were repeated thrice, and the results were calculated using following equation:

$$\text{Water absorption (\%)} = \frac{W_a - W_o}{W_o} \times 100$$

where, W_a is weight of composite after water uptake and W_o is the initial weight of compression molded composite specimen.

Statistical analysis

All experiments were repeated thrice, and the corresponding data was presented as mean \pm STD. Statistical analysis was performed using Microsoft Excel 365 ProPlus version and the mean \pm STD were calculated from each experiment.

Results and discussion

Production of fungal ligninolytic enzymes

Ligninolytic enzyme consortia (LiP, MnP and Lac) was obtained from *Schizophyllum commune* after 5-days of incubation in SSF of sugarcane bagasse. The ligninolytic consortia with maximum enzymatic activities of 1763.1 U/mL for MnP, 1836.4 U/mL for LiP and 318.9 U/mL for laccase was used for the delignification of lignocellulosic material (rice husk). The use of microbial-based and enzymatic methodologies comprises several advantages i.e., low sugar losses, high delignification and green environmental impacts [37].

Enzymatic delignification of rice husk

Rice husk was enzymatically modified using ligninolytic enzyme mixture (MnP, LiP and Lac) and the respective results are given in Table 2. After enzymatic modification of rice husk, the lignin contents were reduced from 22.70% to 13.54% with maximum delignification of 40.35%. Moreover, the cellulose percentage was also increased from 32.06% to 72.56% after enzymatic modification as shown in Table 2. Taniguchi

et al. [38] reported similar results i.e., 41% delignification of rice husk after 60 days of incubation treated with *Pleurotus ostreatus*. Shi et al. [39] reported 28% of delignification of cotton stalks using *Phanerochaete chrysosporium*. However, the presently developed in-house consortia of ligninolytic enzymes from indigenous white-rot fungus (*Schizophyllum commune*) showed better results in a much shorter time-period (i.e., 5-days of incubation) with maximum cellulose exposure.

Bacterial treatment of delignified rice husk

Bacterially treated delignified rice husk showed a significant change in their surface morphology. SEM micrographs showed a random deposition of bacterial cellulosic microfibrils over the delignified rice husk. The cellulosic microfibril deposition over the delignified rice husk lead towards the improvement in water uptake and mechanical characteristics of biocomposites as described in the later part of the study. Earlier, Asgher et al. [34] and Chawla et al. [40] successfully modified lignocellulosic fibers by culturing *Acetobacter xylinum* and showed that the fibers reinforced with BC into the interface between the fiber and the matrix. Additionally, BTRH composites exhibited a fibrous morphology because of the presence of microcellulosic content and consequently leading towards the improved mechanical characteristics of biocomposites.

Characterization of biocomposites

Structural composition – FTIR

The major structural constituents in all newly developed biocomposite specimens were investigated using FTIR spectroscopy. FTIR spectrum of PVA-NRH and PVA-NRH-GLY-CHT showed particular functional groups bands at 1595 cm^{-1} and 1730 cm^{-1} which indicate the presence of lignin components in the biocomposites (Fig. 2a). The broad stretching bands at 3337 cm^{-1} and 3428 cm^{-1} indicate the presence of -OH functional group of cellulose which are closely related to the -OH stretching bands of PVA. In pure PVA, the visible peaks around 3295 cm^{-1} and 1650 cm^{-1} indicate the presence of residual acetate functional groups [41, 42]. The characteristic broad and sharp peak at 1090 cm^{-1} in pure PVA and PVA-BTRH biocomposites was assigned to the stretched vibrations of C-O functional groups [3]. The broad large peak at 3437 cm^{-1} and 3462 cm^{-1} indicate the existence of -OH functional group of cellulose in BTRH and 1595 cm^{-1} arises

Table 2 Percent of cellulose and lignin in enzymatically modified rice husk treated with ligninolytic mixture

Lignin content before treatment (%)	Lignin content after treatment (%)	Delignification (%)	Cellulose content before treatment (%)	Cellulose content after treatment (%)
22.70 \pm 0.23	13.54 \pm 0.51	40.35 \pm 0.83	32.06 \pm 0.23	72.56 \pm 0.21

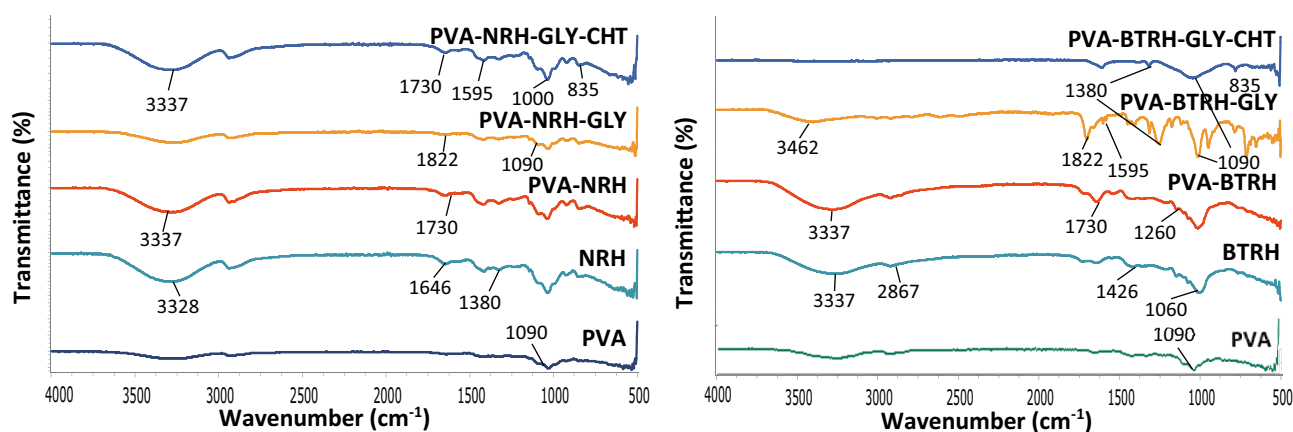


Fig. 2 **a** FTIR spectra of composite counterparts PVA, NRH and their composite specimens PVA-NRH; PVA-NRH-GLY and PVA-NRH-GLY-CHT; **b** FTIR spectra of PVA, BTRH and their composite specimens PVA-BTRH; PVA-BTRH-GLY and PVA-BTRH-GLY-CHT

from C-O functional groups of cellulose [43]. The characteristic band of BC reinforced delignified biocomposite showed a broad peak at 2867 cm^{-1} which indicate the vibrational stretching of aliphatic CH bonds among cellulose molecules (Fig. 2b) whereas, broad peak around 1090 cm^{-1} indicate the presence of ether (C-O-C) functional linkage [1]. The sharp peak around 1380 cm^{-1} indicate the presence of secondary alcoholic and primary aliphatic functional groups among PVA-BTRH-GLY and PVA-BTRH-GLY-CHT. The characteristic peaks around 2867 cm^{-1} and 1060 cm^{-1} in BTRH indicate the presence of asymmetric stretching of C-O-C functional groups [44].

Surface morphologies – SEM

SEM micrographs of newly developed biocomposites have been presented in Fig. 3 (left half). The SEM microstructure of pristine (NRH) exhibited few cracks and pores, and the surface was relatively rough than bacterially treated delignified rice husk. The porous/ cracked surface of PVA-NRH biocomposites was due to the deprived cellulosic dispersion in PVA matrix. The cracked surface of PVA-NRH was significantly improved by the addition of plasticizer in PVA-NRH-GLY biocomposite. The distribution of cellulose in watery environment should enhance together with the degree of cellulosic modification [45]. The SEM micrographs of 2% chitosan reinforced biocomposites (PVA-NRH-GLY-CHT and PVA-BTRH-GLY-CHT) showed compact and smoother surface as compared with other biocomposite materials (Fig. 3).

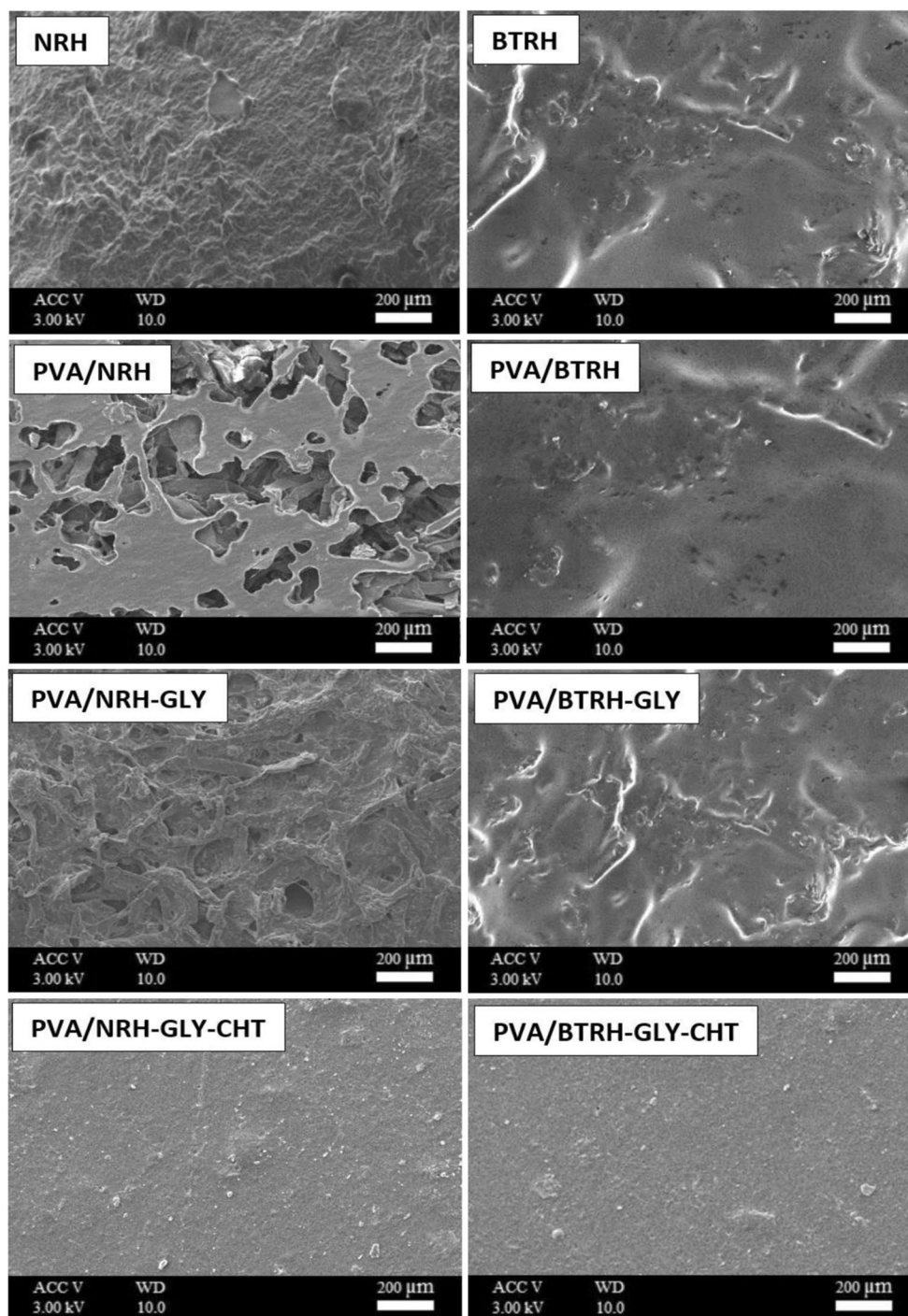
The adhesion seems quite poor in PVA-NRH and PVA-NRH-GLY biocomposites as compared with BTRH as shown in Fig. 3 (right half). Furthermore, the delignified rice husk showed smoother surface where micro-filamentous cellulosic deposition could be clearly observed. Several

researches have reported the modification in chemical, physical and micro-structural properties of graft-copolymerized matrix [1, 46]. The bacterial cellulose covers the composite surface creating a fibrous network indicating the improved interfacial adhesion between BTRH and the PVA matrix [47]. The slight modification in the surface of BTRH-PVA-based biocomposites are because of the existence of PVA in BTRH matrix. Increase in bacterial cellulosic microfibril content in biocomposite sample lead towards the non-uniform integration of material indicating improved interfacial adhesion [48].

Mechanical characteristics of biocomposites

The mechanical characteristics of newly developed biocomposites have been summarized in Table 3 and Fig. 4. The mechanical profile of PVA films exhibited the Young's modulus of $1392 \pm 0.47\text{ MPa}$, tensile strength of $22.34 \pm 0.41\text{ MPa}$ and percent elongation of $118.46 \pm 2.54\%$. The PVA-NRH biocomposites showed the percent elongation of $12.3 \pm 0.31\%$, tensile strength of $28.30 \pm 0.54\text{ MPa}$ and Young's modulus of $1656 \pm 0.46\text{ MPa}$. The tensile strength of PVA-BTRH was improved to $37.47 \pm 0.52\text{ MPa}$ as compared to PVA and PVA-NRH composite, while Young's modulus reached $2824 \pm 0.93\text{ MPa}$. The characteristics of biocomposite, especially mechanical are highly influenced by the existence of extra spaces among cellulose molecules that weakens hydrogen bonding, ultimately leading towards reduction in mechanical strength [49]. The entanglement and interaction between composite counterparts were weak, the addition of glycerol/maleic anhydride, that hold excellent plasticization/compatibilization efficacy, led towards improved entanglement between the two components

Fig. 3 SEM microstructure of NRH, BTRH and their bio-composite specimens i.e., PVA/NRH, PVA/BTRH, PVA/NRH-GLY, PVA/BTRH-GLY, PVA/NRH-GLY-CHT and PVA/BTRH-GLY-CHT



ultimately leading towards improved mechanical characteristics of newly developed biocomposites [50, 51]. The mechanical parameters of biocomposites were further improved by the addition of chitosan filler (10 wt.% of total biomass) in native as well as bacterially treated delignified rice husk. In Fig. 3, rough and crystalline structure of biocomposites could be observed with less porosity which indicate excellent filler effect of chitosan [52].

Water absorption kinetics

All newly developed biocomposites were investigated for their water absorption capacities at ambient environmental conditions using complete-dip method (Fig. 5). PVA-NRH showed a maximal of 24% water absorption after 30 min which was increased to 112% after 120 min of immersion period. Comparatively, PVA-NRH-GLY exhibited a significant decrease in water absorption capacity after the same

Table 3 Mechanical properties of biocomposites prepared from native and bacterial cellulose reinforced delignified rice husk

No	Sample ID	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)
1	PVA	22.34 ± 0.41	1392 ± 0.47	118.46 ± 2.54
2	NRH	N/A	N/A	N/A
3	PVA/NRH	28.30 ± 0.54	1656 ± 0.46	12.30 ± 0.31
4	PVA/NRH-GLY	49.32 ± 0.26	3246 ± 0.30	5.34 ± 0.44
5	PVA/NRH-GLY-CHT	62.48 ± 0.91	3753 ± 0.32	2.20 ± 0.01
6	BTRH	N/A	N/A	N/A
7	PVA/BTRH	37.47 ± 0.52	2824 ± 0.93	8.20 ± 0.33
8	PVA/BTRH-GLY	58.81 ± 0.73	3417 ± 0.43	3.30 ± 0.12
9	PVA/BTRH-GLY-CHT	71.12 ± 0.54	3846 ± 0.20	3.80 ± 0.04

PVA: poly (vinyl alcohol), NRH: native rice husk, BTRH: bacterially treated delignified rice husk, GLY: glycerol/maleic anhydride (plasticizer/compatibilizer), CHT: chitosan (filler), N/A: not available

Numbers on X-axis represents

- 1: PVA/NRH
- 2: PVA/NRH-GLY
- 3: PVA/NRH-GLY-CHT
- 4: PVA/BTRH
- 5: PVA/BTRH-GLY
- 6: PVA/BTRH-GLY-CHT

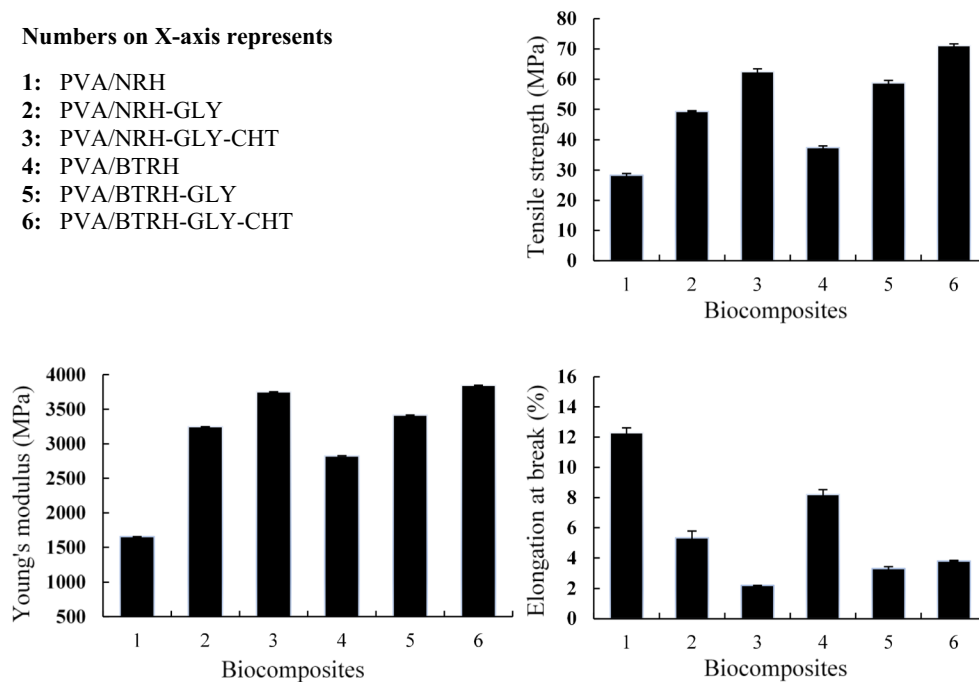


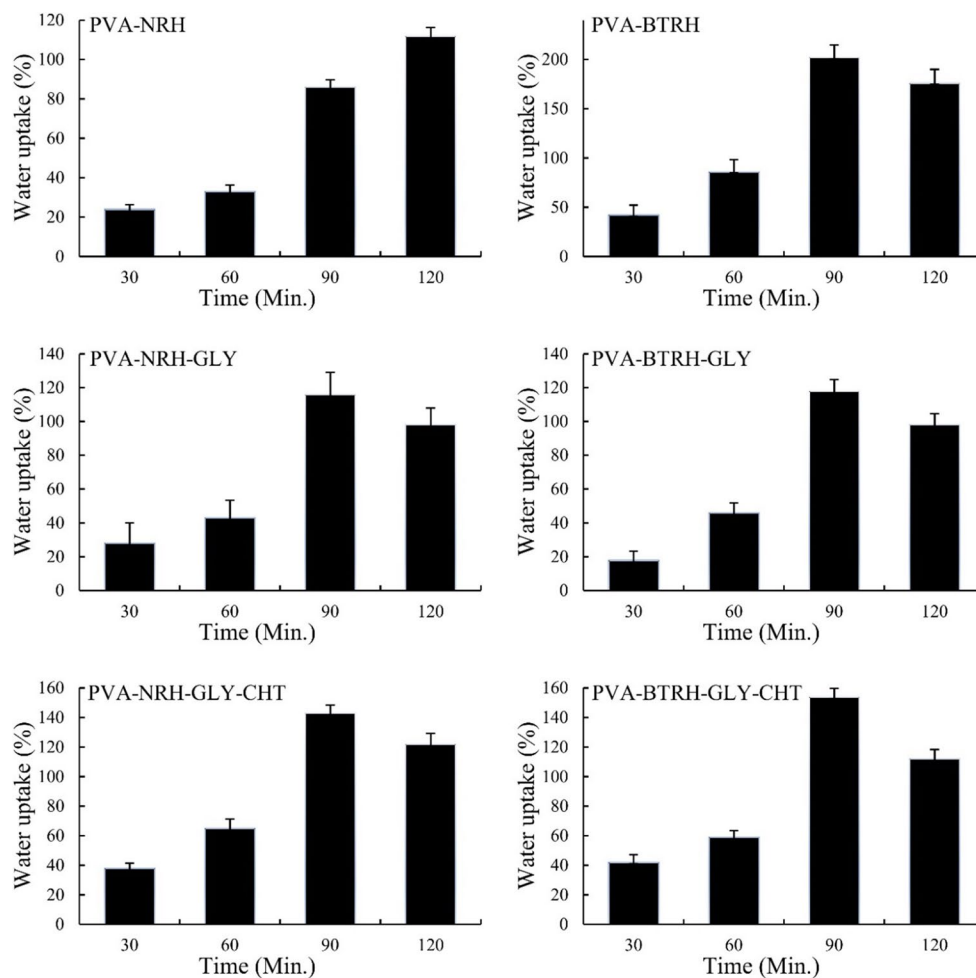
Fig. 4 Graphical representation of mechanical characteristics (i.e., tensile strength, Young's modulus and percent elongation at break) of newly prepared biocomposite specimens

immersion time period. This can be attributed to the fact that addition of plasticizer possesses an intense effect on water uptake capacity of biocomposites when it is utilized in optimum concentration. The chances behind decreased water absorption capacity of the composite specimens are due to the existence of microvoids in biocomposites which are responsible of water uptake [53]. Saturation point was attained after 130 min of immersion due to high water absorption kinetics [54] The water absorption was decreased after 120 min of immersion, which could be attributed to the presence of silicon-cellulosic membrane at the surface of rice husk which could impart partially hydrophobic

characteristics, ultimately reducing water sorption capability of biocomposite [55].

Composite specimens from delignified rice husk (PVA-BTRH) showed much higher water absorption capacity (202%) as compared with PVA-NRH and PVA-NRH-GLY. This can be predicted to the hygroscopic nature of cellulose and the presence of -OH functional group in it. However, biocomposites with the addition of plasticizer (PVA-BTRH-GLY) exhibited 118% of water absorption capacity. When we increase the fiber mass in biocomposite matrix, this causes increased aqueous uptake potential due to higher cellulose impregnation over the substrate, ultimately leading

Fig. 5 Determination of water absorption of all biocomposites developed from native as well as delignified rice husk i.e. PVA-NRH, PVA-NRH-GLY, PVA-NRH-GLY-CHT and PVA-BTRH, PVA-BTRH-GLY, PVA-BTRH-GLY-CHT



to the swelling and increased aqueous transport through the matrix interface [56]. The addition of chitosan filler in the biocomposite specimens PVA-NRH-GLY-CHT and PVA-BTRH-GLY-CHT showed moderately increased aqueous uptake capacities i.e., 143% and 154% respectively. This could be attributed to the fact that chitosan is hygroscopic in nature having higher capability of hydrogels formation that lead to the higher water transport through the specimens [57, 58].

Conclusions

In this research, ligninolytic enzymatic consortium (MnP, LiP and Lac) produced by *S. commune* IBL-06 was used for the delignification of native rice husk. An enhanced level of cellulosic content was recorded (from 32 to 72%) after the treatment of ligninolytic enzymes. Further modifications were performed using cellulosic microfibrils integration from a bacterial spp. *Acetobacter xylinum*, and the biocomposites were developed with novel features for

potential biotechnological applications. Moreover, a novel method was established for the development of microbial cellulose-reinforced delignified rice husk-PVA-based composite materials. Significantly improved morphological and mechanical characteristics were recorded in newly developed biocomposites as demonstrated by the characterization analysis. In conclusion, this technology exhibited potential to revalorize the lignocellulosic based biomaterials for functional products development.

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Author contributions Muhammad Asgher: Conceptualization; Supervision; Resources; Writing—review & editing; Project administration. Irum Nasir: Methodology; Investigation; Data curation; Validation. Nimrah Khalid: Methodology; Investigation; Validation. Sarmad Ahmad Qamar: Methodology, Writing – Original draft; Software; Formal analysis; Visualization; Correspondence.

Compliance with ethical standards

Conflict of interest Authors declared that they have no conflict of interest.

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