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PII: S0959-6526(17)31404-X

DOI: [10.1016/j.jclepro.2017.06.235](https://doi.org/10.1016/j.jclepro.2017.06.235)

Reference: JCLP 9985

To appear in: *Journal of Cleaner Production*

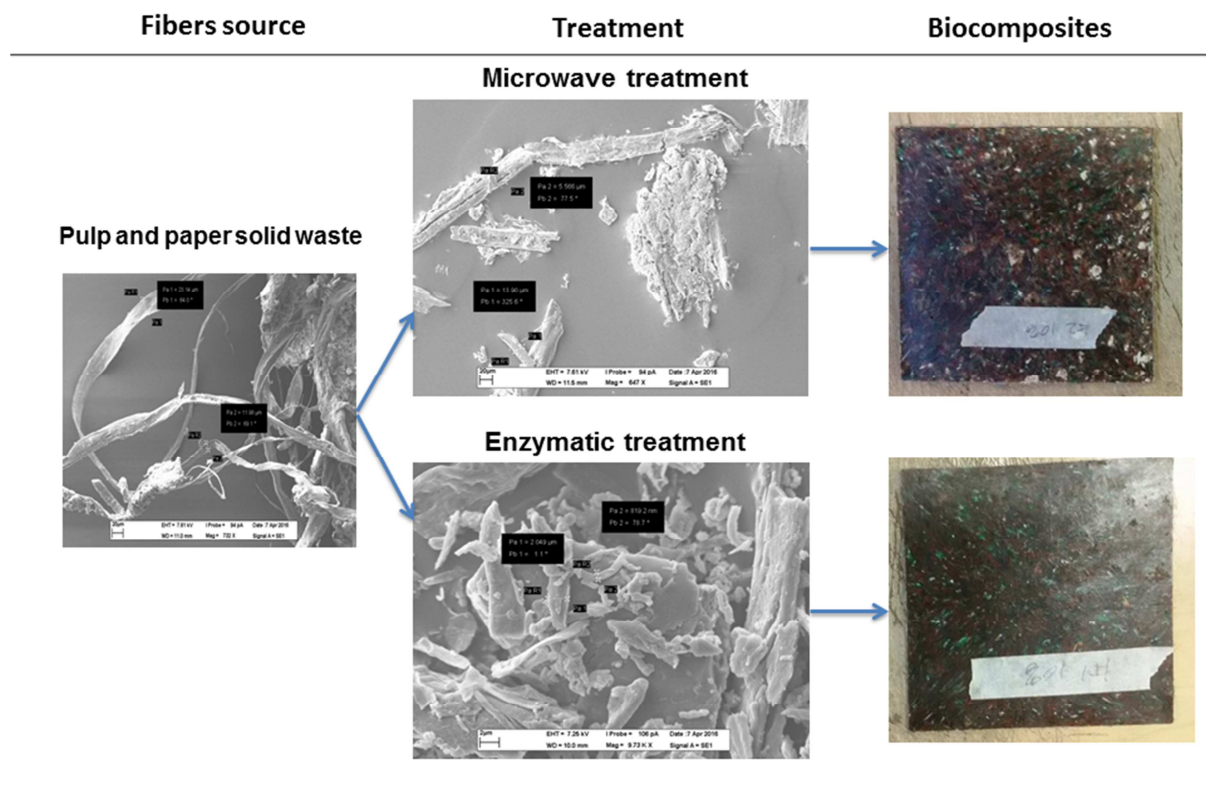
Received Date: 25 October 2016

Revised Date: 28 June 2017

Accepted Date: 28 June 2017

Please cite this article as: Laadila MA, Hegde K, Rouissi T, Brar SK, Galvez R, Sorelli L, Ben Cheikh R, Paiva M, Abokitse K, Green synthesis of novel biocomposites from treated cellulosic fibers and recycled bio-plastic polylactic acid, *Journal of Cleaner Production* (2017), doi: 10.1016/j.jclepro.2017.06.235.

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ACCEPTED MANUSCRIPT

Green synthesis of novel biocomposites from treated cellulosic fibers and recycled bioplastic polylactic acid

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Abbreviations: Polylactic acid, PLA; Recycled polylactic acid, PLAr; Pulp and paper solid waste, PPSW; Microwave treatment, MWT; Solid state fermentation, SSF; 2, 2-azino bis (3-ethylbenzthiazoline-6-sulfonic acid), ABTS; Carboxyl-methyl-cellulose, CMC; 3,5-dinitrosalicylic acid, DNS; Filter paper cellulase assay, Fpase. Analysis of variance, ANOVA; Response surface methodology, RSM.

Abstract

This study investigated mechanical properties of biocomposites developed from recycled polylactic acid (PLA) from packaging industry and treated cellulosic fibers from pulp and paper solid waste. Microwave and enzymatic treatments were used for extraction and surface modification of hydrophilic cellulosic fibers. Enzymatic treatment was specifically performed for activation of hydroxyl groups and improvement of adhesion between matrix and fibers including controlling the length of cellulosic fibers with size reduction of around 50 % (142 and 127 μm for primary and mixed biosolids, respectively) as compared to microwave treatment. Microwave treatment produced cellulosic fibers of 293 and 341 μm , for primary and mixed biosolids, respectively. Mechanical properties of biocomposites with 2 % (w/w) of treated cellulosic fibers (Young's Modulus 887.83 MPa with tensile strain at breakpoint of 7.22 %, tensile stress at yield 41.35 MPa) was enhanced in comparison to the recycled PLA (Young's Modulus 644.47 ± 30.086 MPa with tensile strain at breakpoint of 6.01 ± 0.83 %, tensile stress at yield of 29.49 ± 3.64 MPa). Scanning electron microscopy revealed size reduction of cellulosic fibers. X-ray diffraction and Fourier transform infrared spectroscopy confirmed strong mechanical properties of novel biocomposites.

Keywords: biocomposite; cellulosic fiber; microwave treatment; enzymatic treatment; mechanical test; polylactic acid

1. Introduction

Biocomposites are composed of polymeric matrix and reinforcement. Numerous studies have shown that synthetic fibers, such as glass and carbon fibers are commonly used as reinforcement for composite materials due to their strong mechanical properties. Recently, a wide range of attractive alternative materials that can replace synthetic fibers, such as natural or cellulosic fibers are increasingly being used as 'eco-friendly materials' (Faruk et al., 2012). Natural fibers offer several advantages over synthetic materials as reinforcement composites, such as good mechanical and physical proprieties, high stiffness and tensile strength (Pickering et al., 2016). Furthermore, they are renewable, biodegradable and abundantly available in nature (Karaduman et al., 2013).

There are several types of natural fibers, such as jute, hemp, kenaf, flax, sisal and ramie, which possess excellent potential as reinforcement for composites (Saheb and Jog, 1999). However, natural fibers in their native form have many disadvantages, such as poor compatibility with the matrix. The poor compatibility in biocomposites is due to hydrophilic nature of the cellulosic fibers in poor adhesion and moisture absorption. Thus, pretreatment of natural fibers is necessary to improve the biocompatibility between matrix and fibers, more so by the activation of hydroxyl groups on these fibers. The most common methods of pretreatment are physical, chemical, and enzymatic treatments (Henriksson et al., 2007). Chemical modification of fibers, such as treatment with alkali, acid and organic solvents are mostly useful due to its efficiency in improving compatibility between the matrix and the fibers (Kabir et al., 2012). Additionally, physico-chemical processes, such as microwave irradiation (MWI) or thermal pretreatment are efficient in terms of degradation and solubilization of extracellular polymers. Nevertheless, these methods have many drawbacks such as toxicity of chemicals and higher costs. On the other

hand, treatment of natural fibers using enzymes, such as laccase and cellulolytic enzymes have been used for the modification of natural fibers (Henriksson et al., 2007). These methods are environment friendly, and highly efficient using controlled reactions (Kharazipour et al., 1997). However, combined physical-enzymatic-chemical-pretreatment showed promising results in terms of delignification, fiber extraction and indirect reduction of the fiber size (Saha et al., 2011).

Several fungi are known to produce cellulose modifying extracellular enzymes, of which *Trametes versicolor* and *Trichoderma reesei* have been extensively documented for production of laccase and cellulolytic enzymes, respectively (Pazarlioglu et al., 2005). Studies have shown that cellulase can modify and degrade lignocellulosic materials and convert them to simple sugars (Perez et al., 2002). Laccase is used for the degradation of phenolic compounds (Rencoret et al., 2014), activation of cellulosic fibers and improvement of adhesion between fibers and matrix in composite materials (George et al., 2014). The fact that cellulose is naturally covered by lignin-comprising phenolic compounds, its removal by biocatalytic degradation using enzymes, such as laccase improves the interaction of cellulose fraction with polylactic acid leading to adhesion between fibers and matrix.

Pulp and paper solid sludge (PPSW) constitutes one-third of the total waste produced in Canada (Das et al., 2016). However, only 25 % of this PPSW is recycled, and this leads to environmental issues (Oral et al., 2005). PPSW can also be converted to enzymes through bioconversion. In addition, PPSW contains higher amount of cellulose (Das et al., 2016) so that it could be used as a potential source for extraction of renewable cellulosic fibers. Polylactic acid (PLA) is gaining attention as a bioplastic because of its unique properties, such as biodegradable and renewable, higher hydrophobicity, tensile strength, and rigidity. PLA can be used in automobiles, packaging,

and pharmaceutical industries (Bitinis et al., 2013). However, these biopolymers exhibits some disadvantages, such as the molten state, thermal degradation, residence time in the extruder and shredding process, which decreases the mechanical and physical properties of PLA after each recycling cycle (Pillin et al., 2008). Nevertheless, these properties can be improved by incorporating cellulosic fibers from PPSW (Mukherjee and Kao, 2011). In addition, recycled PLA (PLAr) becomes resistant to biodegradation with increased recycling. The degradability of PLAr could be prolonged up to 24 weeks (unpublished data) in comparison to the pure PLA, which degrades completely in 5 weeks (Ashter, 2016). However, the degradation duration can be decreased to 4-8 weeks by blending PLAr with different percentage of cellulose fibers ranging from 2-30 % (Park, 1995).

In the present study, two types of treatment for cellulosic fibers were investigated: physicochemical method of extraction using microwave irradiation (MWI) with dilute sulfuric acid and an enzymatic treatment for surface activation of extracted fibers. Further, different proportions of these treated cellulosic fibers were used as reinforcement to fabricate biocomposites with PLAr to study the effect of the treated cellulosic fibers on the mechanical properties of the biocomposites.

2. Materials and methods

All the chemicals used were of high purity and they were purchased from Fisher Scientific (Ottawa, Ontario, Canada). Pulp and paper solid waste (PPSW) (Kruger Wayagamack Inc, Trois Rivieres, Quebec, Canada) was used as a source for cellulosic fibers. PLAr ($\leq 600 \mu\text{m}$) was procured from Gaudreau Environment (Victoriaville, Quebec).

2.1. Laccase production

Fungal strain, *Trametes versicolor* (ATCC-20869) was used for the production of laccase enzyme using PPSW as a carbon source (410 g per kg dry substrate of total carbon). PPSW was washed and dried at 60 °C for 24 hours and used as a substrate in the solid state fermentation (SSF).

T. versicolor was freshly grown from the stock on potato dextrose agar (PDA) at 30 °C for 3 days and it was used as inoculum. The SSF was carried out in PPSW supplemented with Tween-80 at 0.5 % (v/v) (moisture adjusted to 75 % (w/w) with sterilized water). The growth medium was inoculated with the fresh culture from the PDA plate and it was grown at 30 ± 1 °C for 15 days (Pazarlioglu et al., 2005).

2.2. Cellulolytic enzyme production

The fungal strain, *Trichoderma reesei* (NRRC-207F) was used to produce cellulolytic enzymes (endoglucanase, β -glucanase, β -glucosidase and cellulase) using hemp fiber as a sole carbon source (70 % w/w of cellulose). The fibers were cut into small pieces of 1 to 2 cm in length and used as a substrate for fermentation (Awafo et al., 1996).

The SSF medium with 20 g of the hemp fibers was taken in 500 mL Erlenmeyer flasks. The moisture was adjusted to 75 % (w/w) with sterilized water and the pH was adjusted to 6.5. The medium was inoculated with *T. reesei*; 10^6 - 10^7 spores per mL from PDA plate and it was grown at 30 ± 1 °C for 15 days (Wen et al., 2005).

2.3. Enzyme extraction

Laccase enzyme extraction was performed in 50 mM sodium phosphate buffer (pH 6.5). The SSF medium was mixed with buffer at a ratio of 20:1 (v/w) for 1 h with constant stirring and it was centrifuged at 7000g for 20 min at 4 °C. Likewise, cellulolytic enzyme was extracted in sterile distilled water having 0.1 % Tween-80. The SSF medium was mixed with the extraction

buffer at a ratio of 4 g per 100 mL with constant stirring for 30 min and centrifuged at 11000g for 30 min at 4 °C. The supernatant was used as a source of crude enzyme for fiber activation and analysis (Awafo et al., 1996).

2.4. Laccase assay

Laccase activity was determined according to the method described by Gassara et al. (2010). In brief, the reaction mixture consisted of 1.5 mM 2, 2-azino bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) in 100 mM phosphate-citrate buffer (pH 3.5). The reaction mixture was incubated in 0.2 mL reaction volume for 20 min at 45 °C. The oxidation of ABTS was determined at 420 nm using spectrophotometer. One unit of laccase activity was defined as the quantity of enzyme required to oxidize 1 μ mol of ABTS per min.

2.5. Endoglucanase assay

The endoglucanase assay was performed according to the method described by Zhang et al. (2007). The reaction mixture contained 50 mM sodium acetate buffer (pH 5) with 1 % carboxyl-methyl-cellulose (CMC). About one mL reaction mixture was incubated with 0.28 mL of enzyme solution at 50 °C for 30 min. The reaction was stopped by adding 3 mL of 1 % 3,5-dinitrosalicylic acid (DNS). The amount of reducing sugar was measured by spectrophotometer at 540 nm to calculate the endoglucanase activity.

2.6. Exoglucanase assay

The exoglucanase assay was performed according to the method described by Zhang et al. (2007). The reaction mixture contained 50 mM sodium acetate buffer (pH 5) with 2 % Avicel suspension. About 1 mL reaction mixture was incubated with 1 mL enzyme at 40 °C for 30 min. The reaction was stopped by adding 3 mL of 1 % DNS. The final mixture was incubated for 5 min at 100 °C. The activity of β -glucanase was determined using spectrophotometer at 540 nm.

2.7. β -glucosidase assay

The β -glucosidase assay was performed according to the method described by Zhang et al. (2007). In brief, the reaction mixture contained 15 mM cellobiose in 15 mM of citrate buffer (pH 4.8). About 1 mL reaction mixture was incubated with 1 mL enzyme at 50 °C for 30 min. The reaction was stopped by adding 3 mL of 1 % DNS. The final mixture was incubated for 5 min at 100 °C. The activity of β -glucosidase was determined using spectrophotometer at 540 nm.

2.8. Filter paper cellulase assay (Fpase)

The reaction mixture contained 125 μ L of enzyme solution in 250 μ L of 50 mM sodium citrate buffer (pH 4.8) with Whatman filter paper (Whatman no. 1. 0.25 mm pore size, 1.5 cm diameter). The mixture was incubated at 50 ± 1 °C for 30 min. The cellulase activity was calculated using the concentration of reducing sugar. One international unit of Fpase activity is the amount of enzyme that forms 1 mmol of glucose (Jung et al., 2015).

2.9 Cellulose extraction from pulp

The microwave treatment (MWT) was carried out for both primary and mixed PPSW collected from the pulp and paper industry wastewater treatment plant to extract cellulosic fibers. Primary biosolids was collected from the pulp and paper wastewater plant after pretreatment or primary treatment. The mixed biosolids is a mixture of primary sludge and secondary sludge (obtained after biological treatment) in the 4:6 ratio.

2.10. Ball mill grinding

Primary and mixed PPSW was milled in a planetary ball mill (PM100; Retsch Corporation) at 25 °C. Grinding was performed in a 500 mL stainless steel jar with 15 g of primary or mixed PPSW using stainless steel balls with a diameter of 2.4 mm. The number of balls was fixed to 800, which was equivalent to 45 g (Schwanninger et al., 2004).

2.11. Microwave treatment (MWT) and cellulose extraction

The primary and mixed PPSW samples were treated within the frequency range of 300 MHz to 300 GHz for both mixed and primary PPSW. The pretreatment was performed at different time intervals (30, 60, 90 and 120 min) at various temperatures (80, 100, 120, 140 °C) using dilute sulfuric acid (0.5-2 %) (v/v). After MWT, cellulosic fiber sample was centrifuged at 9000g for 30 min at 25 °C and the supernatant was discarded. The sample was washed several times with distilled water, till the pH reached 7. The washed sample was then dried in an oven at 60 °C for 24 h and used for further experiments (Raj et al., 1989).

2.12. Statistical Analysis of microwave treatment

The cellulose extraction conditions from pulp were optimized using statistical design (*Statistical Analysis System Software Version 7*) for higher yield. The central composite design was used to study size reduction and extraction of cellulosic fibers for primary and mixed cellulosic fibers using response surface methodology (RSM), with 3 factors (temperature, time and sulfuric acid concentration) and one response (length of cellulosic fibers) using 20 runs as represented in Supplementary Table S1 (Saha et al., 2011). This statistical design was mainly used to find the optimal conditions for size reduction and extraction of cellulosic fibers.

All the experiments were carried out in duplicates and the average and standard deviation were calculated. Analysis of variance (ANOVA) was used considering *P-value* < 0.05 as significant (Rouissi et al., 2013).

2.13. Enzymatic treatment

The cellulosic fibers extracted after MWT were subjected to an enzymatic surface modification. The aim of this treatment was to activate hydroxyl groups on cellulosic fibers. About 4 g of cellulosic fibers from primary and mixed biosolids (after MWT) was used for surface

modification using different combinations of cellulolytic enzyme (15 U/g) and laccase (20 U/g) in 200 mL working volume (100 mM phosphate-citrate buffer pH 4.8). The reaction was performed at 30 °C with constant shaking at 100 rpm for 7 days. The samples were then periodically drawn for various analysis (Tabka et al., 2006).

2.14. Matrix and cellulosic composite preparation and treatment

Treated cellulose fibers and PLAr was prepared using dry blending of the two. About 30 g each of matrix/cellulose was used for formulation and preparation of standard samples using a different percentage of cellulosic fibers (2, 5 and 10 %) (w/w). The samples were prepared by pressing at 200 °C, 0.8447 MPa for 5 min. All experiments were performed in five replicates and the results are presented as mean and \pm standard deviation

2.15. Analysis of mechanically and enzymatically modified fibers and biocomposites

Particle size was measured using laser scattering particle size distribution analyzer (model DL-3147-165, output 5 mW, Wavelength 650 nm) for both primary and mixed PPSW, the fiber size was measured before and after MWT and enzymatic treatment.

Scanning electron microscopy (SEM), (Carl Zeiss EVO 50) was used to investigate the size and morphology of fibers extracted from PPSW before and after microwave and enzymatic treatment. The samples for SEM were prepared by mounting PPSW on SEM grid using (SPI module sputter coater) with gold.

The samples were prepared as filaments and the changes in the functional groups of the cellulose at different stages of microwave and enzymatic treatment were analyzed using Fourier transform infrared spectroscopy (FTIR) (NICOLET IS50 FT-IR).

X-ray diffraction (XRD) (Siemens D5000) was used for characterization of molecular structure for primary and mixed biosolids. The experiment was performed using fine powder of the samples at 40 kV per 30 mA lamp power using Cu lamp ($\lambda=0.154059$ nm).

The biocomposites were prepared using PLAr and untreated and treated primary and mixed cellulosic fibers. The tensile strength test (model Instron 5565) and the impact test (model Tinius Olsen 104) was performed to analyze the mechanical proprieties of the novel biocomposites. The analyses were completed (5 replicates for each experiment) according to ASTM standards D638 (type IV) and ASTM D256 for the tensile strength and impact tests, respectively. An ANOVA was used to evaluate the significance of the difference between the biocomposite formulations by using *Statistica Software version 7*. The difference was considered significant at P -value < 0.05 (α was fixed to 0.05). Bonferroni's posthoc analysis was applied for the comparison between the biocomposite formulations (Hochberg, 1988).

3. Results and discussion

3.1. Enzyme production and activity

The enzymes used for the cellulose modification were produced using PPWS and hemp fibers as feedstock by SSF. The laccase production using PPWS as sole carbon source and Tween-80 as inducer showed maximum production after 12 days of fermentation at 50.4 ± 6.9 U per g dry substrate.

Fig. 1 depicts the production profile of cellulolytic enzymes from *T. reesei*. Hemp fibers comprising 78 % cellulose were found to be an excellent substrate for cellulolytic enzyme production. Under optimal fermentation conditions (75 % moisture content, pH 6.5, 14 days of incubation at 30 °C), the maximum production of endoglucanase, exoglucanase, β -glucosidase, and cellulase was 26 ± 0.1 , 5.5, 1.2 ± 0.01 and 15.2 ± 0.5 U per g dry substrate, respectively.

There are numerous reports on the production of cellulolytic enzymes using various substrates (Awafo et al., 1996). However, hemp fibers are a low cost and abundantly available substrate. It contains a high amount of cellulose compared to other common feedstock, such as corn stover and rice straw, used for enzyme production by *T. reesei* (Oomah et al., 2002). The enzyme production in this study was closer to other substrates, such as corn stover pretreated with sodium hydroxide (Fang and Xia, 2015).

3.2. Cellulosic fiber extraction using ball mill grinding and MWT from pulp

Ball mill grinding of the primary and mixed biosolids reduced the size from 1200 μm to 894 μm and 2500 μm to 1746 μm , respectively for primary and mixed biosolids. The cellulose fibers were further extracted from the size reduced PPWS using MWI and dilute sulfuric acid (0.5 - 2.5 %) (v/v) for improving extraction yield which can be considered as an eco-friendly method in comparison to other methods which used concentrated sulfuric acid 80-100 % (v/v) as reported by Shafiei et al. (2015).

This extraction was performed for PPWS primary and mixed wastes, which contained a high amount of cellulosic fibers according to Gassara et al. (2010). The extraction yielded around 75 % cellulose after combined physical and chemical treatment (Chen et al., 2011b).

As reported by Graupner et al. (2016), use of cellulosic fibers as reinforcement of composite is a crucial factor influencing the mechanical properties of biocomposites. Optimal conditions for cellulose fiber extraction with reduced size using primary and mixed PPWS were studied using surface response methodology (RSM) comprising variables such as, temperature, time and concentration of dilute sulfuric acid. The RSM results for primary and mixed biosolids are presented in Supplementary Table S1. The variation of fiber size from 894 μm (run No. 1) to 293 μm (run No. 16) for primary biosolids and 1746 μm (run No. 1) 341 μm (run No. 14) for mixed

biosolids show the importance of the parameter optimization. This study indicated that primary biosolids yield smaller sized cellulose (293 μm) in comparison to mixed biosolids (341 μm) after MWT. The optimum temperature and time to obtain decreased size cellulose fiber from primary biosolids was 100 $^{\circ}\text{C}$ and 40 min, respectively at 2 % acid. For mixed biosolids, however, all the three parameters, *viz.*, temperature, time and acid concentration for treatment increased to 120 $^{\circ}\text{C}$, 95 min and 2.48 %, respectively. The variation in MWT parameters and yield of smaller cellulose fiber might be attributed to degradation of fiber-coated substances, such as hemicellulose, pectin and lignin. The decomposition of the coating started from 200 $^{\circ}\text{C}$ up to 315 $^{\circ}\text{C}$ (Moran et al., 2008). In fact, MWT using a low concentration of sulfuric acid was efficient in terms of energy compared to other reported treatments, which were performed at a high temperature or high concentration of chemicals (Chen et al., 2011a). Electrical energy (E) consumption was calculated for MWT of both primary and mixed biosolids according to the Equation 1:

$$E = \frac{P \times t}{1000} \quad (1)$$

Where, E: electrical (kJ) energy, P: power (in this study, 500 W) and t: pretreatment time (s). The electrical energy consumed was approximately 4.5 and 2.8 MJ, for optimized MWT using low concentration of sulfuric acid (0.5-2.5 %) (v/v) for primary and mixed biosolids, respectively. Thus, net energy savings were obtained as compared to the steam explosion and aqueous methods which consumed around 7.2 and 4.26 MJ energy, respectively for pretreatment and wood size reduction under similar conditions (Zhu and Pan, 2010). Thus, this method can be used as an alternative for the pretreatment and size reduction of natural fibers.

The validation of the results analyzed by ANOVA is shown in Supplementary Table S2. The two ANOVA models had p-value 0.0002 and 0.0157, which was lower than the minimum limit value

of 0.05, indicating statistical significance. According to the p-value, the parameter which influenced the optimal condition for length of cellulosic fibers in the case of treated mixed and primary PPSW with MWT was the concentration of sulfuric acid and temperature. The results are in concordance with the findings reported for temperature and sulfuric acid treatment for cellulosic fibers by (Tahezadeh and Karimi, 2008). Thus, the optimal conditions for cellulose extraction were 150 °C, 95 min, 2.48 % of sulfuric acid and 140 °C, 150 min 2 % of sulfuric acid for mixed and primary biosolids, respectively.

3.3. Effect of enzymatic treatment on cellulosic fibers

Enzymatic treatment was performed for both primary and mixed cellulosic fibers with laccase and cellulolytic enzymes for reducing the size of the fibers and activation of interfacial hydroxyl groups on these fibers. These two enzymes have been widely reported for treatment of natural fibers for their specific action, where the cellulases modify the amorphous region of cellulosic fiber and laccase aids in the removal of phenolic content in the lignin and hemicellulosic part of the lignocellulose (Henriksson et al., 2007). The enzymatic treatment using cellulolytic enzymes and laccase was investigated for primary and mixed biosolids of fiber lengths, 293 and 341 μm , respectively. The size variation of primary and mixed cellulosic fibers after enzymatic treatment is presented in Fig. 2. After 7 days of incubation, the size of cellulosic fibers was reduced from 293 to $142 \pm 17.6 \mu\text{m}$ for primary biosolids and 341 to $127 \pm 5.65 \mu\text{m}$ for mixed biosolids. This size reduction could be attributed to the removal of hemicellulose and phenolic compounds, such as hydroxyl groups thereby reducing the size of cellulose (Heap et al., 2014). The enzymatic treatment also prevented aggregation of fibers in the biocomposites as it adversely influenced the mechanical properties of biocomposites (Bledzki et al., 2009). In addition, the hydrolysate of the treated primary and mixed biosolids showed 2.74 and 2.93 mg per mL reducing sugar

respectively, attributed to the action of cellulase. The synergistic effect of laccase and cellulolytic enzymes on the primary and mixed biosolids was further analyzed by various analytical techniques, *viz.*, SEM, FTIR and XRD and is discussed in the latter sections. Laccase and cellulolytic enzymes are highly efficient in PPSW cellulosic fiber treatment. Further, laccase and cellulase removed most of the coating substances on the surface, such as hemicellulose, pectin and lignin-producing fibers with higher roughness and hydrophobicity for various applications, such as reinforcement of composite and replacement of carbon fibers (George et al., 2014).

3.4. FTIR spectra

The structural changes in the cellulose fibers from primary and mixed biosolids were analyzed by FTIR at different stages, *viz.*, before and after MWT and enzymatic treatment. The FTIR spectra for the effect of various treatments on primary and mixed biosolids are shown in Fig. 3 (A) and Fig. 3 (B), respectively. Firstly, the peaks at 1636 cm^{-1} for primary biosolids (Fig. 3 A) and 1664 cm^{-1} for mixed biosolids (Fig. 3 B), characteristic of the carboxyl functional group or acid esters, were decreased by degradation of hemicellulose after MWT. Secondly, the vibration peak at 1236 cm^{-1} corresponding to the acetyl functional groups present in lignin, was also decreased, thus indicating the degradation of lignin present in the cellulose extracted from both primary and mixed biosolids. On comparing the spectrum before and after MWT treatment, the appearance of a vibration peak at 2900 cm^{-1} corresponding to the C-H groups present in cellulose (Tang et al., 2015) was seen (Fig. 3) for primary and mixed biosolids, respectively. This indicated the effect of MWT while using a lower concentration of sulfuric acid on the degradation of coating substances, such as hemicellulose lignin and pectin which could influence the size of cellulosic fibers compared to the initial length. Thus, the MWT was effective in

extracting the cellulosic fibers which are present in a higher percentage in PPSW, and hence it can be used as a reinforcement for PLAr.

The peaks at 2923 cm^{-1} and 3330 cm^{-1} , corresponding to hydroxyl groups for primary and mixed biosolids were substantially decreased indicating the fiber activation after treatment with laccase and cellulolytic enzymes through the reduction of hydroxyl groups. These results are in agreement with previous studies that showed the effect of cellulolytic enzymatic treatment on the reduction of hydroxyl groups (Cao and Tan, 2004). Nevertheless, there was a noticeable change in the peak at 2923 cm^{-1} representing cellulose. This phenomenon could be explained by the degradation of cellulose by cellulase. The peak at 1509 cm^{-1} , which corresponded to benzene structure of the lignin that was also decreased in primary and mixed biosolids, which indicated the degradation of lignin by the enzymatic treatment.

The combined treatment of enzymatic treatment and MWT is highly efficient for the extraction and activation of hydroxyl groups in cellulosic fibers. Likewise, the changes in the hydrophilic hydroxyl groups after enzymatic treatment indicated increased resistance to moisture in cellulosic fibers. Further, it also reflected the degradation of coating substances, such as hemicellulose, lignin, and pectin from the cellulosic fibers (Cao and Tan, 2004).

3.5. SEM analysis

The SEM analysis of cellulosic fibers for primary and mixed biosolids before and after MWT and enzymatic treatment is shown in Fig. 4 and Fig. 5, respectively. The mechanical properties of biocomposites depend on the length of fibers, which can affect the adhesion between fibers and matrix. Thus, smaller size favors better adhesion of matrix to the fibers. As evident from the Fig. 4 and 5, there was a remarkable change in the structural morphology after MWT and

enzymatic treatment. The size of mixed and primary biosolids in millimeter was substantially reduced to micrometer after treatment with enzymes and MWT.

3.6. XRD analysis

The structural changes in the cellulosic fibers analyzed by XRD after the enzymatic treatment is depicted in Fig. 6 (A) and (B) for primary and mixed biosolids, respectively. The principal peaks for primary biosolids and mixed biosolids represented the crystalline regions, which was present at a higher percentage in cellulosic fibers (Park et al., 2010). An increase in crystallinity for cellulose derived from both primary and mixed biosolids after the enzymatic treatment in comparison to untreated primary and mixed biosolids was due to a decrease in the amorphous region as also reported by Pickering et al. (2011).

Hence, the enzymatic treatment influenced amorphous region that affected the adsorption of cellulosic fibers, which can prevented the swelling, increased the hydrophobicity of cellulosic fibers and improved the binding between fibers and PLAr as also reported in previous studies (Kalia and Vashistha, 2012).

3.7. Mechanical properties

Generally, the mechanical properties of PLAr decrease after one cycle of injection. Hence, different percentage of untreated and treated cellulosic fibers were used to test the effect of the amendment and the size of fibers.

The mechanical properties of the cellulose PLAr biocomposite with different percentage of the composition are shown in Fig. 7 and 8, respectively for primary and mixed biosolids. The biocomposites with 5 % treated cellulosic fibers for both primary and mixed biosolids showed higher load (around 250 N and 180 N respectively) when compared to PLAr and 10 %, 2 % untreated/treated fibers.

Mechanical test (Young's Modulus and tensile strength) was measured for each biocomposite formulation of 2, 5, and 10 % of primary and mixed untreated and treated cellulosic fibers to understand stiffness and elasticity of the biocomposites. The mechanical test results of different cellulose fibers and PLAr compositions are summarized in Table 1. The statistical validation of these tests by Bonferroni's posthoc analysis is presented in Supplementary Table S3, S4 and S5. As seen in Table 1, the Young's Modulus of PLAr + 2 % cellulosic fibers for primary biosolids was highest (887.8 ± 36.6 MPa) with about 37.8 % improvement as compared to PLAr with 5 % or 10 % untreated and treated cellulosic fibers, indicating higher stiffness for PLAr + 2 % cellulosic fibers as compared to PLAr alone (658.1 ± 23.1 MPa). Bonferroni's posthoc analysis showed that the Young's Modulus for PLAr + 2 % of treated primary cellulosic fibers was highly significant (887.8 ± 36.6 MPa) (p-value, 0.000004) (Supplementary Table S3)

For PLAr + 2 % primary cellulosic fibers, the tensile stress was higher (41.35 ± 1.764 MPa) as compared to PLAr (29.4 ± 3.6 MPa) alone. The tensile strain at breakpoint for PLAr + 2 % primary cellulosic fibers (7.2 ± 0.6 %) was higher than PLAr (6.1 ± 0.8 %) and PLAr + 2 % untreated primary cellulosic fibers (6.9 ± 0.6 MPa).

Thus, PLAr + 2 % primary cellulosic fibers were shown to have enhanced tensile stress and tensile strain at breakpoint compared to PLAr. These results showed the effect of primary cellulosic fibers as reinforcement compared to polypropylene (30 MPa tensile stress and 3 % tensile strain at break and polypropylene-abaca fibers (44 MPa tensile stress and 5 kJ/m² impact stress and 1.3 GPa Young modulus) (Bledzki et al., 2010). Additionally, Bonferroni's posthoc analysis showed that developed biocomposites with 2 % of treated primary cellulosic fibers was comparable to the control except for the biocomposite, PLAr + 10 % treated mixed cellulosic fibers, which was less significant (p-values, 0.000271 and 0.000007 for tensile stress at break

point and tensile stress at yield, respectively) than the control (Supplementary Table S4 and S5). Furthermore, the formulation of primary treated cellulosic fibers improved mechanical properties of PLAr as compared to polypropylene. These results were comparable to pure polylactic acid reinforced by cellulose kraft fibers and polypropylene reinforced by abaca fibers (Bledzki and Jazzkiewicz, 2010).

By the addition of 2 % (w/w) untreated and treated mixed and primary cellulosic fibers to PLAr matrix, the mechanical properties of biocomposites, such as tensile stress, Young's Modulus and the tensile strain at breakpoint increased compared to PLAr and 5 %, 10 % of untreated and treated cellulosic fibers. This indicated that, at 5 % and 10 % of cellulosic fibers, mechanical properties decreased except for 10 % treated primary cellulosic fibers as shown in Table 1. These results were further confirmed by the impact test for different formulations, where highest value was obtained for PLAr + 2 % untreated and treated cellulosic fibers with $9.3 \pm 2.5 \text{ kJ/m}^2$ and $12.5 \pm 2.5 \text{ kJ/m}^2$ as compared to PLAr with $5.6 \pm 2.57 \text{ kJ/m}^2$ value. These values are higher than the properties of pure polylactic acid and PLAr. This can be attributed to proper adhesion between cellulosic fibers and matrix, the crystallinity of fibers before and after enzymatic treatment, the percentage of reinforcement on the matrix and the fiber size.

As reported for the enzymatic treatment, the activation of hydroxyl groups is one of the most important parameters for compatibility between the reinforcement and the matrix (Kalia and Vashistha, 2012). There was a decrease in the adsorption of hydroxyl groups proving the efficiency of the enzymatic treatment for both primary and mixed biosolids. This activation was clearly shown by the increase in mechanical properties for PLAr with different percentage of cellulosic fibers. However, the percentage of treated cellulosic fibers was a very important parameter in this formulation (Kalia and Vashistha, 2012). If the percentage was higher, the

mechanical properties decreased as shown in Table 1, except in the case of 5 % and 10 % treated cellulosic fibers. On the other hand, the size of treated cellulosic fibers was also affected as confirmed by mechanical test, more specifically for PLAr with 2 % primary cellulosic fibers when compared to the mixed cellulosic fibers.

Thus, the mechanical properties for PLAr + 2 % treated primary cellulosic fibers was the highest as compared to PLAr, polypropylene (PP), polyhydroxyalkanoate (PHB) as reported earlier by Bledzki et al. (2009). The mechanical properties were comparable to pure polylactic acid and PLA reinforced by natural fibers. The adhesion between the treated fibers and PLAr improved due to enzymatic modification as also reported by Spiridon et al. (2016). It also proved that enzymatic treatment was specific and rapid as compared to chemical modification. Strong mechanical properties of novel biocomposites demonstrated the applicability of the biocomposite in several fields, such as automobile and food packaging.

4. Conclusion

The effect of treated cellulosic fibers on mechanical properties of PLAr was tested using the microwave and enzymatic treatment. The microwave irradiation method was efficient for the cellulose extraction and size reduction of cellulosic fibers from pulp and paper solid waste. The enzymatic treatment and subsequent adhesion of cellulosic fiber to PLAr in biocomposite improved the mechanical properties of the biocomposite was obtained for PLAr + 2 % treated primary cellulosic fibers with Young's Modulus 887.83 MPa, tensile strain at the breakpoint of 7.22 % and tensile stress at yield of 41.35 MPa. Further, the study showed that the proposed combined physical and enzymatic treatment for pulp and paper solid waste could be one of the most reliable methods for the reinforcement of the biocomposites, and this could open other valorization options for these residuals.

Acknowledgement (s)

The authors are sincerely thankful to the Natural Sciences and Engineering Research Council of Canada (Discovery Grant 355254 and NSERC CRD Grant), and CRIBIQ for financial support. We would like to thank Mr. R. Fortin and Colin Jacob Vaillancourt from Gaudreau Environment for providing rPLA samples. Likewise, the support of Ozymes Inc. is equally appreciated for valuable comments during the experimental planning from industrial perspective. Financial assistance by the 'Fonds de recherche du Québec-Nature et technologies (FRQNT)' and INRS-ETE has been thankfully acknowledged by K Hegde.

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Figure Legends

Fig 1. Enzyme profiling for: (A) exo and endo-glucanase, β -glucosidase and (B) Fpase cellulase.

Fig 2. Comparative analysis of the size of cellulosic fibers before and after enzymatic treatment

Fig 3. FTIR spectra of: primary (A) and mixed (B) biosolids before and After MWT and enzymatic treatment.

Fig 4. SEM micrograph of treated and untreated mixed biosolids before (A and B) and after (C and D) microwave treatment (MWT).

Fig 5. SEM micrograph of treated and untreated primary biosolids before (A and B) and after (C and D) enzymatic treatment.

Fig 6. XRD spectra for: primary biosolids (A) and mixed biosolids (B) before and after enzymatic treatment.

Fig 7. Tensile stress profile for biocomposites using primary untreated and treated biosolids

Fig 8. Tensile stress profile for biocomposites using mixed untreated and treated biosolids

List of Tables

Table 1. Results of mechanical tests for biocomposite formulations

Table 1. Results of mechanical tests for biocomposite formulations (Abbreviations : Recycled polylactic acid, PLAr; Cellulosic fibers, fibers; MegaPascal, MPa)

			Young's Modulus (MPa)	Tensile stress at break point (%)	Tensile stress at Yield (MPa)
Primary biosolids	Untreated	PLAr + 2% of fibers	679.7 ± 118.4	6.9 ± 0.6	37.4 ± 5.1
		PLAr + 5% of fibers	571.8 ± 59	7.1 ± 1.7	29.5 ± 3.5
		PLAr + 10% of fibers	592.6 ± 77.9	3.8 ± 0.8	24.6 ± 7.2
	Treated	PLAr + 2% of fibers	887.8 ± 36.6	7.2 ± 0.6	41.3 ± 1.7
		PLAr + 5% of fibers	649.1 ± 112.1	5.3 ± 3.1	41.1 ± 5.9
		PLAr + 10% of fibers	714.1 ± 52.2	8.2 ± 0.8	34.4 ± 6.3
Mixed biosolids	Untreated	PLAr + 2% of fibers	687.3 ± 56.6	6.1 ± 2.3	33.7 ± 8.8
		PLAr + 5% of fibers	598.7 ± 73.4	6.1 ± 0.7	25.7 ± 3.7
		PLAr + 10% of fibers	590.2 ± 67.8	4.8 ± 2.8	23.6 ± 8.6
	Treated	PLAr + 2% of fibers	759.8 ± 51.7	7.1 ± 0.2	37.5 ± 3.9
		PLAr + 5% of fibers	601.1 ± 40.1	5.3 ± 2.9	31.7 ± 10.3
		PLAr + 10% of fibers	567.9 ± 42.9	1.7 ± 0.1	5.2
PLAr			644.4 ± 30.08	6.1 ± 0.8	29.4 ± 3.6

Fig. 1

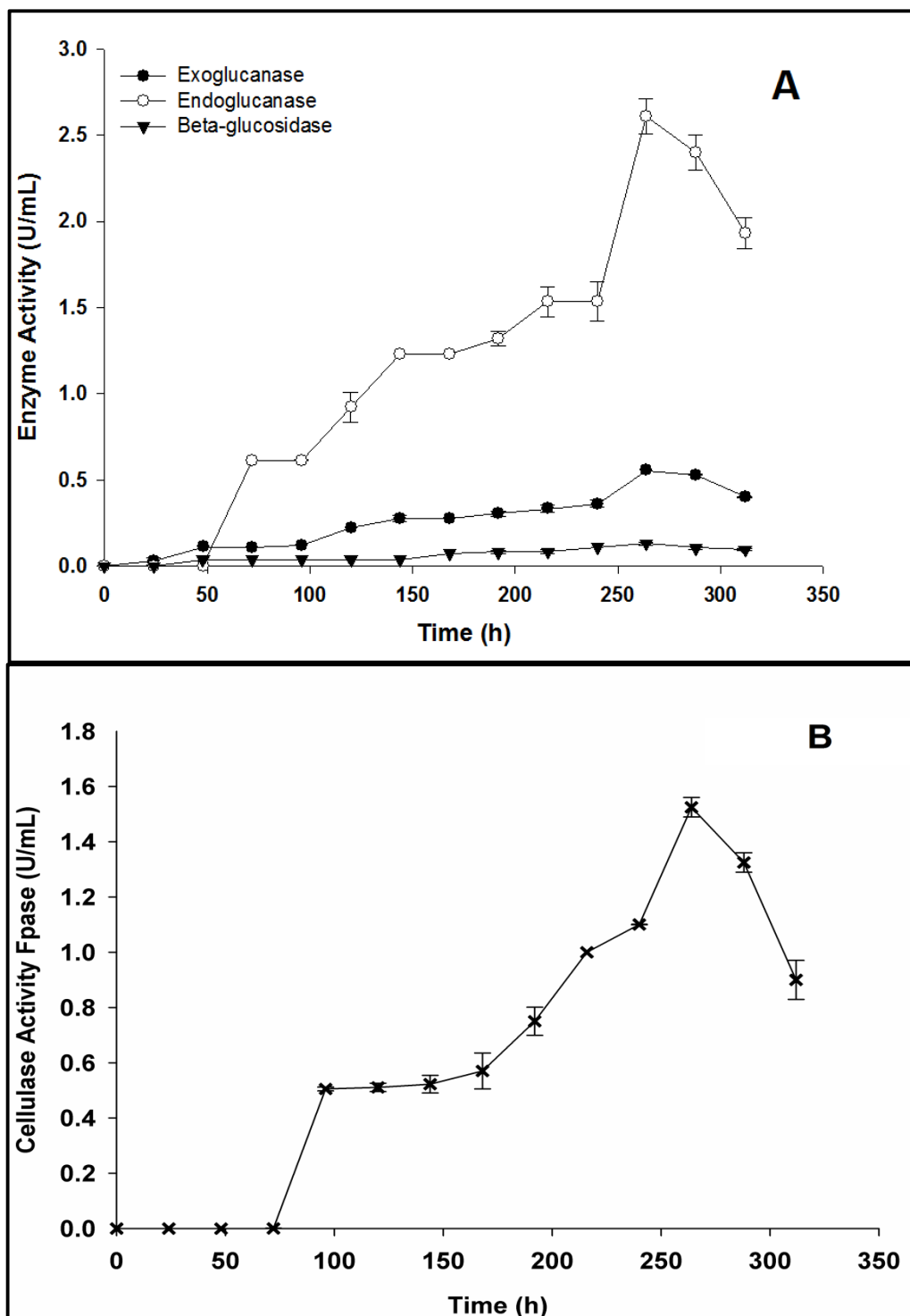


Fig. 2.

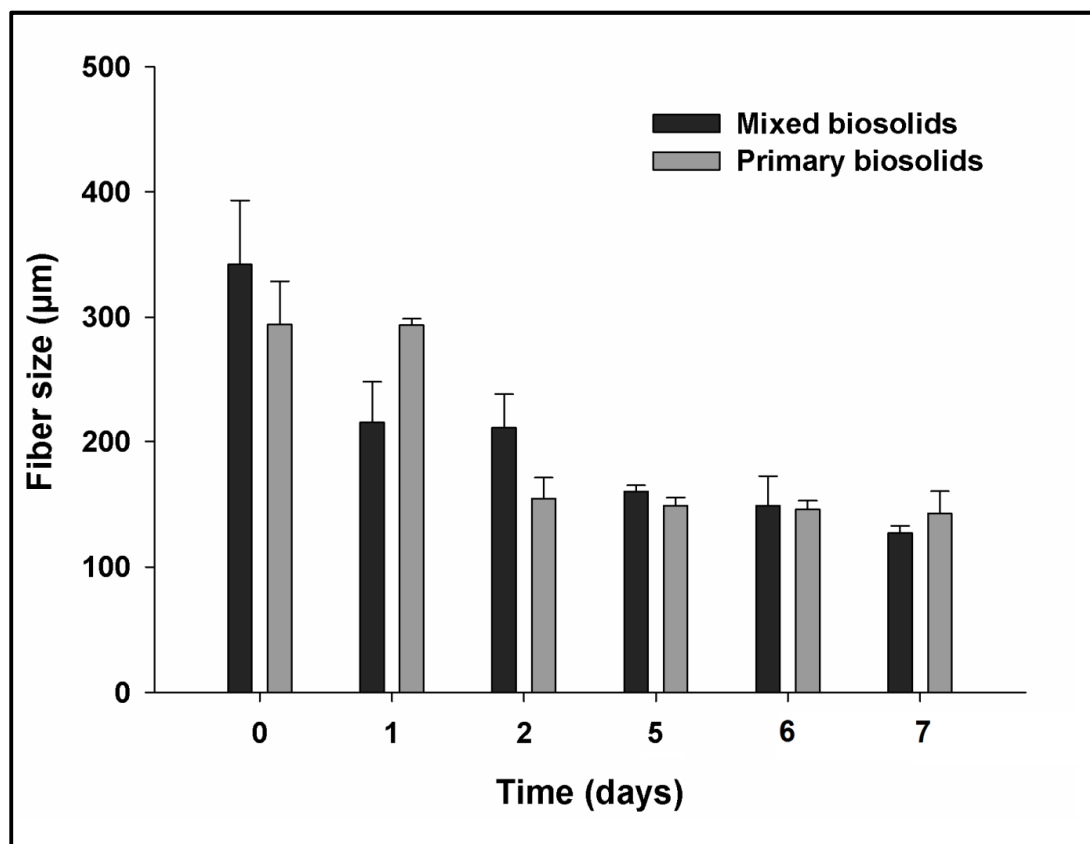


Fig. 3.

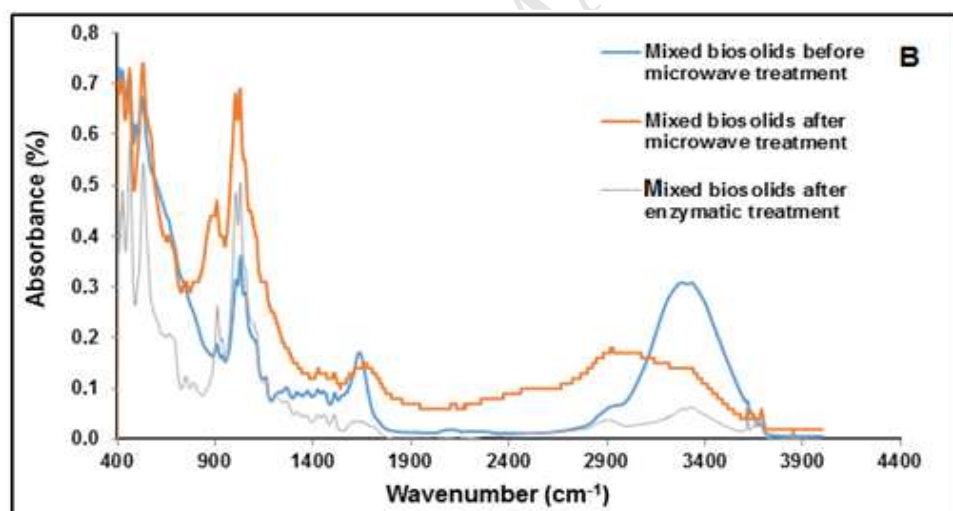
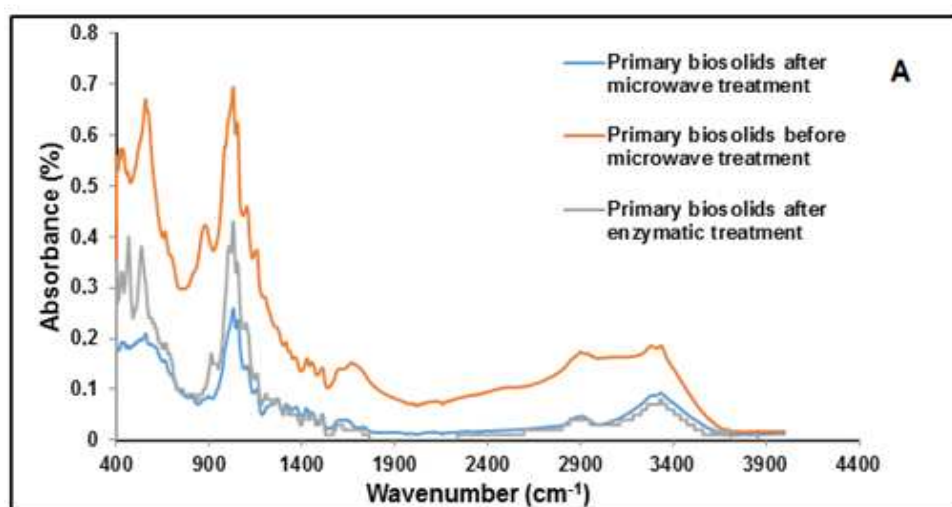


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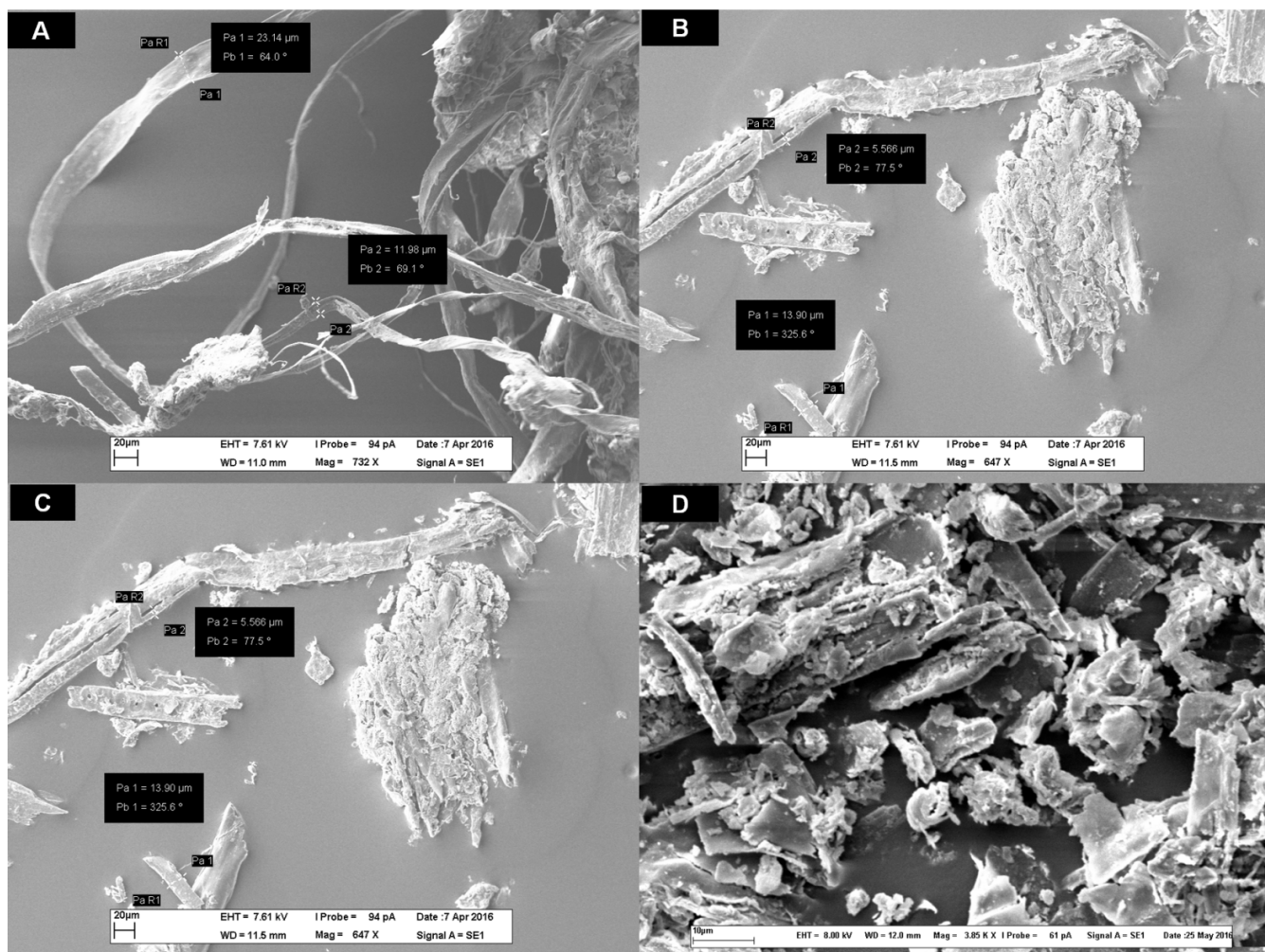


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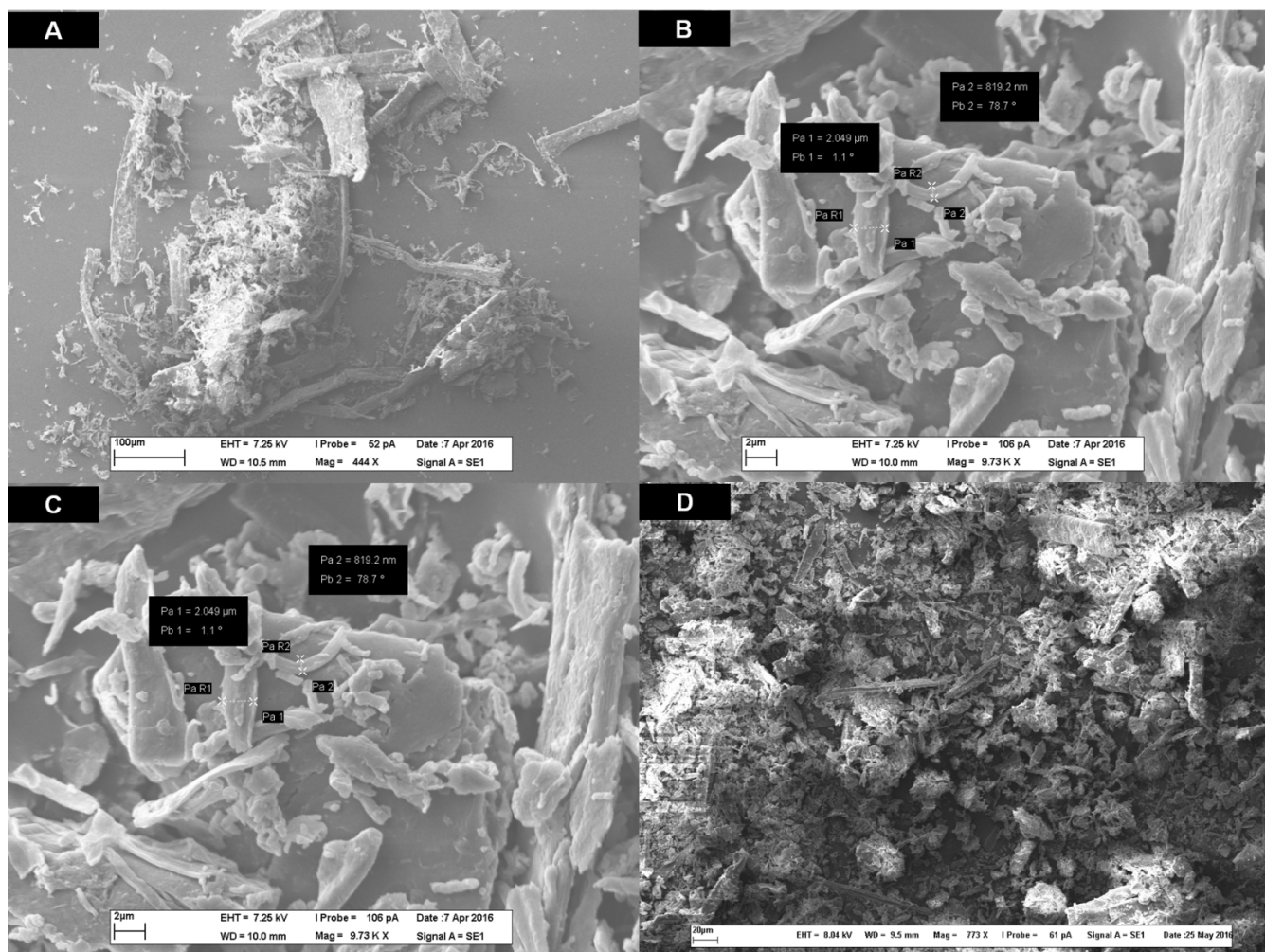


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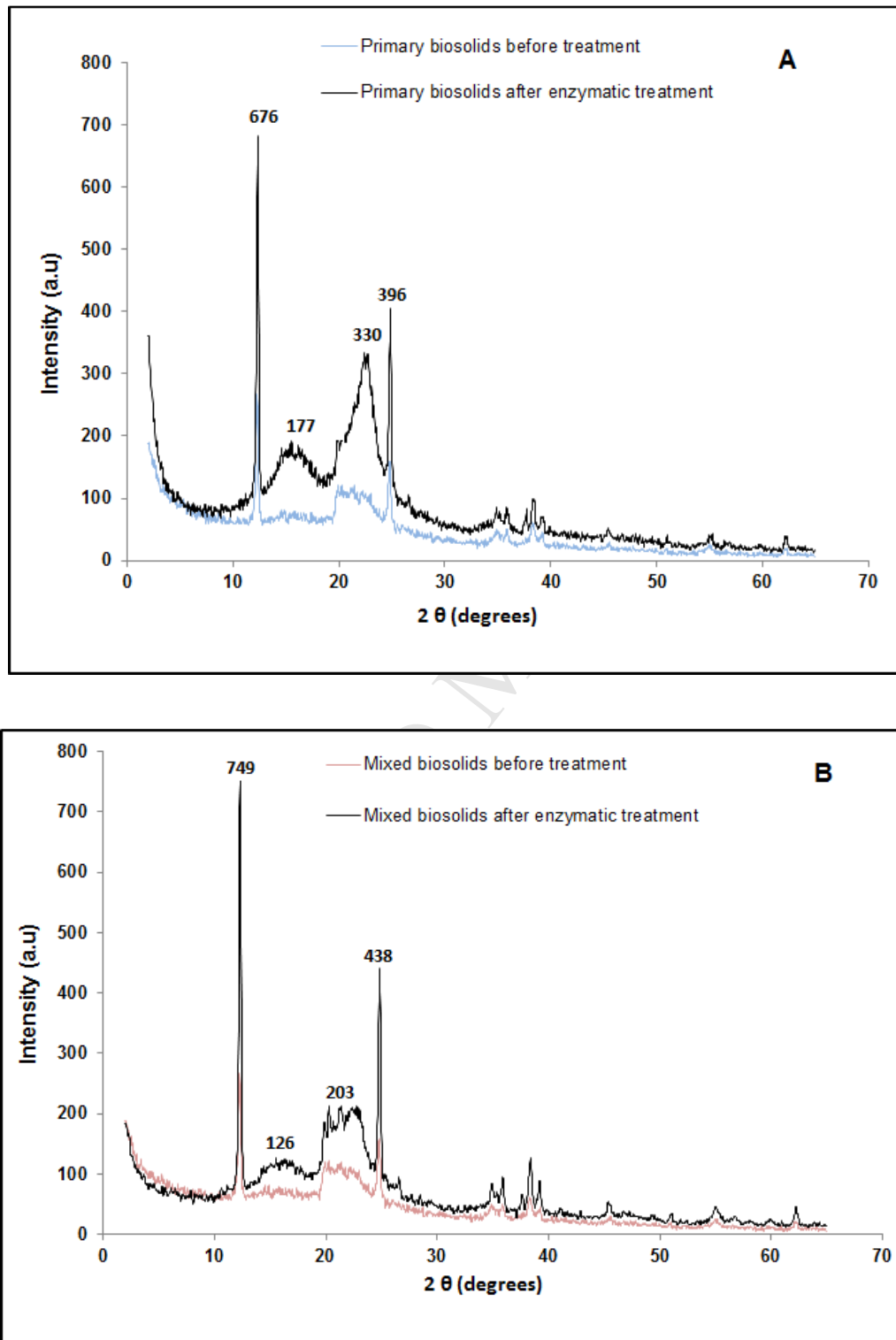


Fig. 7.

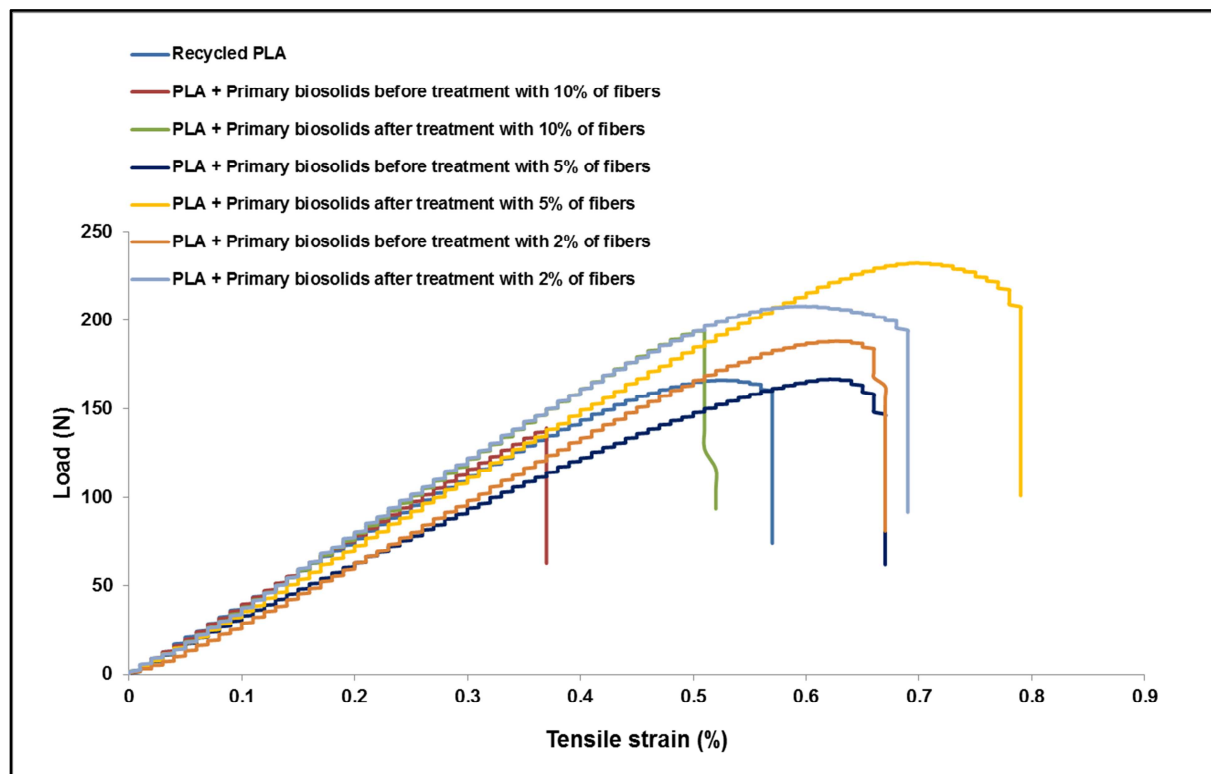
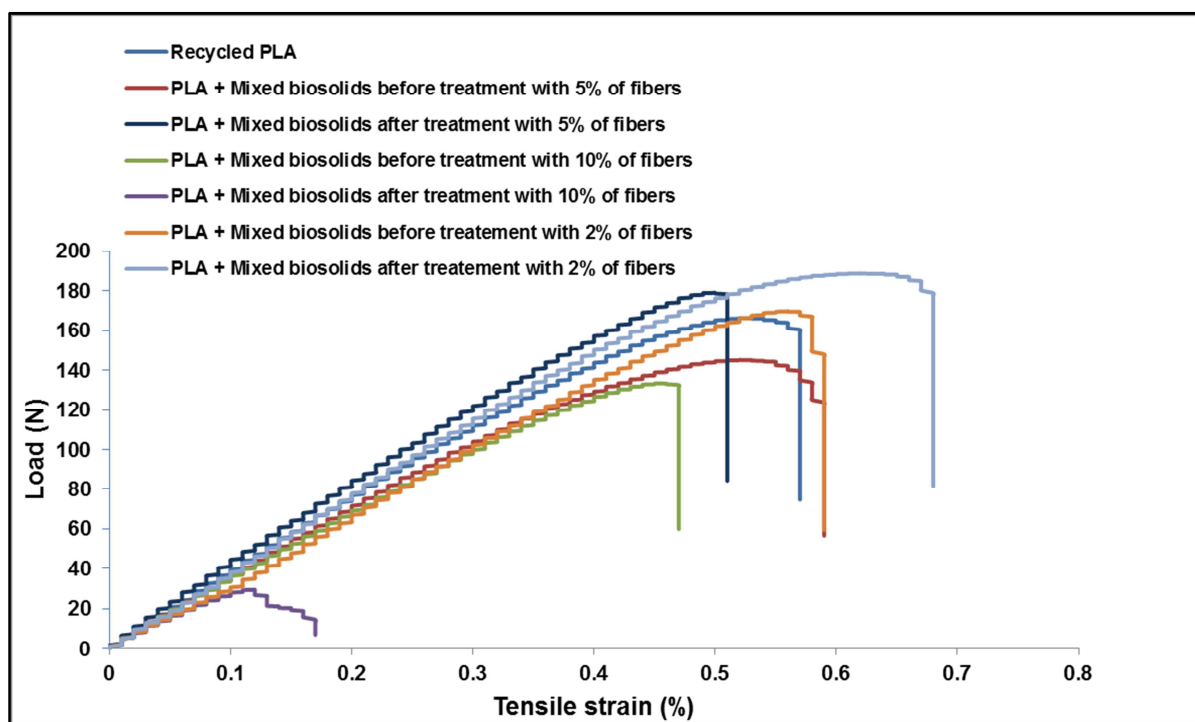


Fig. 8.



Highlights

- Biocomposite was synthesized from recycled polylactic acid and cellulosic fibers
- Combined physical and enzymatic process for biocomposite synthesis was developed
- A green process for surface modification of waste cellulosic fibers is proposed