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Enhanced biodegradation resistance of biomodified jute fibers

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ABSTRACT

A bio-catalyzed process has been developed for treating jute fibers to enhance their tensile strength and resistance against biodegradation. Lipolytic bacteria were used in the process to transesterify jute fibers by replacing hydrophilic hydroxyl groups within cellulose chains with hydrophobic fatty acyl chains. Transesterification of some of the hydroxyl groups within the fiber was confirmed with FTIR, UV–vis spectroscopy, ^{13}C solid state NMR, gas chromatography and analytical determination of ester content. Biomodified fibers exhibited remarkably smaller affinity to water and moisture and retained 62% of their initial tensile strengths after being exposed to a composting environment over 21 days. The corresponding figure for untreated fibers was only 30%. Efficacy of the process reported herein in terms of tensile strength and biodegradation resistance enhancement of fibers achieved after treatment appears to be comparable with similar chemical processes and better than the enzyme-catalyzed alternatives.

1. Introduction

Because of their hydrophilicity, lignocellulosic fibers (LCFs) usually absorb water amounting to several times their dry weight when they are exposed to moist environments. The swelling that results from water absorption exposes the cellulose chains, an essential constituent of the fibers, to cellulase producing microorganisms. This, in turn, leads to a rapid and significant strength loss making LCFs unsuitable in many engineering applications, e.g., in manufacturing geotextiles and composites. The hydroxyl groups present in the cellulose chains are a major contributor to the water affinity of LCFs. Chemical modification of these hydroxyl groups therefore has been used as a strategy for LCFs less hydrophilic and consequently less biodegradable. LCFs modified by acetylation, esterification, grafting, and transesterification, for instance, were found to lead to greater tensile strength, hydrophobicity, and resistance against biological, chemical and physical degradation (Mohanty, Khan, & Hinrichsen, 2000; Rowell, 2005; Saha, Manna, Sen, Roy, & Adhikari, 2012; Sun, Fang, Tomkinson, Geng, & Liu, 2001; Zbidi, Sghaier, Nejma, & Zbidi, 2009). These chemical processes often use toxic or hazardous reagents, require elevated pressure and temperature, or generate potentially hazardous by-products. A biomediated process could therefore be a greener alternative.

Purified enzymes such as pectinase, polygalacturonase, laccase, cellulase, and hemicellulase have been used for modification of natural fibers to enhance hydrophobicity and strength properties by removing amorphous hydrophilic substances from the matrix and enhancing fibril separation (Gubitz & Paulo, 2003; Zhang & Fan, 2010). Purified lipase has been used to graft, transesterify and esterify cellulosic fibers to make them less hydrophilic (Li, Xie, Cheng, Nickol, & Wang, 1999; Sereti, Stamatis, Pappas, Polissiou, & Kolisis, 2001; Xie & Hsieh, 2001; Yang, Wang, & Kuo, 2004; Zhang & Fan, 2010). These enzyme-catalyzed processes use purified enzymes and need an organic solvent based medium. As a result, they are expensive. Further, some of these processes also use hazardous chemicals such as ε-caprolactone, vinyl acrylate, vinyl propionate, vinyl neodecanoate, and acrylonitrile (Li et al., 1999; Sereti et al., 2001; Xie & Hsieh, 2001).

Although pure cellulose has been transesterified using purified lipase, hazardous chemicals has been used in the process and recovery of the enzyme after treatment is not easy. In this study lipase producing bacteria were used directly to develop an inexpensive process for treating jute fibers to make them stronger and more degradation resistant. The reagents used in the process are all non hazardous. The strain of bacteria isolated from a riparian environment was found to produce lipase as well as cellulase. The lipase produced by the strain was found to transesterify the fibers.

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Two challenges had to be addressed in the development. Since the bacteria produced cellulase along with lipase, the bio-catalyzed process had to be optimized considering the influence of lipase and cellulase on fiber chemistry. Second, the aqueous environment required for the sustenance of microbes also was expected to hydrolyze the newly formed ester bonds. To circumvent this difficulty, the microbes were grown via solid state fermentation using minimal water content. Additionally, potassium carbonate catalyst in phosphate buffer was used to minimize hydrolysis of ester bonds (Schuchardt, Sercheli, & Vargas, 1998).

The objective of the present study is the use of lipase producing microbes to transesterify the jute fibers for improvement of the tensile strength, hydrophobicity and degradation resistance against microbial attack. No previous report on such an approach for transesterification of lignocellulosic fibers using lipase producing microbes in a controlled aqueous environment was found in the literature. Further, as shown later, the process is expected to be extremely inexpensive compared to similar chemical and enzyme-catalyzed processes found in the literature. The process could be useful in manufacturing degradation resistant technical textiles, composites, cellulose ester films and plastics.

2. Materials and methods

2.1. Bacteria

The bacterium, identified as *Bacillus megaterium* RB-05 [GenBank Accession Number HM371417] (Chowdhury, Manna, Saha, Basak, Roy, Sen, & Adhikari, 2011), used in this study was isolated from the sediments of river Rupnarayan near Kolaghat, West Bengal, India. Reports on lipolytic and cellulolytic activities of *B. megaterium* can be found in the literature (Sekhon, Dahiya, Tewari, & Hoondal, 2006). Another lipolytic bacteria *Variovorax paradoxus* MTCC1193 obtained from Microbial Type culture collection, Chandigarh, India was used to demonstrate non-specificity of the proposed bio-process to a particular microbial species. Lipase producing potential of the bacteria was investigated by growing them in lipase selective tri-butyrin agar medium (Himedia). Cellulase producing potential was investigated by cellulase selective medium containing carboxy methyl cellulose as sole carbon source.

2.2. Neem oil enriched mineral salt (NOEMS) medium

An emulsion was prepared with neem oil in the presence of various inorganic salts. Every liter of this emulsion contained 50 ml neem oil, 10 g glucose, 3 g NH_4NO_3, 0.14 g KH_2PO_4, 2.2 g K_2HPO_4, 0.14 g NaCl, 0.6 g $MgSO_4$, 1.38 g K_2CO_3, 2.3 mg $ZnSO_4$, 17 mg $MnSO_4$, 10 mg $CuSO_4$, 4 mg Na_2MoO_4, 10 mg EDTA, 0.4 mg $NiCl_2$, and 6.6 mg NaI. The batches of the medium were prepared by adjusting the pH between 6 and 10 using HCl (1 N) or NaOH (1 N). Only 10–50 μl of HCl or NaOH was used at this stage per liter of NOEMS. At such small concentrations HCl or NaOH are non-toxic and non-hazardous. The resulting solution was finally autoclaved at 103 kPa for 15 min.

2.3. Fiber treatment

Jute fibers (*Chorcorus olitorius*) classified as TD5 according to Bureau of Indian Standards procedure (BIS, 2003) were first washed in distilled water to remove dust particles and water soluble fiber constituents, e.g., pectin, soluble hemicelluloses. They were subsequently kept immersed in 0.5% NaOH solution for 24 h for removing amorphous materials within the fibers matrix, typically lignin, pectin, hemicelluloses to better expose the cellulose chains to the bio-catalyzed treatment process described below. Removal of amorphous constituents with dilute alkaline solution leads to disentanglement of fibrils and increased tensile strength, whereas treating LCFs with strong alkali solution leads to chemical degradation of the fibrils and reduced tensile strength (Saha et al., 2010). In this study the maximum increase in fiber tensile strength was obtained when the fibers were treated with 0.5% alkali solution before biomodification. The alkali treated fibers were washed with distilled water to bring the fibers surface to neutral pH. Multi parameter PCSTester 35 (Oakton Instruments, USA) was used in all pH measurements reported in this paper.

The solid state fermentation process involved inoculation of 1 ml of *B. megaterium* RB-05 culture having initial bacterial load 2×10^6 cfu/ml with 2 g of alkali treated jute fibers and NOEMS medium keeping jute fiber to medium ratio 1:1 (w/v) and jute fiber to water ratio 1:1 (w/w). Bacterial growth was found to get impeded when smaller amounts of water were present. The fibers were then incubated under a range of temperature between 25 °C and 45 °C for incubation period of 0–6 days. The NOEMS medium containing fibers were incubated separately under identical conditions but without RB-05 for control measurements. The jute fibers were recovered and washed with 10 ml distilled water by vortexing and were compressed to remove bacterial cells, excess NOEMS medium, and enzymes. The bacterial cells in 1-ml of wash liquor collected from each batch of treatment were counted following spread plate colony counting technique. The remaining wash liquor was centrifuged at 6000 rpm for 6 min at 4 °C to remove the bacterial cells and this cell-free extract was saved to investigate the lipase and cellulase concentration. Before chemical and physical analysis the biomodified jute fibers were further washed 2–3 times with 70% ethanol to remove excess oil and bacterial cells from the fibers and were dried.

2.4. Lipase activity

Three different protocols are currently used for lipase activity determination: titration, colorimetry or spectrophotometry. In this paper we measured lipase activity by the tri-butyrin titration method (Kulkarni & Gadre, 1998) was used. This protocol uses a substrate prepared by dissolving 2 g polyvinyl alcohol (PVA) (Merck) in 100 ml in distilled water at room temperature and emulsifying 90 ml of the solution with 10 ml of tributyrin (Himedia). 5 ml of this emulsion was mixed with 4 ml of Tris–HCl (0.1 M, pH 7.5) (Himedia) and 1 ml cell-free extract and incubated at 30 °C for 30 min in a shaker water bath for lipolysis. The reaction was terminated by adding 2 ml of chilled (−20 °C) acetone and ethanol mixed in 1:1 proportion (v/v). The butyric acid generated in the process was titrated with NaOH (0.01 M) in the presence of 0.1 ml of phenolphthalein indicator (Kulkarni & Gadre, 1998). Equivalent molar amount of butyric acid was estimated from the amount of NaOH used in titration considering 1 ml of 0.01 M NaOH to be equivalent to 0.8811 mg of butyric acid. One unit of lipase is defined as the amount of enzyme which produced 1 μM of butyric acid per min at 30 °C.

The cell free extract was examined further with plate grooving method for lipase detection. An agar plate containing 1% tri-butyrin was grooved with a 5 mm grooving tool. Cell free extracts (10 μl) and control lipase (Sigma Aldrich) solution (100 U/ml) were placed within separate groves and incubated at 35 °C over 12 h. Lipase, if present, would degrade the tri-butyrin ester bonds leading to the formation of a clear zone at the margin of the groove.

2.5. Cellulase activity

A substrate was prepared by dissolving 2 g carboxy methyl cellulose (Merck) in 100 ml distilled water. One part of the cell-free extract collected after fiber treatment was mixed with four parts of the substrate (v/v) and incubated at 50 °C for 30 min for cellulolysis. The reaction was terminated by adding 4 ml of 3,5-dinitrosalicylic

Acid (DNS) (Merck), heating it in a boiling water bath for 15 min and finally cooling it back to room temperature. The color of the solution was stabilized by mixing it with 1 ml of 10% potassium sodium tartrate (Merck) solution and measuring the optical density of the solution at 540 nm using a spectrophotometer (Systronics 106). The amount of glucose released was estimated by comparing the optical density with the glucose standard curve. One unit of cellulase is defined as the amount of enzyme producing 1 μM of glucose per minute at 50 °C (Ghosh, 1987).

2.6. Ester content

Ester content in untreated, alkali treated and biomodified jute fibers was estimated according to Tanghe, Genung, & Mench, (1963). 1 g of biomodified jute fiber was mixed with 40 ml of 75% ethanol and kept at 55 °C for 30 min before allowing the mixture to cool to room temperature. An identical volume of 75% ethanol was separately heated and kept at 55 °C without fibers for 30 min and allowed to cool down to room temperature. Subsequently 25 ml each of 0.5 N sodium hydroxide was added to the mixtures with and without fibers. They were then reheated to 55 °C, kept at that temperature for 15 min, allowed to cool down to room temperature and left undisturbed for 3 days. The excess sodium hydroxide in the mixtures was subsequently titrated with 0.5 N hydrochloric acid using phenolphthalein as indicator. After neutralization, 1 ml of 0.5 N hydrochloric acid was added to each of the two mixtures before leaving them undisturbed for another 12 h. The excess acid was finally neutralized with 0.5 N sodium hydroxide solutions. The ester content, *EC* (in %), was obtained from:

$$EC = 0.05 \times \{(S_T - S_B) - (H_T - H_B)\} \times \frac{M}{w} \quad (1)$$

where S_T and S_B are the volume of sodium hydroxide needed for neutralization of the mixture with and without fibers respectively, and H_T and H_B are the volume of hydrochloric acid needed for neutralization of the mixture with and without the fiber sample respectively, w is the weight of the fiber sample and M is the average molecular weight of the predominant fatty acid chain; oleic acid in the present case.

2.7. Fiber characterization

Water absorption (WA) of jute fibers was measured following ASTM D570-98 (ASTM, 1998a,b) and the equilibrium moisture content (EMC) was determined according to ASTM D 4442-1992 (ASTM, 1992). Contact angle was measured at 35 °C by placing a drop of water (1–2 μl) upon a bundle of approximately 1 g of fibers with a micro-syringe and taking a photograph with a CCD camera attached to a contact angle meter (SEO Phoenix 300) (Alix et al., 2009). The tensile strength of 25-mm long fibers samples was measured according to ASTM D 3822-2001 (ASTM, 2007) using a universal tensile testing machine (Hounsfield, Model H10KS) equipped with a 100-N load cell maintaining 5 mm/s axial deformation rate. The diameter of a single fiber was measured using a Microscope (Leica DMLM) using 100× magnification. Fifty fibers were tested from each of the triplicate biomodified batches for estimating the means of tensile strength and elongation at break at 95% confidence level.

A pellet was prepared with dried jute fibers (1–2 mm long) and KBr in ratio 10:100 (w/w) and used to record the FTIR spectrogram (wave number range: 4000–500 cm^{-1}) using 32 scans in a Thermo Nicolet, Nexus 870 spectrophotometer. The intensities of the major spectral peaks were measured and the values were divided by the peak band intensity at 1050 cm^{-1} corresponding to aromatic C—O stretching because aromatic C—O bonds are expected to remain relatively unaffected during alkali treatment and subsequent biomodification (Colom, Carrillo, Nogués, & Garriga, 2003)

The X-ray diffractogram (XRD) of 1-g powder samples of treated and untreated jute fibers was obtained at 30 °C using RIGAKU ULTIMA III X-ray diffractometer for 2θ range between 10° and 50° maintaining a scanning speed of 2° per minute using Cu Kα radiation. Percent crystallinity index (*CrI*) was the data using

$$CrI = \left\{ \frac{I_{22.5} - I_{18.5}}{I_{18.5}} \right\} \times 100 \quad (2)$$

where $I_{22.5}$ and $I_{18.5}$ are the maximum intensities at $2\theta = 22.5°$ and 18.5° representing the relative amount of crystalline and amorphous constituents, respectively (Elenga, Dirras, Goma Maniongui, Djemia, & Biget, 2009).^{13}C cross-polarization magic angle spinning (CP-MAS) solid state NMR spectra were recorded on a Bruker AV-300 FT NMR spectrometer operating in a 7.01-T magnetic field by tightly packing 1 ± 0.2 mg of fiber, pre-washed with acetone and oven dried at 70 ± 5 °C, in a 4-mm Zirconia rotor sealed with KEL-F cap. A double resonance CP-MAS probe head capable of handling high radio frequency power and spinning samples at the 'magic angle' tuned to 75.48 MHz (13C) Larmor frequency and matched to 50 Ω circuit impedance was used. A contact time of 13 ms was uniformly used for all the samples utilizing 20–180 ppm spectral window (SW) with 10–15 Hz line broadening prior to Fourier transformation. The 13C chemical shifts were referenced externally to tetramethylsilane (TMS). The CP-MAS data were processed with Bruker TOPSPIN (Version 3.0) software.

The fatty acid methyl esters (FAMEs) were prepared following Zheng, Kates, Dubé, and McLean (2006) and were analyzed by gas chromatography using Inst-Perkin Elmer Clarus 500 gas chromatography equipped with a Carbowax® 20 M capillary column and an FID detector. The column was heated to 150 °C, kept at that temperature for 2 min and heated farther to 230 °C at 4 °C/min heating rate. The column kept at this temperature for 5 min. The volatilized FAME was detected using hydrogen as the carrier gas at 0.8 ml/min flow rate, a split ratio of 1:50, 240 °C injector temperature and 260 °C detector temperature. The GC chromatogram obtained was compared with the GC chromatogram of standard Supelco-37-Component FAME mix to identify fatty acids.

For assessing the resistance of fibers against biodegradation, 1-g of jute fiber from each of the treatment batches was kept buried within 100-g of soil prepared by mixing garden soil, silica sand and cow dung in 2:1:1 weight ratio maintaining its pH between 6 and 7 and moisture content at 31 ± 5% in a glass jars (BIS, 1992). The jars were incubated at 35 ± 5 °C and 85 ± 5% RH for 21 days as recommended in BIS (1992). Fiber samples recovered periodically from the jar were washed, dried and subjected to tensile strength testing. Similar testing protocol based on burial of substrates in animal refuse compost and organic soil is used internationally as well (ASTM, 1998a,b).

3. Results and discussion

3.1. Lipase

Lipolytic living cells are known to produce lipase during lipid digestion (Voet & Voet, 2010). Sekhon et al. (2006) found *B. megaterium* (AKG-1) to produce lipase while decomposing the ester bonds of edible vegetable oils. The strain of *B. megaterium* (RB-05) used in this study was also found to produce lipase while breaking neem oil ester bonds. The lipase production by RB05 was confirmed by the plate grooving method, which showed development of clear zones around all the grooves except for the control solution prepared using distilled water instead of the cell-free extract (Fig. 1).

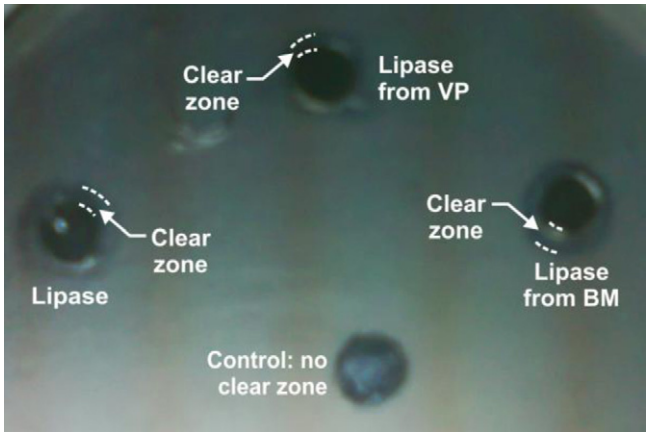

Fig. 1. Lipase assay.

3.2. Infrared and UV spectroscopy

FTIR absorbance spectral band representing O—H stretching vibration (Region A, Fig. 2) becomes less intense following biomodification of the alkali treated jute fibers possibly due to replacement of O—H groups by fatty acyl chains. Spectral response representing C=O stretching vibration (Region B, Fig. 2) of ester of hemicellulose and lignin disappeared following alkali treatment because of partial removal of pectin, and waxes as well as galacturonic acids present in fiber matrix (Saha et al., 2012). The response in this region intensified after biomodification due to the formation of new ester bonds during transesterification. Spectral response representing glycoside C—O—C and C—O stretching of ester groups of lignin and hemicellulose (Janardhnan & Sain, 2011) also become less intense after alkali treatment and reappeared following biomodification (Region C, Fig. 2). Similarly, spectral response representing the bending response of the fatty acyl chain COOH groups attenuated following alkali treatment because of partial removal of pectin, waxes and galacturonic acids and intensified after biomodification because of incorporation of fatty acyl chains (Region D, Fig. 2). These observations therefore are indicative of transesterification of jute fibers during biomodification.

Another lipase producing bacteria *V. paradoxus* MTCC 1193 (VP) has been used for the biomodification of the LCFs to demonstrate the generic nature of the bio-catalyzed process. The FTIR spectral response (Fig. 2, Region B) indicates the occurrence of biomodification in this case as well.

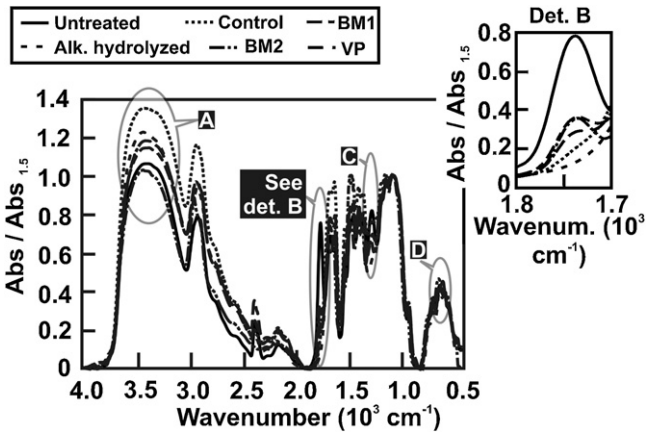

Fig. 2. FTIR spectrograph of jute fibers (BM1: spectrum for fibers treated with *Bacillus megaterium* RB-05 in a small scale batch process; BM2: spectrum for fibers treated with RB-05 in large scale; VP: spectrum for fibers treated with *V. paradoxus* in a small scale batch process.

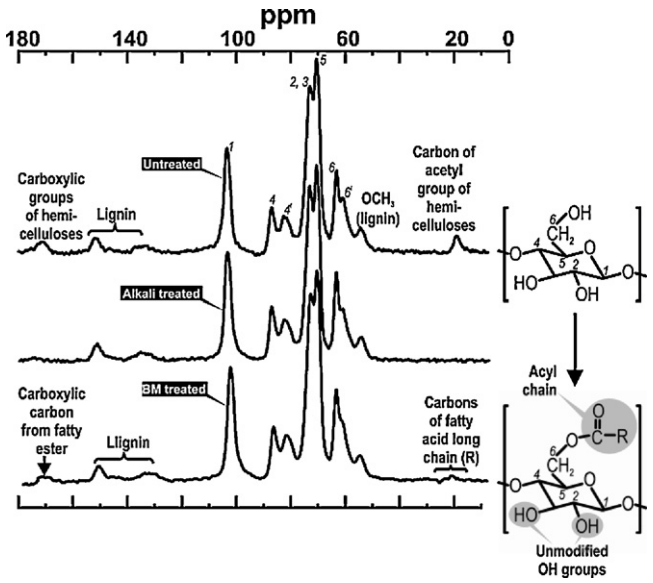

Fig. 3. ^{13}C solid state NMR spectra.

The biomodified fibers, thoroughly washed with 70% ethanol to remove excess oil, were hydrolyzed in 1% NaOH solution (1:10 fiber to solution ratio) for 1 h at100 °C to investigate the nature of chemical change further. UV–visible spectroscopic data (Systronics Ltd.) of hydrolyzate filtrate showed absorption maxima at 288 nm and 292 nm closely matching with the absorption maxima of standard oleic and stearic acids. Since oleic and stearic acids are the main active ingredients in neem oil used in biomodification, their presence in hydrolyzate filtrate provides further support to transesterification inferred from FTIR data. FTIR spectral response in Regions B and D of Fig. 2 was also found to attenuate for biomodified jute fibers subjected to alkali-hydrolysis. Biomodification of the fiber chemistry was further supported by NMR spectroscopy and Gas chromatography.

3.3. Solid state ^{13}C NMR spectroscopy

Peaks in CP/MAS ^{13}C NMR spectra at 18 and 171 ppm representing the methyl and carboxylic carbons of acetyl groups attached to hemicelluloses (Tronc et al., 2007; Wikberg & Maunu, 2004) were found to disappear following alkali treatment (Fig. 3). Removal of hemicelluloses inferred from this observation is consistent with the interpretation of the disappearance of absorption peaks at 1746 cm^{-1} in the FTIR spectra for alkali treated LCFs presented earlier. Peaks in the region between 12 and 37 ppm and at 170 ppm were found to reappear in the NMR spectra of the biomodified jute fibers. The response in the 12–37 ppm region is due to the carbon of methyl groups of long fatty acid chain, which replaced the hydroxyl groups during transesterification of LCFs. The response at 170 ppm, on the other hand, is due to the carboxylic acid groups of the fatty acid ester formed during biomodification. The peaks from 60 to 113 ppm in the NMR spectra represent cellulose carbon present in LCFs. Attenuation of the peak at 60 ppm representing amorphous cellulose following biotreatment is indicative of transesterification of amorphous cellulose (Tronc et al., 2007). The other peaks in the region between 60 and 113 ppm remained unaffected because crystalline cellulose represented by these peaks is relatively inert to chemical changes. Similar inertness of cellulose for sisal fibers was reported elsewhere (Martins, Forato, Mattoso, & Colnago, 2006).

Table 1
Characteristics of jute fibers (mean ± 95% confidence intervals).

Fiber	TS[a] (MPa)	EB[b] (%)	WA[c] (% weight)	EMC[d] (% weight)	*CrI* (%)	CA[e]	EC[f] (%)
Untreated	340 ± 35	1.4 ± 0.4	225 ± 9	9.3 ± 0.4	50	45 ± 7	36
Alkali treated	519 ± 42	1.5 ± 0.5	121 ± 8	5.3 ± 0.2	57	76 ± 5	01
Biomodified (BM[g])	650 ± 51	1.6 ± 0.2	93 ± 5	5.7 ± 0.7	52	98 ± 9	14
Biomodified (VP[h])	619 ± 71	1.5 ± 0.2	105 ± 11	6.7 ± 0.7	54	90 ± 4	8

[a] Tensile strength.
[b] Elongation at break.
[c] Water absorption.
[d] Equilibrium moisture content.
[e] Contact angle.
[f] Gravimetric ester content.
[g] RB-05 treatment.
[h] *Variovorax paradoxus* treatment.

The carbon within the methoxy (—OCH_3) group of lignin represented by the NMR response at 54 ppm and other methyl and alkyl carbons of lignin represented by the peaks between 130 and 152 ppm (Martins et al., 2006) remained unaffected following alkali and biomodification. The observation indicated incomplete removal of lignin during the treatments.

3.4. Gas chromatography

The retention times of 18.8, 22.0, and 22.2 min found in gas chromatograms corresponded with 16:0 palmitic acid, 18:2 linoleic acid and 18:1 oleic acid, respectively. The fact that these fatty acids derived from biomodified jute are also found in the neem oil used in this study for fiber treatment provides additional support to transesterification reaction inferred from FTIR and ^{13}C NMR data.

3.5. Ester content

The sharp reduction in ester content is apparent following alkali treatment compared to untreated fibers is possibly due to removal of hemicelluloses (Table 1). The ester content was found to recover partly after biomodification: an observation consistent with the inferred transesterification of some of the hydroxyl groups within the fiber because of incorporation of fatty acyl chains of neem oil.

3.6. Crystallinity

X-ray diffractograms of untreated and biomodified jute fibers presented indicate crystallinity to increase following alkali treatment from 51% to 57% (Table 1) due to the removal of amorphous materials, e.g., hemicelluloses and lignin, during alkali treatment (Saha et al., 2010). Subsequently, crystallinity decreased marginally after microbial treatment possibly because of incorporation of fatty acyl chains predominantly within non-crystalline cellulose chains and residual hemicelluloses during transesterification. Similar marginal reduction in crystallinity has been observed earlier after transesterification of sisal fibers (Saha et al., 2012) and polyester fibers (Tserki, Matzinos, Pavlidou, & Panayiotou, 2006). In contrast, for jute fibers marginal increase of crystallinity has been reported following transesterification (Saha et al., 2012).

3.7. Tensile strength

Biomodified jute fibers were characterized with approximately twice the tensile strength compared to untreated fibers (Table 1) and the maximum increase in tensile strength was observed when jute fibers were incubated with bacterial culture over 2 days at 35 °C with NOEMS pH of 8. The results presented in Fig. 4 indicate the enhancement of tensile strength with bacterial cell count, lipase, and cellulose production with time and pH respectively. After 2 days the strength enhancement was found maximum possibly due to lower production of cellulase than 3 days. Steep increase in cellulase production was observed as NOEMS pH exceeded 6 and incubation duration exceeded 1 day and degrade the cellulose main chain in the LCFs. Although cellulase production decreased again at NOEMS pH of more than 9, bacterial cell count and lipase production also decreased at such levels of alkalinity. Further, the ester bonds formed during biomodification are expected to hydrolyze above pH 8.0 (Saha et al., 2012). Consequently, the fiber tensile strengths did not increase when initial NOEMS pH of more than 8 was used. Fiber tensile strength and bacterial cell count increased marginally as incubation temperature increased from 25 °C to 35 °C before slightly decreasing as the temperature increased further to 45 °C (Fig. 5).

Since NOEMS initial pH as well as incubation duration and temperature were found to have very similar effects on tensile strengths of treated jute fibers and bacterial cell count, bacterial cell count appears to be a key factor controlling the tensile strength following biomodification. Chemically induced transesterification reportedly led to similar increases in tensile strengths for cellulose

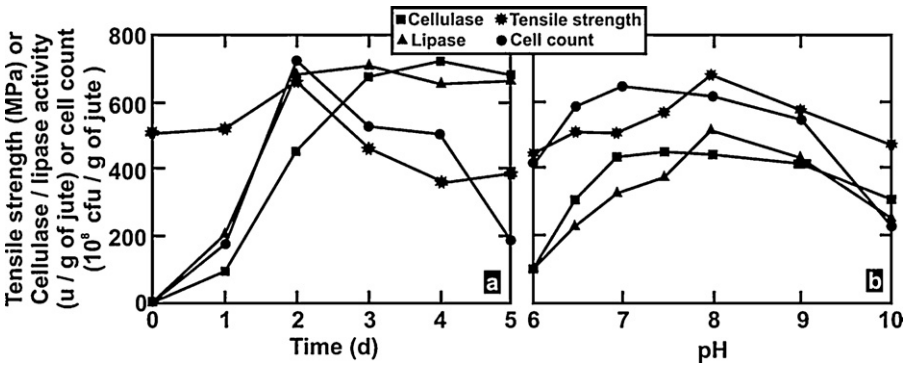

Fig. 4. Bacterial growth, cellulase and lipase production and tensile strength (a) as functions of time at 35 °C and pH 8 and (b) as functions of pH for 2-d incubation at 35 °C.

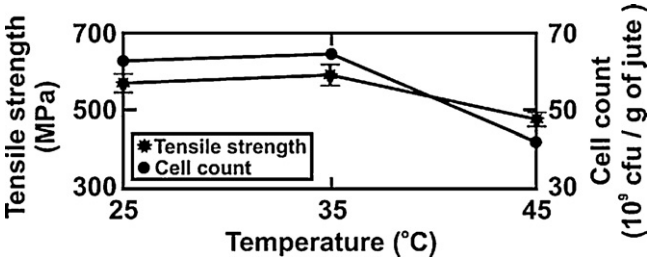

Fig. 5. Tensile strength and bacterial population as functions of temperature for 2-d incubation at pH 8.

(Chauvelon et al., 2000; Crépy, Chaveriat, Banoub, Martin, & Joly, 2009) and lignocellulosic fibers (Gubitz & Paulo, 2003).

In untreated fiber, cellulose chains are entangled within amorphous matrix comprised of hemicelluloses and lignin mainly by hydrogen bonding. Fibril entanglement because of the presence of amorphous matrix of hemicelluloses and lignin in untreated fibril bundle is expected to hinder development of efficient load path. As apparent from the FTIR data presented earlier, partial removal of hemicelluloses and lignin after alkali treatment led to reduction of hydrogen bonding within the fibers possibly disentangling the cellulose chains. It therefore appears that the relatively low tensile strength of untreated fibers increased after alkali treatment because of development of efficient mechanical pathways due to fiber disentanglement. FTIR data presented earlier also indicated that the extent of hydrogen bonding within the fiber did not alter appreciably after biomodification although the tensile strengths of biomodified fibers were marginally higher than those of alkali treated fibers. The modest increase in fiber tensile strength after biomodification is probably due to the plasticizing effect of fatty acyl chains incorporated into the jute fibers (Crépy et al., 2009).

3.8. Affinity to water and moisture

Following biomodification water absorption of jute fibers decreased from 225% to 93% (Table 1). Equilibrium moisture content similarly decreased from 9% to 6% after biomodification. Compared to the contact angles of untreated and alkali treated jute fibers, biomodified jute fibers were also higher (Table 1). These changes are likely to be due to partial replacement of hydrophilic hydroxyl groups within the cellulose chains by hydrophobic acyl chains, which restricted the entry of water molecule into the fibers. Similar reduction of hydrophilicity of cellulosic fibers resulted from enzymatic modification as well (Mohanty et al., 2000; Xie & Hsieh, 2001; Yang et al., 2004; Zhang & Fan, 2010).

3.9. Biodegradation

Biomodified jute fibers were found to retain 62% of their initial tensile strengths after 21 days of soil burial (Fig. 6). In comparison, untreated and alkali treated jute fibers retained only 30% and

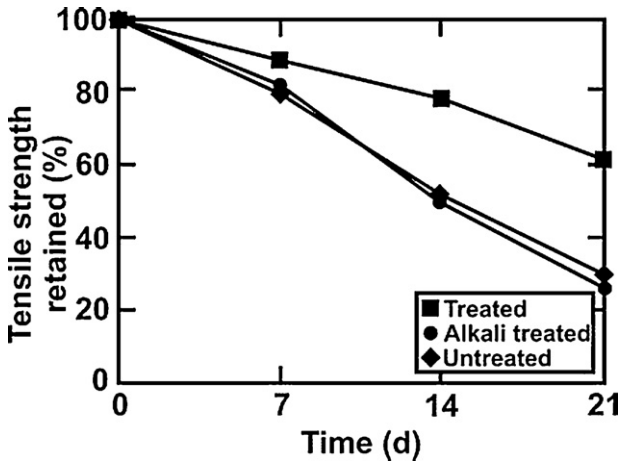

Fig. 6. Tensile strength retention during soil burial.

26%, respectively. The difference in the resistance against biodegradation is caused by the reduced swelling tendency of the fibers arising from partial replacement of hydrophilic hydroxyl groups by hydrophobic acyl chains restricting the exposure of degradation susceptible cellulose chain glycosidic bonds to cellulolytic microorganisms. It should be noted that while the short term tensile strengths of alkali treated jute fibers were only 20% lower than that of biomodified fibers, the tensile strength of alkali treated fibers after 21-d soil burial were less than half of that of biomodified fibers.

3.10. Scaling up feasibility

In laboratory scale development, of the biomodification process only 1 g jute was treated as fiber level. To check whether the process could be scaled up, 1 kg of jute fibers was treated. The FTIR spectral response of jute fibers treated in this 1-kg batch is similar to that obtained during the developmental phase in which the treatment was applied to 1-g batches (Fig. 2). It appears from this result that the process developed in this research is scalable although optimization of process parameters may be required at each step.

3.11. Relative efficacy

Comparison of reagent costs for the process reported in this paper with those for similar chemical or enzyme-catalyzed processes for treating lignocellulosic substrates reveal the potential for a very significant cost savings if the process developed in this research can be adapted industrially (Table 2). Since the costs for substrate, factory space, equipment, depreciation, maintenance, waste handling, personnel and energy for these processes are not expected to differ significantly from each other they were not included in the comparison. Further, the efficacy of the whole cell-catalyzed process in terms of enhancement in tensile strength and

Table 2
Comparison of cost and efficacy of LCF treatment processes.

Treatment (reference)	Treatment cost (US$/kg of fiber), time (days)	WA after treatment (% of WA before treatment)	Tensile strength increase	Tensile strength retention after 21-d biodeg.
Veg. oil – phenolic resin transesterification (Saha et al., 2012)	2, 0.5	70%	98%	84%
Vinyl acrylate–pyridine transesterification (Serati et al., 2001)	230, 0.12	50%	21%	Not reported
Enzymatic acylation of hydroxypropyl cellulose (Sun et al., 2001)	27280, 8	Decreased by an unknown amount	Not reported	ditto
Enzymatic cellulose transesterification (Zbidi et al., 2009)	23210, 5	ditto	ditto	ditto
Enzymatic cellulose acetylation (Zhang & Fan, 2010)	8830, 5	ditto	ditto	ditto
Biomodification (this study)	1, 2	58%	91%	60%

biodegradation resistance of biomodified jute fibers is comparable with that of the chemical processes as listed in Table 2 and better than the pure enzyme-catalyzed ones.

4. Conclusions

A whole-cell-catalyzed bio-process has been developed for transesterifying jute fibers for enhancing tensile strength and biodegradation resistance. The short-term tensile strength of the treated fibers was twice as those of untreated fibers and the tensile strength retained by treated fibers after 21-d biodegradation was 4-times as much as that of untreated fibers. The performance of the proposed process appears to be comparable with similar chemical processes and better than those catalyzed by purified enzymes. Transesterification was confirmed with FTIR, UV–vis spectroscopy, ^{13}C solid state NMR, gas chromatography and analytically estimated ester contents. The tensile strength increase was ascribable to fibril disentanglement due to alkali treatment and plasticizing effects of fatty acyl chains incorporated during biomodification. Incorporation of fatty acyl chains made the fibers less hydrophilic and restricted the exposure of biodegradation susceptible glycosidic bonds of cellulose to microbial actions.

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