

Akhila Rajan · Resmi C. Senan · C. Pavithran
T. Emilia Abraham

Biosoftening of coir fiber using selected microorganisms

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Abstract Coir fiber belongs to the group of hard structural fibers obtained from coconut husk. As lignin is the main constituent of coir responsible for its stiffness, microbes that selectively remove lignin without loss of appreciable amounts of cellulose are extremely attractive in biosoftening. Five isolated strains were compared with known strains of bacteria and fungi. The raw fiber treated with *Pseudomonas putida* and *Phanerocheate chrysosporium* produced better softened fiber at 30 ± 2 °C and neutral pH. FeSO_4 and humic acid were found to be the best inducers for *P. chrysosporium* and *P. putida*, respectively, while sucrose and dextrose were the best C-sources for both. Biosoftening of unretted coir fibers was more advantageous than the retted fibers. Unlike the weak chemically softened fiber, microbial treatment produced soft, whiter fibers having better tensile strength and elongation (44.6–44.8%) properties. Scanning electron microscopy photos showed the mycelia penetrating the pores of the fiber, removing the tylose plug and degrading lignin.

Keywords Biosoftening · Coir fiber · Lignin · *Pseudomonas putida* · *Phanerocheate chrysosporium*

Introduction

Coir is a hard fiber obtained from coconut palms, which grows abundantly in tropical countries. In India, coir manufacturing is a traditional industry, which has taken deep roots in the economic structure of the rural areas in the coastal states, especially in Kerala state [1]. Indian coir enters the world market in the form of raw fiber, spun yarn and woven mats, matting, rugs and carpets,

coir rope, etc. [2]. Bright golden colored yarns are considered the best and obtained from the retted coconut husks. The thicker and coarser variety is known as the bristle fiber and is used in the manufacture of brushes and brooms.

Coir fiber is multicellular in nature, and the length ranges from 10 to 35 cm and diameter from 0.1 to 0.3 mm being thickest in the middle of their length. Coir is light, elastic, water resistant, and resistant to mechanical wear. It is a natural cellulosic fiber and contains apart from cellulose, lignins, and other substances, which serve as building materials for the cell structure (Table 1). The percentages of the constituents—cellulose, lignin, and hemicellulose—[3] vary largely, depending upon the age of the nut from which the coir is derived. Morphological studies of coir fibers show a framework of organized aggregates of cellulose molecules called microfibrils embedded in a matrix of non-cellulosic polysaccharides and lignin. The middle lamella and primary cell wall undergo the greatest lignification and the secondary wall the least.

Lignin is the main constituent of coir responsible for the stiffness of the fiber and protects cellulose from degradation, as lignin itself is extremely resistant to chemical and biological degradations. Fungi that selectively remove lignin without loss of appreciable amounts of cellulose are extremely attractive for use in biological pulping processes, to improve the digestibility of highly lignified plant residues [4] for cattle. Haider et al. and Gradziel et al. have shown that certain pure cultures of bacteria are also able to decompose lignin and to assimilate lignin degradation products as a carbon source [5, 6].

The lignin degrading system of white rot fungi involves several extracellular enzyme activities like H_2O_2 requiring lignin peroxidase (LiP) [7], manganese peroxidase (MnP) [8], laccase and H_2O_2 generating enzymes like glucose oxidase [9], glyoxal oxidase [10], pyranose oxidase [11], veratryl alcohol oxidase [12], etc., and at least two of them are produced by most of the white rot fungi [13, 14]. Lignin removal can also be achieved by

A. Rajan · R. C. Senan · C. Pavithran · T. E. Abraham (✉)
Bioactive Polymer Engineering Section,
Polymer Division, Regional Research Laboratory (CSIR), 695 019
Trivandrum, Kerala, India
E-mail: emiliatea@yahoo.com
Tel.: +91-471-2515253
Fax: +91-471-2490186

Table 1 Chemical composition of coir fiber

Lignin (%)	45.4
Cellulose (%)	43.44
Pectin and related compounds (%)	03.0
Hemicellulose (%)	0.25
Ash (%)	2.22

initial adsorption onto the fungal cells and accumulation within the fungal cells [15]. The fungal mycelium accumulates and decolorizes the lignin molecules [16]. The fungal hyphae can directly metabolize and degrade aromatic and aliphatic molecules.

During progression of traditional retting process, in the saline backwaters for 6–10 months, there is a fall in pectin, pentosan, fat, and tannin contents and practically no loss occurs in the cellulose and lignin contents.¹ Salinity of retting water and the periodical change of retting water is an important condition for the production of good quality fiber [17].

Retted fibers have a smooth surface due to the removal of pectin, exposing the waxy surface layer of the fiber and are less resilient and spinnable. Unretted fibers are resilient and not spinnable. They have a rough surface compared to retted fiber, as pith removal is incomplete.

Research and development efforts have been underway to find new and novel uses for this eco-friendly fiber. Chemical production of whiter coir fiber by removal of lignin with sodium hydroxide and subsequent bleaching of the dark fiber with acid produces a weak thin fiber having reduced strength and the treatment adversely affect the spinning properties. Biological treatment to produce partially de-lignified, whiter fiber will be a better, mild, alternative to this problem.

The present paper deals with the development of a bacterial and fungal system for the treatment of raw unretted coir fiber to produce soft, golden yellow fiber having high elongation, tensile strength, and spinnability. Biosoftening aims to achieve a biopolishing effect with the use of specific microorganisms with selected enzyme specificity toward surface cell wall components.

Experimental

Raw material

Retted and unretted coir fibers collected from Coir Board, Alappuzha were used for the study. Average length—17 cm. Average diameter—250 μm .

Microorganisms used for coir biosoftening

Two organisms *Pseudomonas putida* [MTCC 1194, NCIM, NCL—Pune] and *Phanerochaete chrysosporium* [MTCC 787, NCIM, NCL—Pune] were selected for biosoftening of coir.

Growth media for enzyme production and biosoftening

Vogel's mineral medium consisting of Vogel's salt and trace elements [18].

Four milliliters of Vogel's salt (50 \times), 2 ml of trace elements (100 \times), and 4 g of sucrose dissolved in 180 ml and made into 200 ml.

Coir fibers (20 g) are treated in Vogel's mineral medium containing *P. putida* and *P. chrysosporium* for 20 days. One percent of the microorganism is inoculated into 100 ml of the medium with coir fibers for biosoftening.

Isolation and screening of lignin degrading organisms

Isolation of microorganisms was done from the retting sites in Anchengo area (8°40'N, 76°48'E) in Alappuzha, Kerala. Seventy-six pure cultures were obtained after the initial screening. The organisms were further screened using agar plates supplied with 0.1% lignin (acid precipitated lignin from coir pith) and 0.1% carboxymethyl cellulose as the sole carbon source. Five isolates along with four known strains were selected for further study, based on their ability to grow in lignin-incorporated medium. Sample from the medium containing the microorganisms is centrifuged and the supernatant obtained is used for enzyme activity assay.

Assay of lignin degrading organisms

Manganese peroxidase assay

Manganese peroxidase activity was monitored with phenol red as substrate at 30 °C. The reaction was started by the addition of H₂O₂ to final concentration of 0.1 mM, and was stopped after 1 min with 50 μl of 10% NaOH and A_{610} was measured. Activity was expressed as the increase in $A_{610} \text{ min}^{-1} \text{ ml}^{-1}$ [19].

Lignin peroxidase assay

Lignin peroxidase was measured using veratryl alcohol at 25 °C. The reaction was started by adding H₂O₂ to a final concentration of 0.2 mM and A_{310} was monitored [20].

Laccase assay

Laccase activity was determined by monitoring the oxidation at 420 nm of 500 μM 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) buffered with 50 mM sodium acetate buffer (pH 4.0). Enzyme units are given in moles of product formed per minute ($\epsilon = 3.6 \times 10^{-4} \text{ mol}^{-1} \text{ cm}^{-1}$).

Cellulase assay

Ten milligrams or 0.5 ml of citrate buffer (0.05 M; pH 4.8) was incubated at 50 °C for 30 min and 0.5 ml

enzyme was added. The enzyme assay was terminated by adding DNS. The color developed was measured at 540 nm in UV 2100 spectrophotometer.

Optimization of pH

The two organisms were maintained at pH 4–8 in 0.2 M sodium acetate and phosphate buffer along with the retted coir. Control was maintained at media pH (6.5).

Optimization of temperature

Pseudomonas putida and *P. chrysosporium* were inoculated into medium with retted coir and kept at different temperatures viz. 20, 30, 40, 50, and 60 °C to find out the effect of temperature on the softening of coir.

Effect of inducers

One millimolar of MnSO₄, FeSO₄, CuSO₄, veratryl alcohol, citric acid, guaiacol, vanillin, Tween 80, 1% humic acid, and 0.1% lignin were given as inducers to enhance the removal of lignin for biosoftening.

Effect of carbon source

Sucrose, lactose, citric acid, maltose, dextrose, and glycine were substituted as carbon sources in Vogel's mineral medium.

Chemical treatment of coir

Retted coir fibers were soaked in an aqueous solution of 5% NaOH for a period of 72 h. After the stipulated time, the fibers were removed from the solution, washed with fresh water several times, and finally rinsed with distilled water containing a few drops of HCl to remove any excess NaOH sticking to the fiber surface [21].

Analytical methods

The thickness of retted and unretted coir fiber was measured before and after treatment using an ocular microscope (Brnel), tensile-testing was carried out using an Instron 1195 Universal Testing Machine at cross-head speed of 20 mm min⁻¹. A gauge length of 50 mm was employed for the tensile-testing according to the ASTM D790 test procedure (Instron 1195 Universal Testing Machine, Nottingham, UK). The two ends of the specimen are clamped in a tensile-testing machine and an increasing load is applied until the specimen breaks.

Ultimate tensile strength was calculated by the formula,

$$\text{UTS} = \frac{B/L}{Q.\text{area}} = \frac{B/L}{\Pi d^2} \quad (1)$$

where B/L is the breaking load, d is the diameter of the fiber (mm), $Q.\text{area}$ is the quadrant area.

$$\text{Percentage elongation} = \frac{\text{extension at break}}{\text{specimen length}} \times 100 \quad (2)$$

$$\text{Percentage elongation} = \frac{[(50+x)-50]}{50} \times 100$$

where x is the distance in graph/magnification.

Microscopic studies

The microscopic (Leitz, MPS 15, Brazil) view of coir with attached fungal mycelia was done by staining with lacto phenol cotton blue. The cross sections and surface topographies of treated and untreated fibers were studied using a scanning electron micrograph (SEM) (JEOL JSM-5600 LV, Japan). All specimens were sputtered with a 10-nm thick layer of gold prior to SEM observations.

Brightness measurement

The degree of brightness of coir fiber was measured using brightness meter (color touch model from Technidyne, Edison, NJ, USA) with daylight as the light source.

Results and discussions

Isolation and screening

The organisms isolated from the backwaters in coir retting area were screened by its ability to grow on lignin-incorporated media. The organisms that were able to produce extracellular ligninases were selected, and those producing cellulases were discarded, since the organism needs to preferentially remove lignin from the fiber matrix. Coir fibers (20 g) were treated with the selected microorganisms C-43, C-57, C-70, C-71, BF2, *P. putida*, *P. chrysosporium*, *Aspergillus flaviceps*, and *Trametes hirsuta* for a period of 20 days at ambient temperature (30 ± 2 °C) and at neutral pH. Out of these, *P. putida* and *P. chrysosporium* showed more biosoftening and color reduction compared to the other isolates and were selected for further optimization. MnP activity was found to be more in these organisms, which corresponded to its biosoftening and decolorization ability. The important cultural parameters like temperature, pH, carbon source, and inducers were optimized for these selected organisms.

Optimization of pH

The optimum pH for the growth and lignin removal by *P. chrysosporium* was found to be pH 7.0. The treated coir fibers had a lighter color at pH 7.0 than the control pH. *P. putida* also showed decolorization of fiber at pH 7.0 (Fig. 1). The fiber contains about 40% lignin along with cellulose which gives it a brown color. The partial degradation of lignin brings about a lighter colored fiber. Lignin degradation by *P. chrysosporium* is pH dependent [22]. Even some common buffering agents may inhibit lignin degradation by fungi. The enzyme production and its stability will be more at neutral pH, which is also favoring the preferential degradation of lignin in the coir fiber.

Optimization of temperature

The optimum temperature for growth of the *P. chrysosporium* and *P. putida* was found to be at ambient temperature, 30 ± 2 °C. The growth and attachment of the organism to the coir fiber were more at 30 °C compared to the culture at 20 and 40 °C. Higher temperatures like 40 and 50 °C may act adversely on growth and enzyme production of the organism (Fig. 2).

Effect of inducers

The degradation of lignin by microorganisms is mainly due to the secretion of ligninases or peroxidases. Experiments were conducted to improve the enzyme production by various inducers. One millimolar con-

centration of citric acid, guaiacol, vanillin, Tween 80, veratryl alcohol, CuSO_4 , FeSO_4 , MnSO_4 , 1% humic acid, and 0.1% lignin (acid precipitated polymeric lignin) were given as inducers that could enhance ligninolytic enzyme production. FeSO_4 was found to be the best inducer for *P. chrysosporium* for ligninase production. Fe and Mn are found in the active site of MnP. Veratryl alcohol and guaiacol are substrates for laccase enzyme. Humic acid, a natural inducer, showed maximum enzyme production by *P. putida* and also brought about the degradation of lignin (Fig. 3).

Effect of carbon source

Lignin cannot serve as a sufficient carbon and energy source for its own catabolism by *P. chrysosporium*. The fungi degrade lignin only when supplied with an additional carbon source such as cellulose or glucose [22]. The medium when supplied with sucrose and dextrose showed more whitening of the coir fiber due to the increase in the production of oxidative enzymes. Here sucrose present as the media component was found to be essential for lignin degradation. *P. putida* brought about the removal of color from the coir fiber by using the phenolic compounds as carbon source which was released by the degradation of lignin. The present study proved the earlier reports that higher lignin degradation could be achieved only with cosubstrates such as sucrose and dextrose as carbon source for effective lignin degradation [23–26] (Fig. 4).

Enzymatic lignin degradation

Manganese peroxidase was detected in the culture filtrate of fungal sample treated with coir fiber. It is a component of the lignin degradation system of a basidiomycetes fungus, *P. chrysosporium*. MnP is a Mn(II) dependent extracellular enzyme containing a single protoporphyrin IX prosthetic group and oxidizes phenolic lignin model compounds as well as a variety of other substrates. LiP, MnP, and laccase are the major lignin degrading enzymes and at least two of them are reported to be produced by most of the white rot fungi [14, 28].

Manganese peroxidases cleave most of the bonds linking lignin units and trivalent manganese [Mn(III)] of MnP is a strong oxidizing agent, which reacts with lignin leading to bond cleavage. Although the manganese ion is chelated to a small organic acid and somewhat stabilized, the oxidizing reagent is very small compared with an enzyme molecule and can diffuse into the lignin matrix, attacking bonds quite inaccessible to the active sites of the large proteinaceous lignin and MnP.

The bleaching effect of MnP is probably due to the residual lignin oxidation by chelated Mn^{3+} , generated by enzymatic oxidation of Mn^{2+} [27].

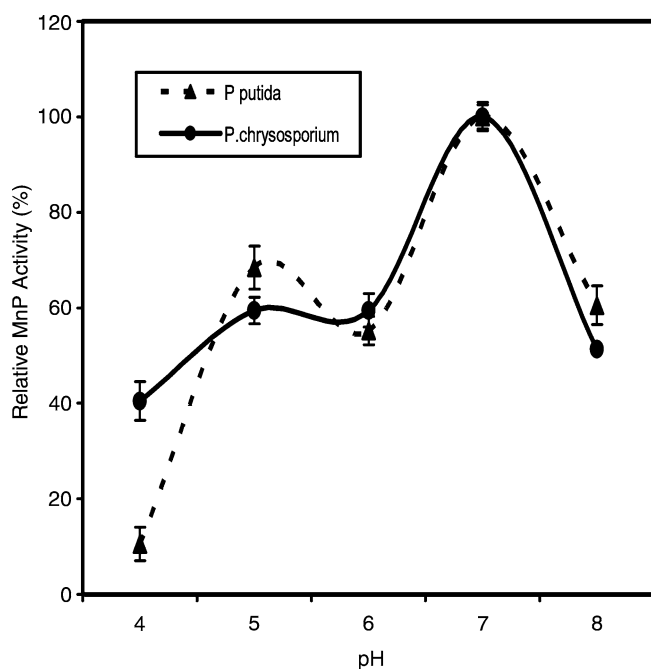
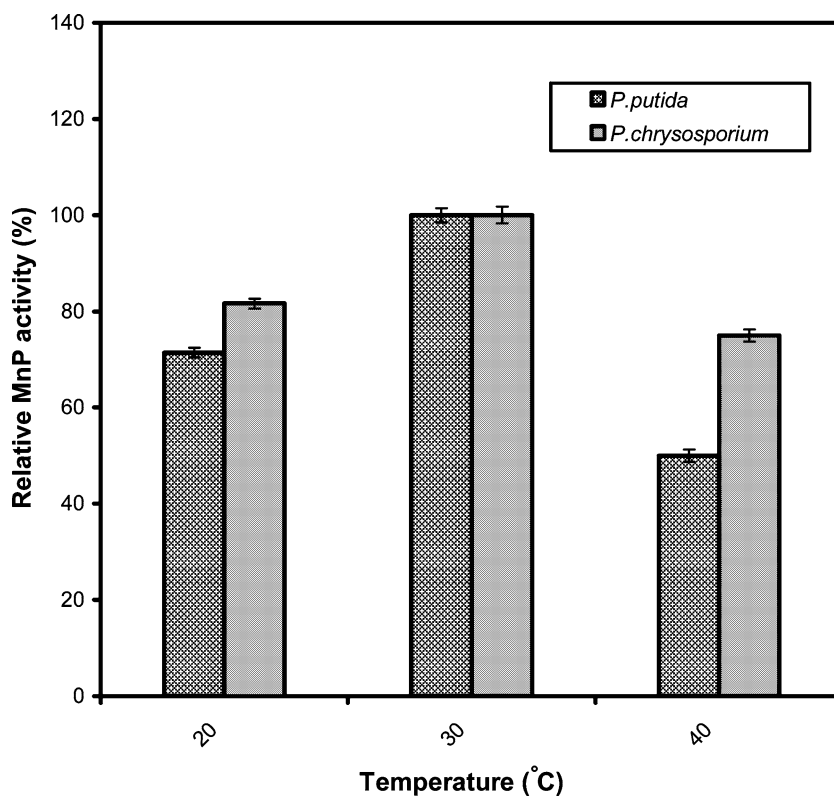


Fig. 1 Optimization of pH for coir biosoftening

Fig. 2 Effect of Temperature on Biosoftening of Coir fiber



Lignin adsorption by *P. chrysosporium*

Lignin does not serve as a sole growth substrate for white rot fungi [22, 28]. Therefore, the fungi degrade

lignin thereby exposing cellulose and hemicellulose for its consumption and to utilize it as a substrate for further growth [29]. Agitation of cultures greatly suppresses lignin degradation by *P. chrysosporium* [30],

Fig. 3 Optimization of Inducers for biosoftening

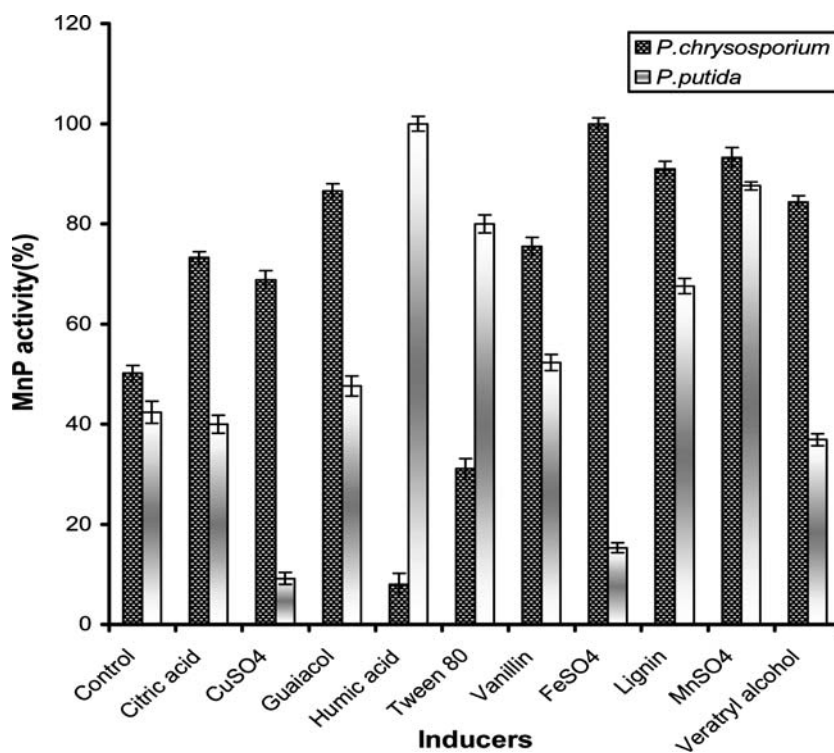
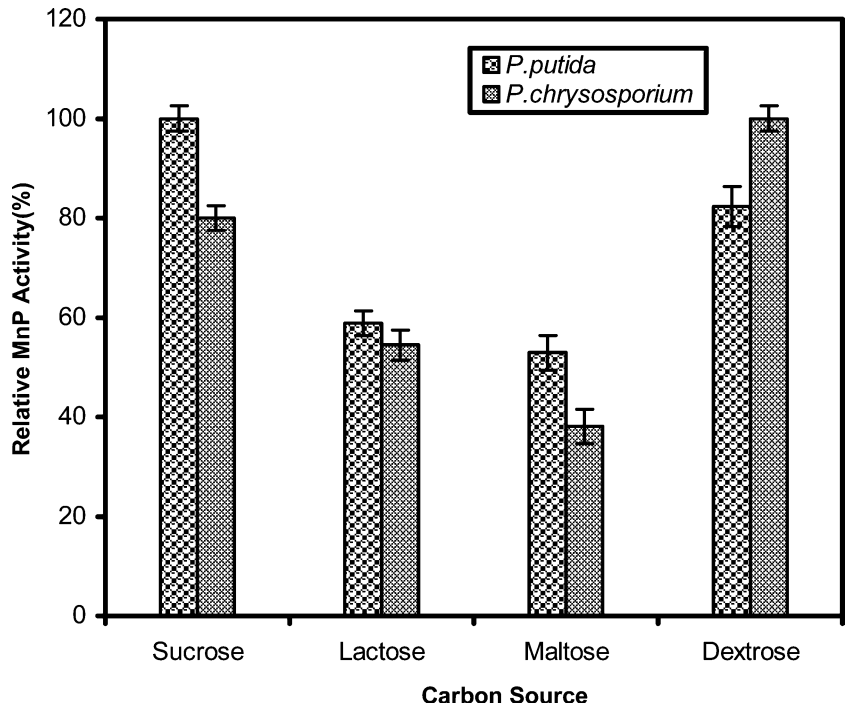


Fig. 4 Effect of Carbon source on Enzyme Production and Biosoftening



which indicates that close contact between lignin monomers and fungal hyphae is required for lignin degradation.

Hyphae get attached to the fiber and selectively adsorb lignin and degrade it to constituent particles. Since the color of fungal cells gets brownish during lignin removal, it shows that lignin is removed by accumulation inside the cells or simple adsorption to the cell surface [15].

Figure 5 shows the penetration of the mycelia through the pores to the lumen of the microfibrils. The pores are formed due to the removal of tyloses (methyl cellulose plugs) which cover the pore on the cell wall.

The microbes remove lignin either by accumulation inside the microbial cells or simple adsorption to the microbial cell surface. *Phanerochaete*, being a filamentous fungus adsorbs the lignin in the mycelia. The mechanism for decolorizing lignin, involves the passive and ionic adsorption of lignin by the mycelial cells which contains chitin and chitosan in the middle phase of its growth [14]. The possible attachment of the fungal mycelia to the coir fiber was examined microscopically (Fig. 5).

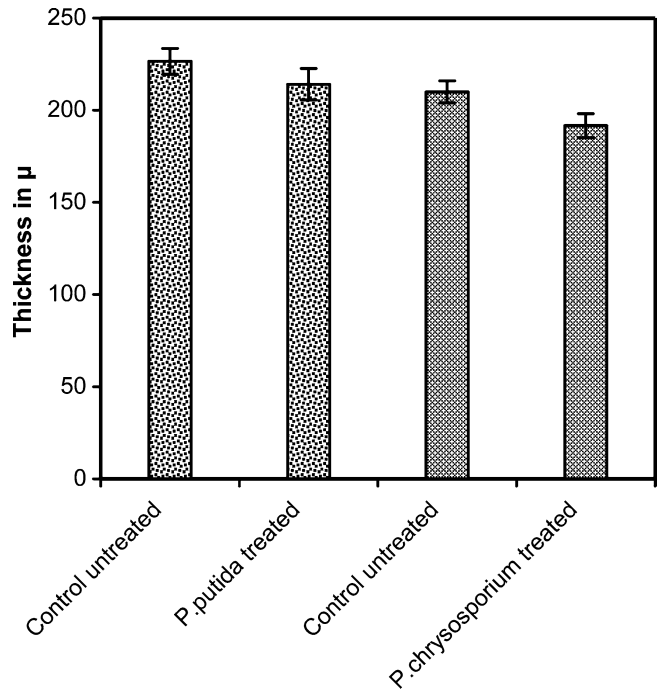
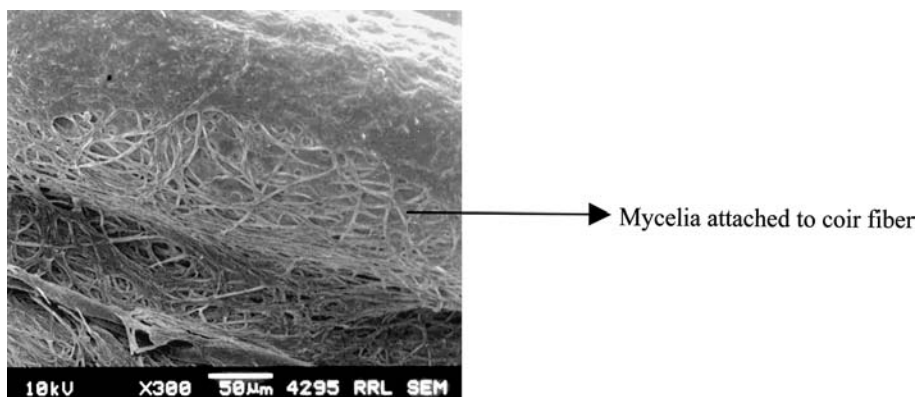


Fig. 5 Effect of Microbial Treatment on Thickness of coir fiber

Table 2 Ultimate tensile strength and elongation of retted and unretted coir fiber

Sample	Retted		Unretted	
	UTS (N mm ⁻²)	Elongation (%)	UTS (N mm ⁻²)	Elongation (%)
Control	163.90	22.40	198.86	34.40
<i>P. putida</i>	199.11	49.60	214.28	44.60
<i>P. chrysosporium</i>	244.90	40.00	270.70	44.80

Fig. 6 *Phanerochaete chrysosporium* attached to coir fiber



Thickness of the fiber

Thickness of the coir fibers was decreased after treatment with *P. putida* and *P. chrysosporium*. Thickness was reduced by 5.2% by *P. putida*, whereas *P. chrysosporium* brought about 8.76% decrease in thickness (Fig. 6). The decrease in thickness is mainly due to the leaching out of the intercellular binding material like lignin and pectin and the removal of cuticle layer. This leads to the smoothing of the fiber and in the reduction in the diameter of the fibers.

Tensile strength of unretted and retted fiber

In the case of unretted coir fiber, the treatment with *P. putida* brought about 7.75% increase in tensile strength, whereas by *P. chrysosporium* there was 36.12% increase in the tensile strength compared to the control sample. The tensile strength of retted coir fiber treated with *P. putida* was 21.4% more than the control retted fiber, whereas *P. chrysosporium* had 49.4% increase in tensile strength compared to raw retted fiber. The comparison is given in Table 2. Chemical treatment of coir fiber with NaOH for 72–76 h increased tensile strength by 15%, and thereafter it showed a gradual decrease. Moreover,

chemical treatment causes dark coloration of fiber compared to enzymatic treatment.

The selective removal of the cementing material will make the compaction of microfibrils by closure of lacuna thereby increasing the tensile strength [22]. Fungal treatment of unretted coir fiber is found to give greater tensile strength to the fiber than bacterial treatment in both retted and unretted coir fibers. This is due to the adsorption of fungal mycelia to the coir fiber and its production of extracellular enzymes like MnP. The increase in tensile strength makes the treated coir fiber more useful than the presently available ordinary retted fiber for a wide variety of applications.

Elongation behavior of coir fiber under stress

Unretted fiber treated with *P. putida* showed 44.60% elongation and *P. chrysosporium* showed 44.80% elongation compared to raw unretted fiber which showed 34.40% elongation. Raw retted fiber used in the study has an elongation of 22.40%, whereas *P. putida* treated fiber had a higher elongation of 49.60% and *P. chrysosporium* treated fiber developed an elongation of 40%. *P. putida* improves elongation of naturally retted fiber. The results show that treatment with either bacteria or

Fig. 7 SEM of cross section of raw coir fiber

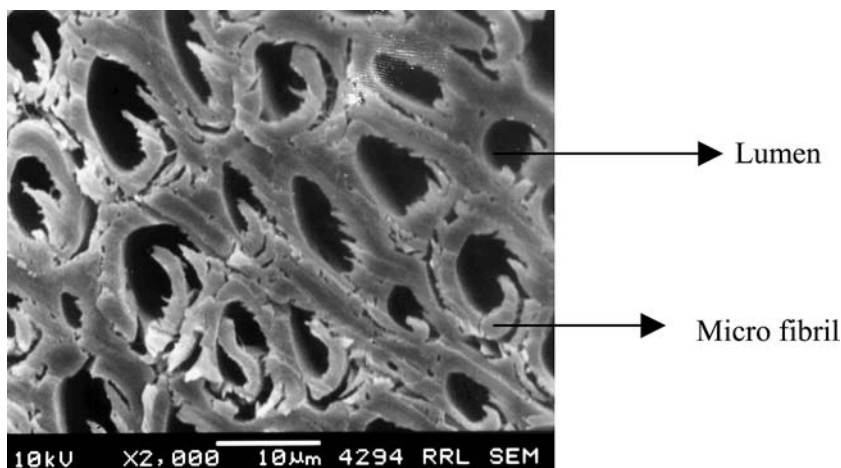




Fig. 8 SEM of biosoftened coir fiber

fungi improves elongation of unretted fiber in the same level (Table 2).

The greater elongation of the fiber improves their stretching property which makes it useful to spin the fiber into fabrics with or without other textile fibers. Elongation is an indication of the ability of yarn or fabric to absorb stretching energy. If the elongation at break of warp yarn is too low, weaving becomes difficult or even impossible. This is the disadvantage of the chemically whitened and thinned fiber, which damages the fibrils.

Microscopic studies

Scanning electron microscopic studies of a cross section of raw coir fiber is shown in Fig. 7.

Cells are crystalline cellulose arranged in a matrix consisting of non-crystalline cellulose lignin complex. The thickness of the outer layer is 3–6 μm . The raw fiber surface is very smooth and tyloses are embedded at regular intervals.

In the case of microbial treated coir fibers, the SEM pictures (Fig. 8) show the exposure of the fibrils indicating the leaching of the intercellular binding material and the cuticle layer.

Brightness measurement

The degree of brightness of microbially treated coir fiber was increased to 10.63 for *P. chrysosporium* and 9.45 for *P. putida* from the control untreated fiber having brightness of 8.91.

Advantages of biosoftened coir fiber

White and soft coir fibers are desirable for dyeing before spinning into yarns. Biological treatment of coir fiber was more advantageous than chemical treatment as it produced soft, whiter, cost effective fibers that have better mechanical strength. Chemical softening is done by leaching of lignin with NaOH under controlled

conditions. Here the fiber gets darkened due to lignin deposition on to the fiber surface and, therefore, require bleaching which will make the fiber brittle. In chemically softened fibers, the strength gets drastically reduced which adversely affect the spinning properties and yarn strength. Biosoftening brings about both softening, thinning, and bleaching of the fiber and avoids the use of caustic chemicals thereby minimizing pollution.

Conclusions

Microorganisms isolated from Anchengo area were screened for biosoftening of coir fiber. The microorganisms selected included five unknown strains and were compared with four known organisms. The unknown strains were C-43, C-57, C-70, C-71, and BF2. The known strains were *P. putida*, *P. chrysosporium*, *A. flaviceps*, and *Trametes hirsuta*. The treatment of the coir fiber was done for a period of 20 days. Most of the Anchengo strains were discarded because of the lack of selective lignin degradation. *A. flaviceps* which produces cellulase make the fiber thin as well as darkens it due to the preferential degradation of cellulose. *P. putida* and *P. chrysosporium* showing more softening of the coir fiber and secretes extracellular MnP was selected. Optimization of physical parameters like pH, temperature, inducers, and carbon sources were done and treatment at this optimum condition gave better results. Neutral pH and ambient temperature (30 ± 20 °C) were found to be the best. Sucrose and dextrose were the best C-source for both *Pseudomonas* and *Phanerochaete*. FeSO_4 was found to be the best inducer for *P. chrysosporium* and humic acid the best inducer for *P. putida*. SEM showed the fungal mycelia penetrating the coir fiber through the pores, thereby selectively removing lignin. The ultimate tensile strength and elongation percentage increased for the microbial treated both retted and unretted coir fibers. Retted coir lost strength and elongation compared to unretted coir. The method is more suitable to treat unretted coir to get a better quality fiber and the fungal treatment is the best giving maximum strength for the fiber, which is suitable for dyeing and spinning. The biosoftened coir fibers can be blended with natural fibers to produce composite yarns for the manufacture of furnishing textiles, fabrics, soft carpets, etc.

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