



Economical and ecofriendly wet processing of coir using 'Trametes versicolor' and Coirret

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ABSTRACT

The possibility of biobleaching and biosoftening of coir yarn in order to reduce the requirement of softening and bleaching chemicals in wet processing of coir was studied in laboratory scale experiments. Treatment of coir yarn for 72 hrs using *Trametes versicolor* (NCIM 993) and Coirret, a bacterial consortium was carried out. The biotreatment could impart an appreciable degree of softness to the yarn and the which was confirmed by the Flexural Rigidity Test. An improvement in softness of the yarn to the extent of 38% using bacterial treatment and 46% using fungal treatment was achieved. An increase of 10% in Brightness Index of the *Trametes versicolor* treated yarn was observed and a reduction in the requirement of bleaching chemicals could also be achieved.

Introduction

Various processes involved in coir processing like dyeing, bleaching, sludge disposal etc are deteriorating the quality of the lakes and other water bodies. The coir fibre and yarn used in coir industry for the production of mats, matting, runners, rugs and carpets are bleached using chemical bleaching methods which use a wide range of chemicals such as peroxides, hypochlorites, chlorites, peracids, sulphites, bisulphites, borohydrides, etc. Adopting biobleaching and biosoftening for wet processing of coir is an alternative to using hazardous chemical treatments. Emulsions of oil, fats and waxes, soaps, sulphonated oil, sulphated alcohols, fatty acid condensation products and quaternary ammonium compounds are the major classes of softening agents. The cheapest method for softening is alkaline treatment, which was effective to a certain extent, but was detrimental to the strength of the yarn. So far, an acceptable chemical softener for industrial use has not been found due to economical as well as environmental concerns. Bio-softening aims to achieve a bio-polishing effect with the use of specific microorganisms, with selected enzyme specificity towards surface cell wall components. The rigid lignin network between fibre cells prevents flexible behavior of the fibre. Softening of the fibre without affecting the fibre strength can be accomplished along with the biobleaching treatment, since the component responsible for the colour and stiffness are one and the same 'lignin'. Biosoftening of unretted coir fibre was more advantageous than the retted fibre. Unlike the weak chemically softened fibre, microbial treatment produced soft, brighter fibres having better tensile strength and elongation properties (Rajan *et al.*, 2005) Biosoftened coir fibre is spinnable and can be blended with other natural fibres like jute, sisal, banana for producing furnishing fabrics, textiles, etc. (Rajan and Abraham, 2007) .

Lignin is the second most abundant natural polymer and forms a matrix surrounding the cellulose, the most abundant biopolymer. Basidiomycetes white-rot fungi are responsible for the most extensive biodegradation of lignin. They have a powerful extracellular enzymatic complex, capable of depolymerizing this aromatic polymer into lower molecular weight compounds. *Phanerochaete chrysosporium* is one of the most characterised white-rot fungus and *Trametes versicolor* has also been studied in details. (Ana Maria *et al.*, 2007).

Trametes versicolor, formerly known as *Coriolus versicolor* is a typical lignin-degrading fungus. Wood pulp biobleaching by lignin degradation has been studied well with this white rot fungus. (Homma, 2007) They produce three main extracellular enzymes involved in ligninolysis; viz. laccase, lignin peroxidase and manganese peroxidase. Though all white rot fungi do not produce all three enzymes, laccase occupies an important place in ligninolysis. (Arora and Gill, 2001) *T. versicolor* or its enzymes have been reported to delignify and bleach kraft pulp and also efficiently dechlorinate and decolourise bleach kraft pulp effluent. (Ana Maria *et al.*, 2007) Bio-softening of the mature coconut husk using Basidiomycetes fungi was faster and more efficient in degrading lignin and toxic phenolics. *Phanerochaete chrysosporium*, *Pleurotus eryngii* and *Ceriporiopsis subvermispota* degrade lignin efficiently without any appreciable loss of cellulose, yielding good quality fibre ideal for dyeing. (Suganya, *et al.*, 2007)

Coirret is a patented bacterial formulation constituting of *Mycoplana. bullata*, *Mycoplana dimorpha* and *Pseudomonas desmolyticum* belong to the family Actinomycetes which possess the ability of degrading phenolic compounds. (Das, A.R., 2001)

The objectives of the present study was to evaluate the biobleaching and biosoftening efficiency of bacterial and fungal cultures on coir yarn and to combine biobleaching and chemical bleaching to check the feasibility of reduction of bleaching chemicals required and thereby reduce surface water pollution.

MATERIALS AND METHODS BIOBLEACHING/SOFTENING

The coir yarn taken for the study was Anjengo yarn, properties of which are given in Table 1.

Table 1 Properties of Anjengo yarn

Yarn Diameter	3 – 6 mm
Length	200 – 300 m/kg
Nature of twist	Hard twist
Quality	Superior

INOCULUM PREPARATION

The pure bacterial cultures of COIRRET viz. *Mycoplana bullata*, *Mycoplana dimorpha* and *Pseudomonas desmolyticum* maintained in the Microbiology laboratory of CCRI were sub-cultured on nutrient agar slants and incubated at 37°C for 5 days to attain the maximum growth. These individual cultures were then inoculated into 200ml nutrient broth and incubated for 72 hours.

Trametes versicolor NCIM 993 was sub-cultured on Potato Dextrose Agar (PDA) slants and incubated at 30°C for 7 days. After seven days, the culture was inoculated into the optimized laccase production media and incubated at 30°C for 4 days. The optimum temperature and incubation period for laccase production by *Trametes versicolor* has been reported as 30°C and 4 days respectively. (Pazarlioglu *et al.*, 2005)

The cell free culture fluid (cfcf) of *Trametes versicolor* was obtained by centrifuging the culture broth in ROTEK RMC– 6 centrifuge and filtering the supernatant through Whatman 40 filter paper.

EXPERIMENTAL DESIGN

The coir yarn was soaked in water (material: liquor ratio of 1:20) maintaining an untreated control. The treatment flasks were inoculated with bacterial and fungal cultures and incubated at 37°C and room temperature respectively. The treatment was continued for 72 hours after which the yarn was carefully taken out of the flask, washed with water and air-dried. The surface of the treated yarn was scanned in the SEM to understand the change in surface morphology. Lignin content of the untreated and treated yarn was estimated. The softness and brightness of the treated yarn was assessed using Flexural Rigidity Tester and spectrophotometer respectively. The fungal treated yarn was chemical bleached and the pH, phenol content, BOD and COD of the bleach liquor were estimated.

Standard recipe for regular bleaching of coir using hydrogen peroxide was adopted for chemical bleaching with a material : liquor ratio of 1:15 and treatment temperature of 85°C for an hour, after which, the yarn was drawn out, washed with cold water and air dried in the shade.

FLEXURAL RIGIDITY ASSESSMENT OF COIR YARN

Flexural Rigidity of the untreated, bacterial treated and fungal treated yarn was tested using the Flexural Rigidity Tester (developed by CCRI). Flexural rigidity test indirectly measures the degree of softness by evaluating the flexural rigidity of the fibre, when subjected to deformation. 25 samples each of raw, control and treated fibre were tied around a PVC pipe of 2 inch diameter to attain the shape of a ring. After 24 hours, the rings were tested using the Flexural Rigidity Tester with and without load (1g) and the ring diameter and deformation of ring on loading was noted. The average radius of the ring and deformation on loading were calculated. The Flexural Rigidity was calculated using the following formula:

$$\text{Flexural Rigidity} = 0.0047 \text{ mg. } (2 \pi r)^2 \cos\theta / \tan\theta \text{ (gcm}^2 \text{)}$$

where, mg = weight of load applied in grams
r = radius of the ring in cm
d = deformation of lower end of ring in cm
 θ = $493d/2\pi r$

SCANNING ELECTRON MICROSCOPIC STUDY OF COIR YARN

The surface morphology of the treated, untreated and control coir yarn were observed using Scanning Electron Microscope (SEM) JEOL JSM 6380LV.

ESTIMATION OF LIGNIN IN FIBRE SAMPLES BY ACID HYDROLYSIS METHOD

One gram of coir yarn/fibre was accurately weighed and cut into small pieces. Weighed coir yarn was packed in a whatman 1 filter paper and refluxed with ethanol-benzene mixture (1:2) in a Soxhlet extraction apparatus for 4 hours. After cooling, the fibre was filtered, washed with ethanol and dried in hot air oven. The dried sample was refluxed with 200ml boiling water for 4 hours in a round bottom flask attached to a Liebig condenser. It was then cooled, filtered and air-dried. The dried sample thus obtained was transferred to a 100ml beaker and carefully macerated into a fine paste with the addition of 5ml 72 % H₂SO₄. Another 20ml 72 % H₂SO₄ was then added to make the final volume 25ml. This mixture was kept at room temperature for 2 hours. The sulfuric acid treated sample was diluted with 580ml distilled water in a 1000ml round bottom flask. Diluted sample was again refluxed for 4 hours. After refluxing, the mixture was cooled and filtered through a sintered G-4 crucible and washed thoroughly to remove all the residual acid. The sintered G-4 crucible with the residues was dried at 105°C in a hot air oven for 5-8 hours for complete moisture removal till a constant weight was obtained. The residue obtained after this treatment is lignin and the lignin content of coir fibre was calculated as follows

$$\text{Lignin content} = \frac{\text{Weight of lignin(in grams)}}{\text{Weight of sample taken}} \times 100$$

(Stephen Y.Lin & Carlton W.Dence(Eds.), 1992)

EVALUATION OF BRIGHTNESS INDEX

Brightness index (TAPPI 452/ISO 2470) of the raw, control and treated samples were measured using Premier Colorscan Spectrophotometer in the visible wave length (360-700nm) range.

PHYSICO-CHEMICAL PARAMETERS OF BLEACH LIQUOR

pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Phenol content of the bleach liquor were estimated using standard methods. (Standard Methods for the examination of water and waste water, 1989).

RESULTS & DISCUSSION

The observations of the biobleaching study are furnished in Fig 1. It was observed that 72 hour treatment with *Trametes versicolor* considerably improved the brightness of the coir yarn. The treatment with bacterial consortium revealed comparatively less bleaching effect. The coir yarn treatment with the cell free culture fluid of *T. versicolor* also displayed the same degree of brightness to the yarn, which confirms the presence of extra-cellular lignin degrading enzymes in the culture fluid.

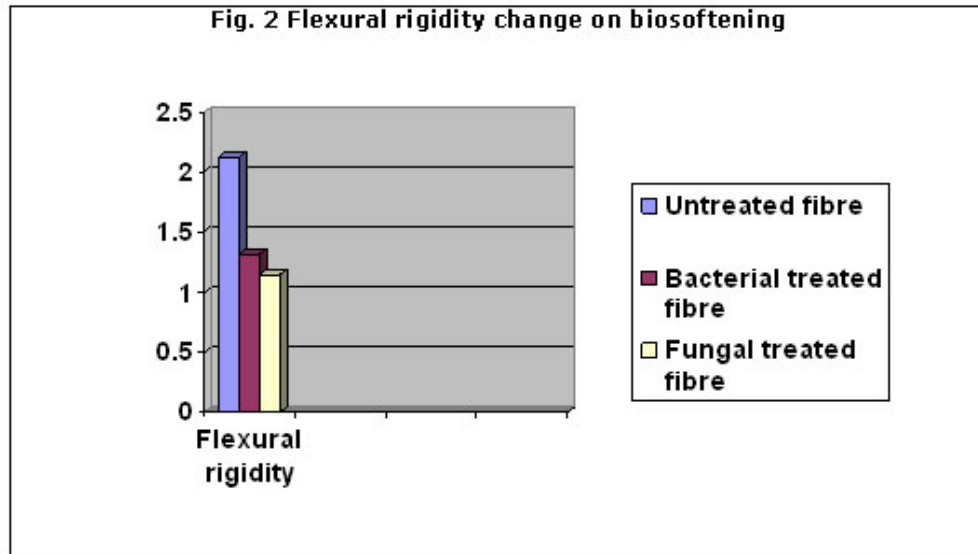
The coir yarn obtained after the chemical bleaching was observed to be rougher in texture than the unbleached yarn. The shade of the chemical bleached yarn was taken as the standard for evaluation of the chemical reduction trials.

Fig. 1 Control (left), Fungal (middle) and Bacterial treatment (right) on coir yarn



ASSESSMENT OF SOFTNESS – FLEXURAL RIGIDITY TEST

Both the bacterial and fungal treatments were found to be capable of imparting softness to the coir fibre. Apart from improved sensory perception in the feel of the fibre, the flexural rigidity test confirmed the softness. FR values obtained were 2.13 gcm² for raw fibre, 1.32 gcm² for bacterial treated fibre and 1.15 gcm² for fungal treated fibre. Almost 38 % reduction in flexural rigidity of the yarn was observed after bacterial treatment whereas the fungal treatment showed a reduction of 46 % without any loss in strength of the fibre. Reduction in flexural rigidity is directly proportional to the improvement in softness. The result can be interpreted as a bar diagram as shown in figure 2.

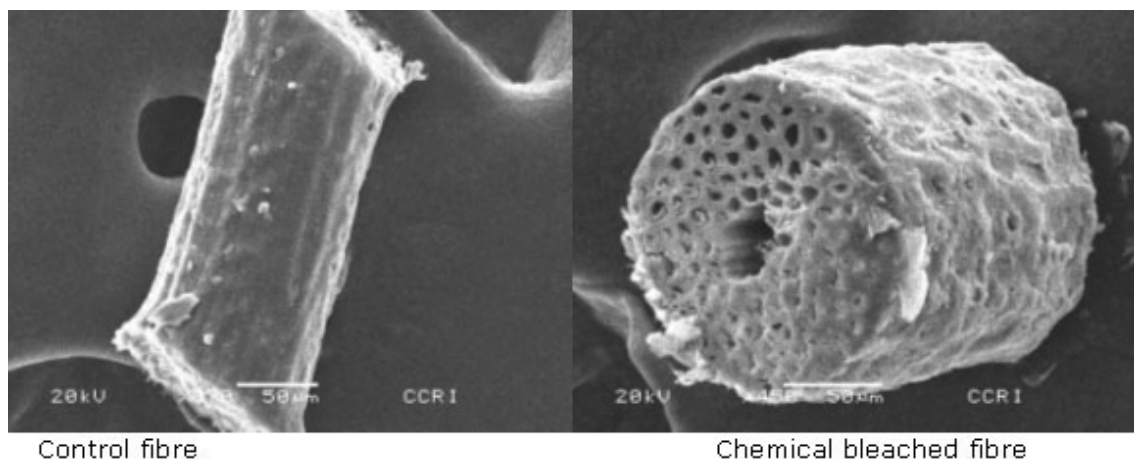


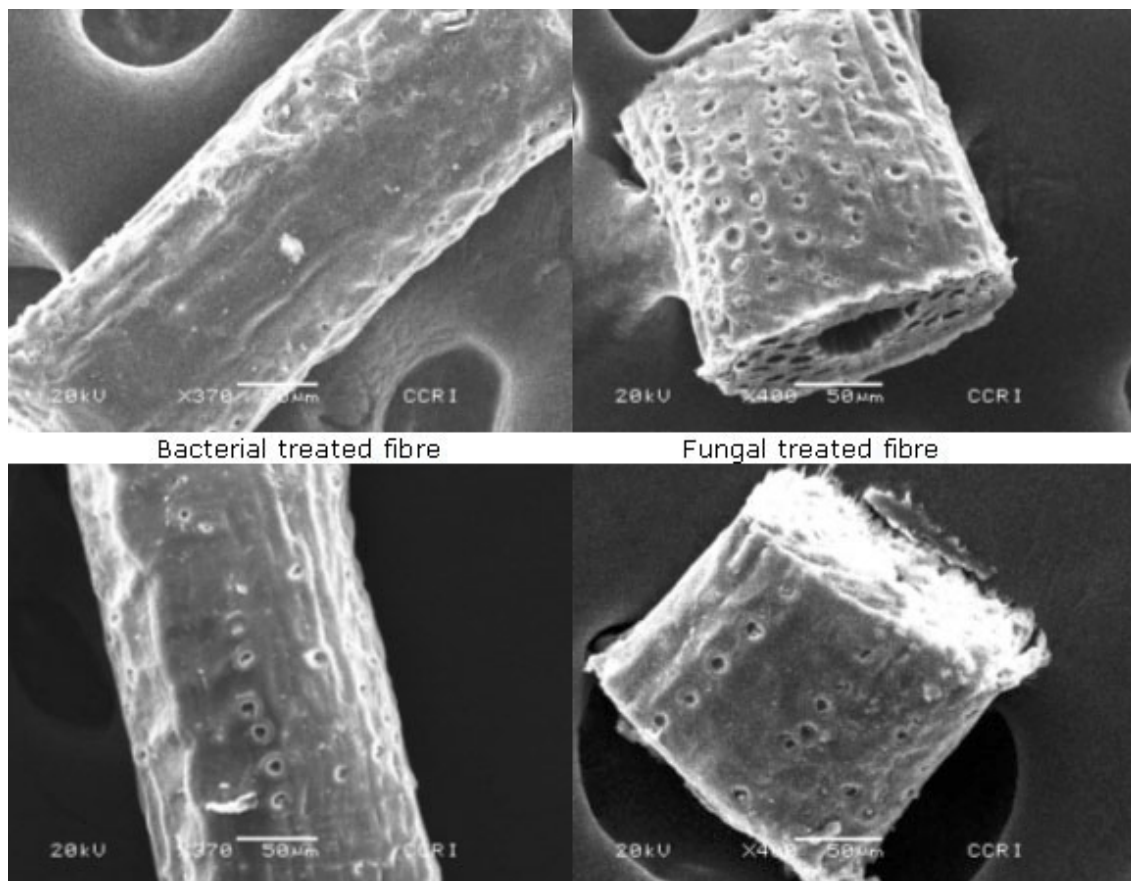
SCANNING ELECTRON MICROSCOPIC STUDY

Samples of untreated, control, biobleached and chemical bleached coir yarn/fibre were scanned at different magnifications to examine the changes in surface structure on bleaching. Details of SEM studies are furnished in Figures 3, 4 and 5. Fig. 3 portrays the normal and cross-sectional view of the untreated fibre. A thin layer of cuticle can be observed all over the surface of the fibre in the case of untreated and control fibres. This layer was absent in the chemical as well as biotreated fibres.

Another important finding was the appearance of pores on the surface of the treated fibre, which was not observed in the control and untreated fibres. The distribution of pores was higher in chemical bleached fibre, followed by fungal and bacterial bleached fibre. This indicated that bleaching modifies the surface topology of the fibre to some extent.

Fig. 3 Scanning Electron Microscope image of Untreated fibre (Normal view & Cross sectional view)





CHEMICAL REDUCTION TRIALS

Bio-bleaching was evident in fungal bleached coir yarn as the brightness was significant over the control yarn. The fungal treated yarn (CRT-A) bleached with the same amount of chemicals was found to be brighter than the chemical bleached yarn. Hence the chemical reduction trials were carried out on the fungal treated coir yarn. Three reduction trials were carried out with 2.4 % , 4 % and 8.8 % reduction in the usage of chemicals. The yarn obtained after the combined bleaching trials retained the same brightness as that of chemical bleached yarn. To confirm the visual assessment, the brightness index of the treated yarn was also evaluated (CRT-B, CRT-C and CRT-D).

EVALUATION OF BRIGHTNESS INDEX

The colour / brightness of the yarn was quantified in terms of Brightness Index using Premier scan Spectrophotometer. Brightness index of the untreated yarn was observed to be 10.093. Fungal treatment increased the brightness index by a factor of 0.97 ie 11.063. Brightness of the chemically bleached yarn was 18.507. The chemical reduction trials (CRT) were assessed in terms of brightness index, so as to retain the same degree of brightness. The brightness index of the fungal treated yarn bleached with the same amount of chemicals was 19.978 which was higher than that of the chemical bleached yarn. The coir yarn after combined bleaching was found to have the brightness index close to that of 18.507, the brightness index of chemical bleached yarn. Data on the brightness index of different samples have been furnished in Table 3.

Table 3 Brightness index of treated yarn

Sample	Brightness Index
Untreated yarn	10.093
Control yarn	9.766
Bacterial treated yarn	9.351
Fungal treated yarn	11.063
Chemical bleached yarn	18.507
CRT - A	19.978
CRT - B	19.197
CRT - C	18.128
CRT - D	18.305

ANALYSIS OF BLEACH LIQUOR

To evaluate the status of the effluent after biobleaching, different physico-chemical parameters of the residual bleach liquor were studied. pH, Biological Oxygen Demand (BOD), Chemical

Oxygen Demand (COD) and phenol content were estimated to analyse the pollution aspects of bleaching. The results obtained are tabulated in Table 4. It was observed that, when compared to chemical bleaching, the biobleach liquor possessed lower BOD, COD and phenol content, thereby confirming that the process leads to reduction in pollution. Combined bleaching or bleaching of fungal treated coir yarn with lesser quantity of chemicals, have considerably reduced the BOD, COD and phenol content of the bleach liquor indicating the advantage of combined bleaching over chemical bleaching.

Table 4 Physico-chemical parameters of bleach liquor

TREATMENT	pH	PHENOL Concentration (mg/l)	BOD (mg/l)	COD (mg/l)
Control	7.65	2.656	38.50	1111.04
Bacterial treatment	7.37	7.584	263.54	912.64
Fungal treatment	4.44	10.784	709.46	1289.60
Chemical bleaching	9.35	74.700	1246.62	7022.40
Combined bleaching	9.60	64.700	844.56	4435.20

Although the use of *Trametes versicolor* for paper pulp biobleaching has been well studied there is no literature available on the application of *Trametes versicolor* for biobleaching and biosoftening of coir yarn. In the present study the biobleaching potential of *Trametes versicolor* on coir yarn was evaluated and the observations confirm its potential for biobleaching and biosoftening of coir yarn.

Flexural rigidity test indirectly measures the degree of softness by evaluating the flexural rigidity of the fibre, when subjected to deformation. Coir yarn treatments with both the bacterial and fungal cultures were observed to impart a smoother feel to the yarn. Softening of 46 % by fungal treatment and 38 % by bacterial treatment was achieved. This was remarkably higher than the softening reported in a study on softening of coir fibre using different chemical softeners where the maximum softening (24.65 %) was obtained using sodium hydroxide. (Anto, et al, 1997) Since the rigid lignin network between fibre cells restricts its flexible behavior, softening might have occurred as a result of the degradation of surface lignin.

SEM studies of coir yarn could help in understanding the difference in surface structure brought about by bleaching. The appearance of pores on the treated yarn is an indication of the removal of lignin bondage between the cellulose strands. The effect of bleaching was greater in chemical bleaching as also the extent of pore formation. However this may not affect the strength of the fibre/yarn, since the degradation takes place only on the surface of the fibre.

In a report on softening of coir yarn conducted at CCRI, caustic soda has been used as the softening agent. Even though caustic soda treatment was found to be quite effective in producing the crimp on the fibre, the colour of the fibre was changed into deep brown. The effluent treatment was another big problem to tackle as the residual alkali needed to be neutralized with huge quantities of inorganic acids making the process hazardous and uneconomical. Therefore, it was not accepted by the coir industry.

(Sarma, 2001). Thus biosoftening of coir yarn has a better edge over chemical softening in the aspects of pollution control and cost. In biological treatment, the toxic lignin degradation products like phenols are not only removed from the coir but are consumed by the microorganisms, thus reducing the toxicity of the effluent. This in turn reduces the COD, phenol content and other pollution parameters of the effluent.

The present study indicates that the pre-treatment of coir yarn with *T. versicolor* followed by chemical processing could be an economical alternative for bleaching in the coir industry. The wet processing technology, which may be adopted by this combined bio-chemical bleaching, will also lead to the reduction in pollution load released as effluent to the water bodies.

The salient findings of this study pave the way to the development of an eco-friendly process for biobleaching and biosoftening that can be adopted in the wet processing of coir.

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