# Application of Microorganisms to Enhance Biodegradation of Phenolic Compounds and to Improve Retting of Colr

Anita Das Ravindranath\* and U.S. Sarma
Central Ceir Research Institute, Kalavoor, Alappuzha 688 522, India
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The coir industry provides a livelihood to the ruml sector, especially to women in Kerala in South India. An uninterrupted supply of the raw material, "the coir fibre", is essential to ensure regular occupation for those engaged in the production of coir. This paper reports a study on the phenolic compounds released during retting of coir by thin layer chromatography and high performance liquid chromatography techniques. Inoculation of phenol degrading strains of bacteria during retting is observed to yield fibre of superior quality within 72 h. Retting of coir can therefore be carried out in tanks resorting to biotechnological methods instead of the conventional practice of steeping coconut husks in the backwaters of Kerala. This would prevent the backwaters from being contaminated with polyphenols like tannins, melanin, catechines, sharoles and quinores which are released from the husks and eliminate environmental pollution.

## Laroduction

Coir is a natural hard fibre extracted from the mesocary of the coconut and supports the small scale industry based in Kerala in India. This industry generates employment to the rural population and mostly women are engaged in the retting and spinning of coir.

# Reiting

Coconut husks are steeped in the backwaters for "reving", a process which extends from 9 to 11 months. The husks being lignocellulosic in nature release polyphenols into the backwaters, creating a disturbance in the ecosystem. The details of a study conducted on the environmental parameters at three typical retting sites after one month and five months of steeping of husks are given in Table 1. The pH of the environmental waters lowered from neutral to the additio range, indicating the release of acidic substances which include polyphenols and pectins. The HOO (brochemical oxygen demand) is an empirical test to determine the relative oxygen requirement of material 4 wastewaters. The test measures the oxygen utilized during a specific incubation period for the

biological degradation of organic materials (carbonaceous demand) and the exygen used to exidise inorganic material. The BCD levels were observed to increase with the progress of retting.

The steeping of coconut husks lends to the deterioration in the quality of the backwaters affecting the aquatic life<sup>3</sup> and therefore extraction of coir fibre by any alternative method would provide a solution to the pollution caused by retting.

A step towards mechanization of the coir industry has led to the development of de-fibering, decorticating and combing machines which can provide an

Table 1 — Environmental factors recorded during the sampling of busk from three retting grounds in Quilon District of Kerala

Site and Month of Retting	pŀí	H.O.D. mg/L	Polyphenols µg/ml,
Thrikkadavur, 1	7.5	0.805	14
Murundal, 1	7.0	2.86	12
Kottakkakam, I	7.5	3.51	S
Thrikkadayur, 5	6.0	7.6	3.72
Murundal, 5	6.5	77	1.26
Kottakkakam, 5	6.5	7.7	2.44

<sup>\*</sup>Author for correspondence

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internative to the conventional "retting" of the husk. This method of extraction could yield coir fibres without retting. However, the quality of the mechanically extracted fibres was inferior to the retted fibre in that it lacked a consistent colour and had a harsh teel, which made it unacceptable to the coir workers.

It was then envisaged to treat the fibre with polyphenol degrading bacterial strains which could remove the polyphenolic substances which are photosensitive and tend to reattach to the fibre, yielding a fibre of inferior quality.

#### Materials and Methods

The polyphenolic substances in the leachates from coconut husk were analysed using TLC and HPLC techniques. Samples of ret liquor were drawn out at different intervals of soaking and chloroform extracts of the samples were spotted on silica gel piates together with standard compounds. The solvent system used for the separation of the compounds were Benzene: Dioxane: Acetic Acid (90: 25: 4) and Benzene: Ethyl Acetate: Acetic Acid (85:15:1). The identification was carried out by spraying with tetrazotized benzidine and exposure of the plates to iodine vapours.

The HPLC analysis was carried out using a Beckman System Gold with a 250 × 4 mm column packed with Viosfer C-8 support. The mobile phase consisted of acetonitrile and water (20:80) and the flow rate was set at 0.5 mL/min. The detector was set at 235 nm in the UV range. The standard compounds used for the study were catechol, resorcinol, pyrogallic acid, tannic acid, ferulic acid, syringic acid and vannilic acid.

#### Experimental

The coir fibre used for the study consisted of one tonne mechanically extracted fibre from mature green husks and baled into bundles of 35 kg each. The bacterial consortia used for the study was 'Coirret'. The microorganisms constituting 'Coirret' belong to the Actinomycetes group. They are Gram negative cells, 0.1 to 0.5  $\mu$ m wide and 1.25 to 4.5  $\mu$ m long in size. The cells are motile by means of subpolar tufts of flagella and possess the property of utilisation of 0.01 to 0.05% of phenolic compounds. The stock culture in saline containing 210  $\times$  10<sup>4</sup> cells per mL was used as inoculum for the development of 'Coirret'. Six kilograms 'Coirret' was treated on the

coir fibre soaked in water in RCC tanks for 12 h. The treatment period was 48 h, after which the fibre bundles were allowed to stand in tap water for a further period of 12 h. The treated fibre was then dried and processed into coir yarn which was subjected to the evaluation of light fastness test on the Xenotest.

# Results and Discussion

The Rf values of 'Coirret' liquor in TLC were 0.31, 0.40 and 0.96, which corresponded to that of resorcinol (0.40) and pyrogaliic acid (0.96). Six peaks at retention times of 2.75, 3.5, 4.09, 5.65, 5.33 and 6.9 minutes were obtained when Coirret liquor was injected into the HPLC. The RT of standard catechol was 3.48 min which is close to that of one peak (3.5 min), indicating its presence in the ret liquor sample. Thus, the major polyphenols identified by TLC and HPLC were resorcinol and catechol.

The observations of the lightfastness test conducted on the Xenotest are furnished in Table 2. The lightfastness rating of the yarn sample from 'Coirret' treated fibre was equivalent to that of the yarn sample from fibre extracted by conventional practice of retting. It is therefore evident that inoculation of polyphenol degrading bacterial strains could result in the removal of soluble photosensitive polyphenols

Table 2 —	- Lightfastness studies or samples of coir	n 'Coirret'-treate)		
Test Method Gesellschaft	Quarzlampen	G.Mbh, HANAU FRG, Light and		
Humidity	70 %	70 %		
Temperature	37 °C	37 °C		
Test	1006 1955	g to de la company de la compa		
Sample	Description	Grade*		
٨	Yarn from untreated fibre	1		
B	Yam from 'Coirret' fibre	II		
С.	Yarn from conventionally retted fibre	1;		

\*Grade I — Fading within 30 minutes of test expressive Grade II — Fading within 145 minutes of test expressive

them from redeposition onto the fibre. This leads to the production of a fibre of reflect quality in a period of 72 h, as against the conventional process of retting which extends from 9 to 11 months. The whole process of the retting of fibre was carried out in RCC tanks which eliminated the apprehensions of pollution caused by traditional retting methods.

### Conclusion

'Coirret' can be applied to mechanically extracted green husk coir fibre to yield fibre of a quality comparable to that obtained by conventional retting reethods. With the adoption of the biotechnological techniques in coir fibre treatment the coir industry can make not only a clean and profitable but also a non-polluting retting process.

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