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Research paper

A Comparative Study on Chemical and Biological Methods for Partial Degradation of Lignocellulose in Coconut Coir Fibers

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Abstract

Coir bristle fibers were known for their lignin content and hardness level; they are commonly used in floor mats and traditional ropes. This research proposes the application to soften and partially degrade the coir fibers through an eco-friendly approach, providing a more sustainable alternative to chemical treatment. The coir bristle fibers were pretreated, washed, and sterilized mechanically to eliminate surface contaminants. The organism that exhibited the property of lignocellulose degradation was further considered throughout this research. The isolated and highly efficient strains were subjected to biochemical characterization followed by their treatment against the identified bristle coir fibers. Based on the incubation time, the post-treatment included neutral washing and drying of the fibers, and a comparative analysis was performed among the two identified strains. The *Bacillus, Pseudomonas spp* were cultured and inoculated into the fibers under controlled conditions, and based on the degradation results obtained, *Bacillus spp* showed better results than Pseudomonas spp. Hence, *Bacillus spp* was considered for a high-throughput technique as a 16S RNA sequence to arrive at species-level identification. This eco-friendly treatment lowers the environmental impact of fiber softening and enhances the physical characteristics of coir bristle fibers for advanced applications. The new method shows a promising route to the biotechnological valorization of coir and paves the way to integrate coir into biodegradable composites and sustainable textiles.

Keywords: *Coir Bristle Fibers; Bacillus SPP; Pseudomonas SPP; Chemical Degradation; Biological Degradation.*

1. Introduction

Generally, Lignocellulose biomass comprises three main components: cellulose (60%), hemicellulose (17-32%), and lignin (10-25%) [25-27]. Lignocelluloses are among Earth's most abundant renewable biomass, as mainly the solid wastes from rice husks, straws, stalks, coconut coir, etc, are rich in hemicellulose. Burning and discarding of these solid wastes are problematic to nature in one way or another. Hence, to overcome this issue, an emerging microbial approach is employed to mitigate global warming while repurposing agricultural and industrial wastes [28], [29]. Lignin is closely associated with hemicellulose molecules by covalent and hydrogen bonds, making the structure highly resistant to enzymatic and microbial hydrolysis [30]. Hence, exploring and developing an efficient lignin degradation technology has recently become the research hotspot [31]. By considering the repurposing of coconut coir fibers without a burden to the environment, this research attempts to benefit society.

As the earlier reports have noted, coir (coconut coir) is a lignocellulose material containing hemicellulose (0.25%), cellulose (43.44%), and a substantially higher percentage of lignin (35-45%) when compared with other fibers as reported [32]. The bristle coir fibers were considered one of the coconut palm's by-products. *Cocos nucifera L.* (Aracaceae) was considered for this research due to its significant value to humanity, dating back centuries. India tops the world coconut map with an area of nearly 1.23 million hectares. India is the largest producer, with 13.968 million nuts compared to the world production of 53.598 million nuts [15]. Coir fiber belongs to the seed fiber group and is extracted from the mesocarp of coconuts. It is hard and stiff fiber [1]. Coir is a natural fiber that holds lignocellulosic material, which is responsible for being stronger and smarter during water absorption. It stands neutral in the emission of carbon dioxide [11]. India produces about 3, 09,000 tons of coir every year. They were commonly used as brushed door mats, agriculture wire, and geotextiles, thanks to their durability, potential biodegradability, water resistance, and furry texture [5].

The dark brown fiber is obtained from matured/ripened coconut shells. Likewise, the white coir was harvested from immature/unripe coconut shells. On the other hand, it comes from coconut shells harvested just before they are ripe. This light brown or white fiber is softer and less intense than brown coconut fiber. Another classification method is based on the length of fiber. Both brown and white coir consist of fibers that are 4 to 12 inches long (10-30 cm). Those at least 20 cm (8 in) long are called sows. Shorter fibers, which have a finer structure, are called mattress fibers. About 300 g of coconut shell provides approximately 80 g of fiber, a third of which is brush bristles.

Coconut fiber is the only natural fiber resistant to saltwater nets for gathering shellfish and ropes at sea. Coir yarn is strong and almost weatherproof. American hop growers prefer to tie their vines' supports [10]. High water resistance, i.e., 6-8 times its weight; Slow decomposition due to high content of lignocellulose; excellent moisture binding ability even after drying; high porosity, retains and releases nutrients for a long time; acceptable electrical conductivity (EC) pH and cation exchange capacity (CEC), Better physical flexibility that can withstand pressure better, A poor heat conductor helps keep the soil temperature under control [17].

Coir is the by-product obtained from the coconut industry and is renewable with its resource-elastic, cellular, cork-like material that forms non-fibrous tissue. The percentage of coconut shell fibers in total weight of the shell is usually 50-60%. A ton of coconut kernels is produced for every 10,000 shells used in the coconut industry. Accumulation of coke in large quantities near coir factories creates solid waste pollution problems; hence, it consists of lignocellulosic compounds. Decomposing these compounds was once considered an impossible task [20].

The microorganisms available in the soil can transform organic matter into CO_2, biomass, thermal energy, or humus-like end products. The main components of organic matter are carbohydrates, lipids, and lignin. These microorganisms can assimilate the organic matter depending on their ability to produce an enzyme for degradation [18]. There are several lignin degraders in fungal species, such as white rot fungi, most of which are basidiomycetes. These fungi mainly remove the outermost lignin and cellulose, leading to further coir degradation. Apart from these fungi, some bacteria were also found to be involved in the degradation of coir. These bacteria can produce lignin-degrading enzymes, such as lignin peroxidases, which aid in coir degradation [4].

2. Materials & methods

2.1. Material collection

The raw material, such as bristle coir fibers, was collected from a coconut farm at Karunya Nagar, Coimbatore, Tamil Nadu, India. The raw coconut obtained was found to be a mixture of both white and brown fibers, including husk. Only the brown mature bristle fibers were separated from the mixture by hand picking and transported to the laboratory in a sterile container. Similarly, soil was collected from the same field where the coconut shell was dumped, and the microorganisms were isolated from it by the serial dilution method.

Soil was serially diluted; the dilutions of 10^{-4}, $10^{-5,}$ and 10^{-6} were considered for processing. About 1 mL of respective dilutions was spread onto the nutrient agar plates and kept for incubation at 37° C for 24 h. Then the obtained organisms were further screened in selective media as soybean casein digest media, ISP5, and Nutrient media. after 24 h the plates were stored at 4°C. These organisms were inoculated onto Soybean Casein Digest media, ISP 5, and nutrient media, to find out the positive selective media for isolated microorganisms. Based on the colony morphology, about four colonies were identified, subcultured, and stored for future research.

2.2. Biochemical tests

Table 1: Biochemical Characterization of Microorganisms Isolated from Soil Samples

S. No	Tests Performed	References
1	Indole production test	26
2	Methyl red test	26
3	Voges proskauer test	2
4	Citrate utilization test	1
5	Catalase test	22
6	Starch hydrolysis test	14

2.2. Pre-coir treatment

Coir fibers procured from the local farm were thoroughly washed with sterile double-distilled water and then acetone. It was dried and conditioned in the hot air oven. The coir fibers were then stored in an airtight container until further use. The bristle coir fibers were subjected to chemical treatment with 5% NaOH solution (Chemical treatment) and kept undisturbed for 7 days. Analytical grade sodium hydroxide was procured from HiMedia, India. On the other hand, bristle coir fibers were buried a few centimeters below the soil level (biological treatment) and left undisturbed for 7 days. After 7 days of incubation, all samples were taken out and washed thrice with distilled water to remove the debris present in the fiber. They were then dried and cut into small pieces with a length of 1 cm each [14].

The Nutrient (NB) and Vogel's broth (VB) were prepared according to the standard procedures. About 10 mL of NB and VB were transferred into to first series of test tubes as set A, B, C, D, E (both comprise two tubes: 1 for NB and 1 for VB). Set E is considered as control. Likewise, about 10 mL of NB and VB were transferred into to second series of test tubes as Set AA, BB, CC, DD, EE (both comprise two tubes: 1 for NB and 1 for VB). Set EE is considered as control. To each tube 1cm (1gm) of chemically treated coir in A, B, C, D & E, respectively. To another set of tubes, AA, BB, CC, DD, EE, 1 gm of biologically treated material was added, along with 1 mL of *Bacillus spp* was added to all tubes except the control. During incubation, the tubes with coir samples were carefully observed for degradation. It was checked at a regular interval of 5 days and kept for 20 days of incubation.

2.3. Post coir treatment

The bristle coir fibers were, after pre-coir treatment (20 days), alkali and soil treated, and then washed with sterile distilled water. The dried coir specimens were primarily subjected to visual observation for any changes, weight reduction, softness, etc. The specimens with changes were further subjected to SEM to get high-resolution images [6].

2.4. Determination of lignin content

The grounded sample (pulp) was treated with sulfuric acid (two-step hydrolysis process) to dissolve its carbohydrates. The residue was filtered, dried, weighed, and considered as an acid-insoluble lignin. A minimal quantity of the lignin dissolved during acid hydrolysis was called acid-soluble lignin, determined by measuring the absorbance at 205 nm of the filtrate from the acid-insoluble lignin determination. The total lignin content arrived at as the sum of acid-insoluble and soluble lignin (ISO 21436/2020)

2.5. SEM analysis of coir degradation

Both alkali and soil-treated coir fibers were subjected to SEM analysis to reveal the topography of Bacillus and Pseudomonas spp, and after partial degradation of lignin in the specimen. The images of 100µm, 20µm, 10µm, and 5µm are to be obtained for correlating the results obtained from this research.

3. Results and discussion

3.1. Isolation of microorganism

The grown-up organisms were subcultured onto nutrient agar plates to obtain pure culture. The culture was found to be distinctive. About two colonies were found to be white, and it is labelled as 1 and 2. The third colony was pink in colour and labelled as 3. Similarly, the fourth colony was found to be yellow and labelled as 4 and shown in Fig. 1. The colonies used for the treatment process and based on their activity, Bacillus and Pseudomonas spp were considered throughout this research.

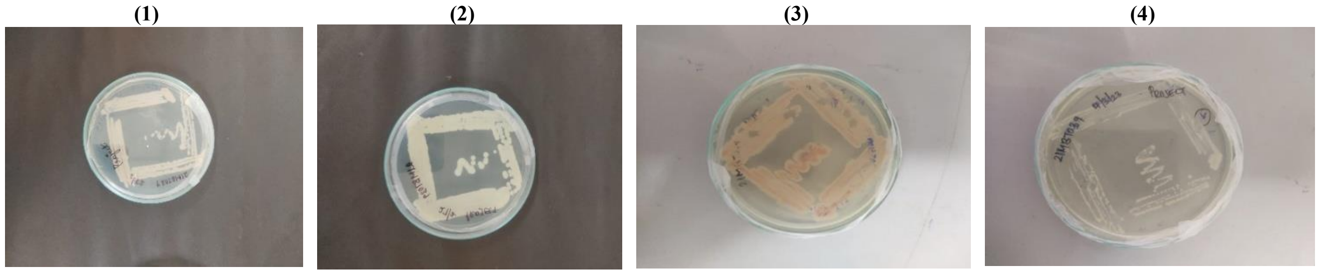

Fig. 1: Visualization of Isolated Colonies Based on Their Morphology and Appearance.

3.1.1. Inoculation of organisms

After inoculation of two selected strains in selected media as Nutrient and Vogel's mineral broth. The samples were incubated in an incubator shaker at 37ºC for 20 days. The organisms inoculated were found to grow within the period of 48 h. The colour of the control in nutrient broth was changed to red colour the other tubes were found to be yellow. The observed colour change was due to the reduction in pH; an increased concentration of the hydrogen ions resulted in the change in pH. Increased hydrogen ions indicated the presence of acidity. It may be due to the reaction between the media and coir in the presence of an organism. Both alkali and soil-treated samples showed no change in pH. The *Bacillus spp. showed better results in the degradation of lignocellulose among* the four isolated strains.

3.1.2. After treatment

After 27 days of pretreatment and incubation, the samples were removed. The coir fibers were washed with distilled water to remove the traces of microorganisms and dried. The colour change was observed in alkali-treated samples, from dark brown to golden yellow. The soil treated sample was found thickened and available in dark brown colour. During drying, the visual differences in all the samples were examined. It was found that NaOH-treated samples soften the fibers when compared with both soil-treated and control samples, as represented in Figure 2.

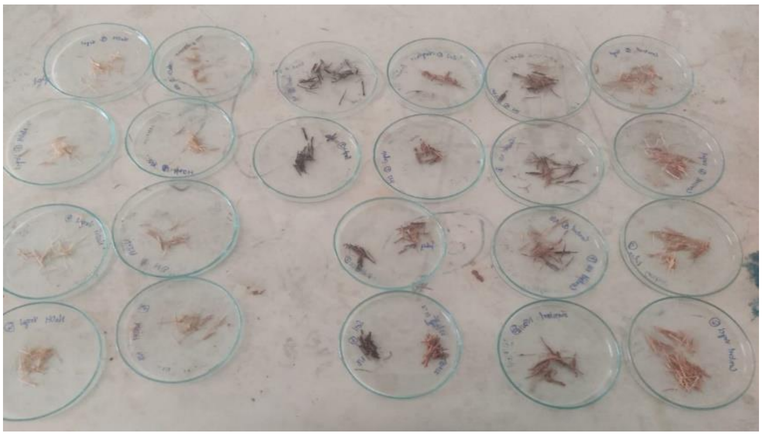

Fig. 2: Observation of Dried Coir Specimens After Incubation.

3.2. Biochemical characterization

Table 2: Biochemical Characterization of Bacillus SPP and Pseudomonas SPP

S. NO.	TEST NAME	*Bacillus cereus*	*Pseudomonas spp*
1)	Indole Production Test	Positive	Negative
2)	Methyl Red Test	Positive	Negative
3)	Voges Proskauer Test	Positive	Negative
4)	Citrate utilization test	Positive	Positive
5)	Catalase test	Positive	Positive
6)	Starch hydrolysis test	Positive	Negative

Based on the positive results obtained against the coir degradation, the two microorganisms were characterized through biochemical tests, listed in Table 2, followed by 16S RNA sequencing. The *Bacillus cereus* strain was biochemically characterized and also performed 16S RNA sequencing, and the same was submitted to the NCBI as PQ273208.1

3.3. Determination of lignin content

The percentage of both acid-insoluble and soluble lignin available in the coir sample was found to be 26 and 23%, respectively. After the treatment process, the presence of lignin was reduced by 23 and 21%, respectively, in alkali and soil treated samples.

3.4. Characterization of coir degradation

The alkali-treated specimen was found to be softened during physical and microscopic analysis. The sample was then subjected to analysis under higher resolution with Scanning Electron Microscopy (SEM). The SEM analysis was conducted with fewer than 100, 10, and 2µm sizes to determine the morphological and porosity changes [22].

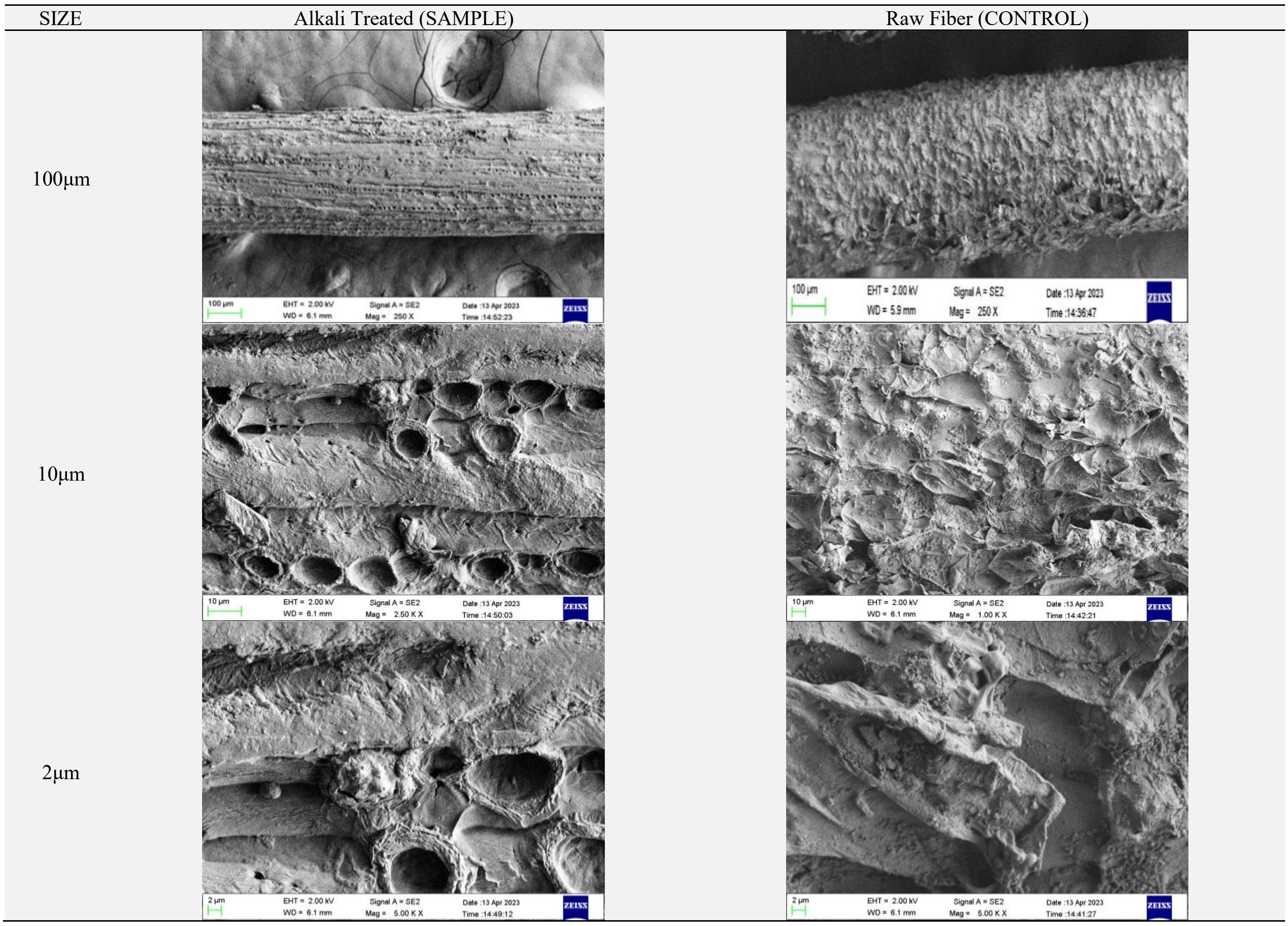

Fig. 2: Characterization of Alkali Treated Fiber and Raw Fiber (Control) at 100, 10, and 2µm.

The SEM analysis of chemically treated samples had shown a pore-like structure on their outer surface. The control does not have any pore-like structure in the outer layer, and it was observed with a thick, uneven surface. However, the alkaline-treated samples have smoother outer faces, and the exterior layer was removed. This will give the microorganism more penetrating capability into the sample for degradation, in the future [16]. When compared with the control, the 100 µm of alkali-treated indicated topographical variations greater than the control. To validate further, the fibers were scanned under 10 µm and 2µm, represented in Fig. 2. Both of them revealed the presence of irregular and less porous, hollow structures in it. The SEM image indicated that the degradation occurred due to its presence it in alkali reaction alone for about 27 days. Typically, the alkali treatment helps in removal of lignin, hemicelluloses, and exposes the cellulose fibers, thus increasing the surface roughness, to ease the future surface reactivity through other treatments.

The biological-treated and *Bacillus cereus-treated* sample had a softer outer surface, and it became accessible for the organism to go deep into the layers of the coir [19]. When compared with the control, the 100 µm showed topographical changes it than the control. To validate further, the fibers were scanned under 10 µm and 2 µm, represented in Fig. 3. Both of them revealed the presence of regular, numerous, and intense porous, hollow structures in it. The SEM image indicated that the degradation occurred due to the reactions of alkali alone for about 7 days. After alkali treatment, *Bacillus cereus* was added, acting as catalysts, promoting oxidation/reduction or microbial degradation with favorable environmental conditions.

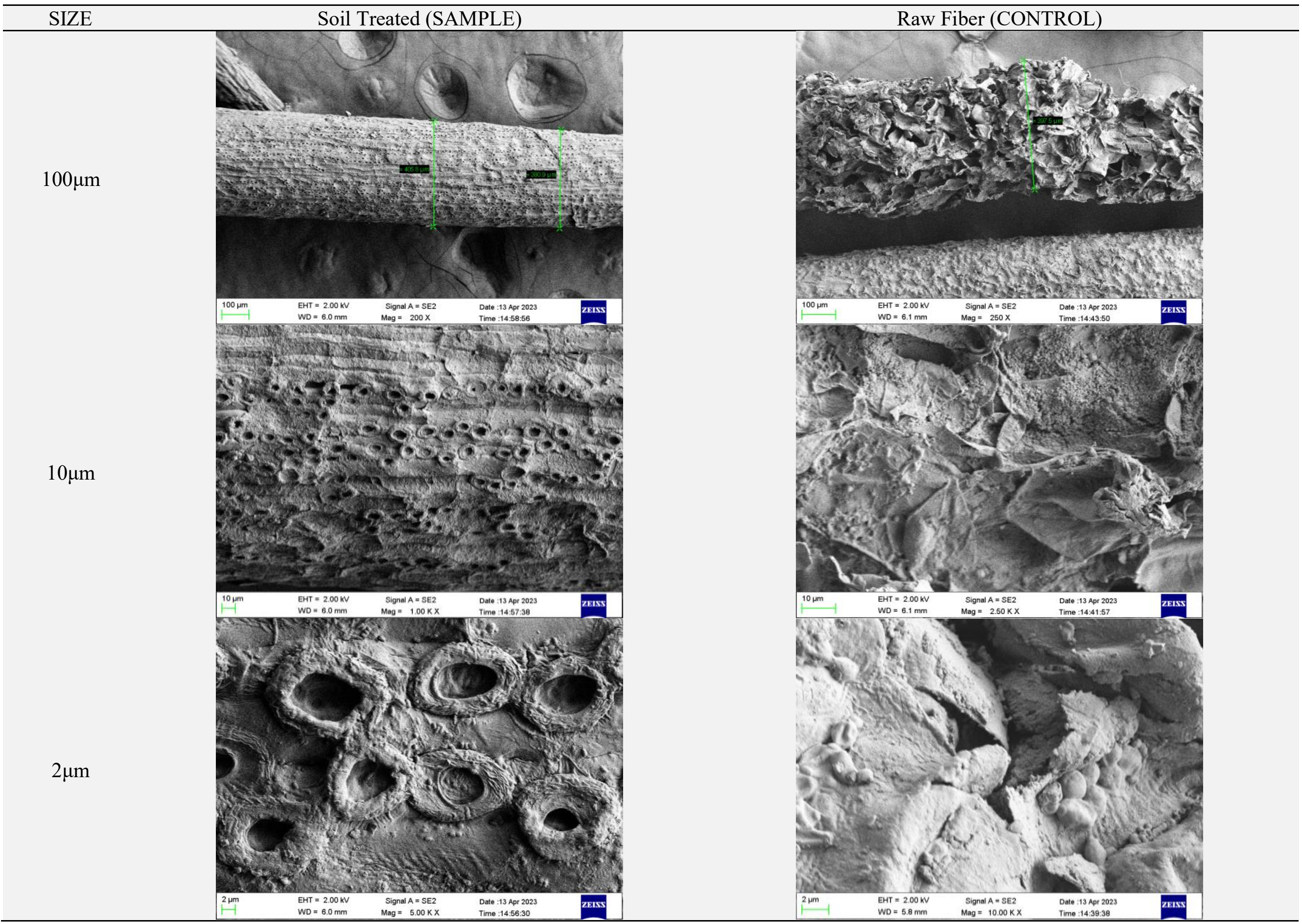

Fig. 3: Characterization of Alkali, *Bacillus cereus* Treated and Raw Fiber (Control) at 100, 10, and 2µm.

4. Summary and conclusion

The present study validated that the alkali treatment, along with *Bacillus cereus*, enhanced the biodegradable property of the coir fiber. The alkali treatment removes non–cellulose components as lignin and hemicellulose. It increased the porosity of the fiber and surface area, which facilitates better interaction with degrading agents in correlation with the earlier studies of George et al., 2012 [33]. Furthermore, allowing the coir with silver nanoparticles contributed increased oxidative reactivity on the surface of fiber, promoting enhanced breakdown under microbial or composting conditions as shown by Rai et al., 2009 [34], may be considered for future research. The synergetic activity of chemical and biological treatment offers a promising strategy for the development of eco-friendly biodegradable coir-based composites or materials for the agricultural and packaging industry.

Additionally, long-term field studies and soil interaction studies should be conducted to evaluate the real-world biodegradability and environmental impact of the modified coir that may be repurposed. Expanding this approach of alkali treatment along with microorganisms could pave a path for broader industrial adaptation and sustainable fiber technologies in the future. As the thickness of the coir decreases, it can be used to make many commercial products, such as doormats, rugs, brushes, sacks, fishing ropes, etc. The biodegraded coir pith, along with microorganisms, can also be converted into biofertilizers for plants to utilize. These microorganisms, along with degraded coir, have nutrients that are required for the growth of plants.

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