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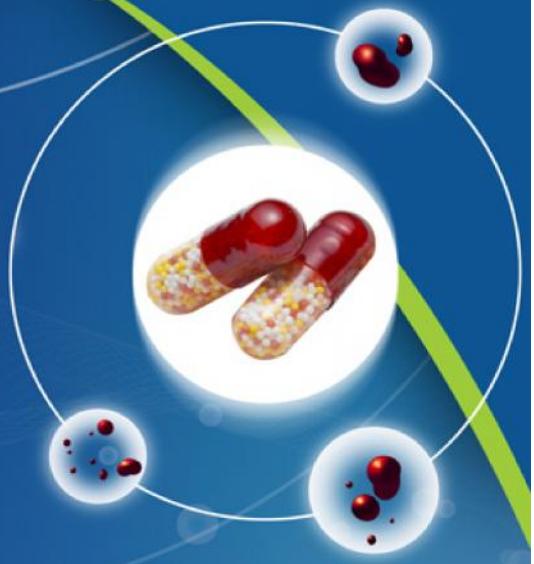

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Research Article

BIOLOGICAL MODIFICATION OF SUNN HEMP FIBERS WITH BACTERIAL CELLULASE

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ABSTRACT

This research articles deals with the bacterial treatment of sunn hemp fibers with bacterial cellulase using Brevibacillus parabrevis and Streptomyces albadancus. Some bacteria produce both bacterial cellulose and cellulase. Both cellulose and cellulase were used for the modification of natural fibers.Bacterial cellulase degrades cellulose in fiber wall structure, initiates wall stripping and fines generation. Refining then delaminate cell walls and causes wall to collapse and starts fibrillation, which provide strength to fibers.The effect of pH on the bacterial modification was also tested. Modified sunn hemp fibers were characterized with different techniques such as FTIR Spectroscopy,Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) Analysis.

Key Words:- *Sunn Hemp, FTIR Spectroscopy, Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) Analysis, Bacterial Cellulase*

INTRODUCTION

Cellulose fiber reinforced polymer composites have received much attention because of their low density, nonabrasive, combustible, nontoxic, low cost and biodegradable properties. A lot of research works have been performed all over the world on the use of cellulose fibers as a reinforcing material for the preparation of various types of composites. However, lack of good interfacial adhesion, low melting point, and water sensitivity make the use of cellulose fiber reinforced composites less attractive.

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Cellulose fibers are emerging as low cost, lightweight and apparently environmentally superior alternatives to glass fibers in composites. The negative environmental impact of the steadily increasing use of plastic and composite materials requires the development of new combinations of materials, possibly with improved properties, but with reduced environmental harm. Consequently, the development of recycling-friendly biodegradable composites has become a top priority. The development of bio based composites gained particular relevance with the increasing availability and diversity of biodegradable polymers. Bio based composites are claimed to offer environmental advantages such as reduced dependence on non-renewable energy/material sources, lower pollutant emissions, lower greenhouse gas emissions,

enhanced energy recovery, and end of life biodegradability of components. Since, such superior environmental performance is an important driver of increased future use of natural fiber composites, a thorough comprehensive analysis of the relative environmental impacts of natural fiber composites and conventional composites, covering the entire life cycle, is warranted.

Biofibers are hydrophilic in nature due to large number of hydroxyl groups. The hydrophilic nature of biofibers often results in poor compatibility with hydrophobic polymer matrices. Therefore, it becomes necessary to modify the surface of biofibers for better binding between fiber and matrix. Some bacteria produce both bacterial cellulose and cellulase. Both cellulose and cellulase were used for the modification of natural fibers [1, 2]. The introduction of bacterial cellulose onto natural fibers provides new means of controlling the interaction between natural fibers and polymer matrices. Coating of natural fibers with bacterial cellulose does not only facilitate good distribution of bacterial cellulose within the matrix, it also results in an improved interfacial adhesion between the fibers and the matrix. This enhances the interaction between the natural fibers and the polymer matrix through mechanical interlocking. Bacterial cellulose coated natural fibers introduced nanocellulose at the interface between the fibers and the matrix, leading to increased stiffness of the matrix around the natural fibers [3, 4]. This chapter describes the pre-treatment of sunn hemp fibers using bacteria *Brevibacillus parabrevis* and *Streptomyces albaduncus* which aimed at improving the interfacial adhesion to biobased polymers and might lead to truly biofiber reinforced composites with enhanced properties and much better durability. The modified biofibers were characterized by scanning electron microscopy, X-ray diffraction and TGA/DTA techniques to determine their surface morphology, crystallinity and thermal behavior.

MATERIALS

Sunn hemp fibers were obtained from the local resouces. The bacteria strain Brevibacillus parabrevis (MTCC No. 2708) and Streptomyces albaduncus (MTCC No. 1764), Yeast extract, Beef extract, Peptone and Agar, Glucose, NaOH and NaCl were used as received.

METHODS

Purification of sunn hemp fibers

Sunn hemp cellulose fibers were washed with detergent in order to remove impurities and then Soxhlet extracted with acetone for 24 hours in order to remove waxes, lignin and other impurities and were dried at room temperature.

Pre-treatment of sunn hemp fibers using bacteria *Brevibacillus parabrevis* and *Streptomyces albaduncus*

For the bacterium growth, the standard growth mediums for bacteria *Brevibacillus parabrevis* (MTCC No. 2708) and *Streptomyces albaduncus* (MTCC No. 1764) were prepared and pH was adjusted to 7.2 and 7.4 with sodium hydroxide. The starter culture was first autoclaved at 121 °C for 45 minutes and then inoculated with the bacterium strain in static conditions at 29+10 °C in an incubator. Glucose (1.5 g and 2.0 g) was added into the culture mediums to produce culture media. After 24 hours of bacteria *Brevibacillus parabrevis* and 3 days of bacteria *Streptomyces albaduncus* incubation in static cultures, some of the suspension materials were used to start agitated cultures. Under these conditions, strings of materials started appearing and were harvested by filtering with gauze. All products were kept in vacuumed desiccators with anhydrous calcium sulfate until characterization. Loose sunn hemp fibers (0.5 g, 5 cm long) were put in two 250 mL Erlenmeyer flasks, one containing 90 mL of culture medium which composed of 4 g/L glucose, 2 g/L yeast extract, 1 g/L beef extract, 5 g/L peptone, 5 g/L NaCl and 20 g/L agar for bacteria *Brevibacillus parabrevis*, and another flask containing 90 mL of culture medium which composed of 4 g/L glucose, 4 g/L yeast extract, 10 g/L beef extract and 20 g/L agar for bacteria *Streptomyces albaduncus*. These formulations were found to promote the

bacterial medication of sunn hemp fiber with stable pH. After autoclaving at 121 °C for 20 minutes, both the flasks were inoculated with 10 mL of a 24 hours and third day old broth of a previous culture of bacteria *Brevibacillus parabrevis* (MTCC No. 2708) and bacteria *Streptomyces albaduncus* (MTCC No.1764), respectively. The fermentation was conducted under agitated conditions on a shaking plate (150 rpm) in an environmental chamber at 30 °C for one week.

Extraction of modified sunn hemp fibers

After the fermentation, the modified sunn hemp fibers were purified in 0.1M NaOH at 80 °C for 20 minutes to remove all microorganisms, medium components, and soluble polysaccharides [5]. After filtration, they were then thoroughly washed in distilled water until neutral pH.

Characterization of modified sunn hemp fibers

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of the original sunn hemp and biologically modified sunn hemp fiber were taken with KBr pellets on PERKIN ELMER RXI Spectrophotometer.

Scanning electron microscopy (SEM)

Scanning electron microscopic studies of original sunn hemp and biologically modified sunn hemp fibers was carried-out on Electron Microscopy Machine (LEO 435 VP). Since cellulose has non conducting behaviour so it was gold plated in order to prevent charge buildup on the sample. Scanning was synchronized with microscopic beam in order to maintain the small size over large distance relative to the specimen. The resulting images had a great depth of the field. A remarkable three dimensional appearance with high resolution was obtained.

Thermogravimetric analysis/differential thermal analysis (TGA/DTA)

Thermogravimetric analysis and differential thermal analysis studies were carried out in nitrogen atmosphere at a heating rate of 10 °C/minute using Perkin Elmer, (Pyris Diamond) thermal analyzer.

X-ray diffraction (XRD)

X-ray diffraction studies were performed under ambient conditions on X-ray diffractometer (Brucker D8 Advance) using Ni-filtered CuKα (1.5418 Å) radiation and scintillation counter as detector at 40 kV and 40 mA on rotation between 5 to 50^o at 2θ scale at 1 second step size and increment of 0.01 degree with 0.5^o or 1.0 mm of divergent and anti-scattering slit. Crystallinity index (C.I.), which measures the orientation of the cellulose crystals in a fiber to the fiber axis, was determined by using the wide angle X-ray diffraction counts at 2θ scale close to $22^o$and 18^o. The counter reading at peak intensity at 22^o is said to represent the crystalline material and the peak intensity at 18^o corresponds to the amorphous material in cellulose material [6,7]. Percentage crystallinity (%Cr) [8] and crystallinity index (C.I.) [9,10] were calculated according to Equations (1) and (2):

$$(1) \qquad \%Cr = \frac{I_{22}}{I_{22} + I_{18}} \times 100$$

$$(2) \qquad C.I. = \frac{I_{22} - I_{18}}{I_{22}}$$

Where, I_{22} and I_{18} are the crystalline and amorphous intensities at 2θ scale close to 22^0 and 18°, respectively.

RESULTS AND DISCUSSION

Bacterial cellulose degrades cellulose in fiber wall structure, initiates wall stripping and fines generation. Refining then delaminate cell walls and causes wall to collapse and starts fibrillation, which provide strength to fibers. Effect of bacterial cellulase on sunn hemp fibers resulted in stripping of fiber surface through hydrolysis of β 1, 4- glycosidic bond which removes subsequent layers or fibrils of the fiber by the mechanism of peeling effect leaving the fiber less hydrophilic and easier to

drain. Increase in drainage has also been attributed to decrease in amount of amorphous and gel like polysaccharide layer on the surface, yet it did not affect the amount of fines as well as removal of fuzz formation increases the commercial value of sunn hemp fibers. Slow kinetics of enzymatic degradation of crystalline cellulose allow fabric and fiber properties to be improved without excessive damage.

Surface Modification of sunn hemp fibers using bacterial cellulase

Surface Modification of sunn hemp fibers using bacterial cellulase from bacteria *Brevibacillus parabrevis* was observed for 3 days, at the pH 7.2 and 1.5 g glucose and for 5 days, at the pH 7.4 and 2.0 g glucose for bacteria *Streptomyces albaduncus*, which results in enhanced smoothness and brightness due to the removal of gum materials and small fibrils protruding from the fiber surface [11, 12].

The effect of pH on the bacterial modification was tested in the range pH 6.7 to 7.7. The optimum pH for modification of sunn hemp fibers by bacteria *Brevibacillus parabrevis* and *Streptomyces albaduncus* was 7.2 and 7.4, respectively. Amount of extracellular protuberant structures on the fiber due to cellulase production by bacteria *Brevibacillus parabrevis* and *Streptomyces albaduncus* increased linearly upto the pH 7.2 and 7.4, respectively, and then decreased. This is due to the fact that high pH deactivated bacteria thereby inhibiting the bacterial cellulase. In general, glucose has been used as carbon source for bacteria *Brevibacillus parabrevis* and *Streptomyces albaduncus*. The optimum glucose concentration used for biopolishing by bacteria *Brevibacillus parabrevis* and *Streptomyces albaduncus* was 1.5 g and 2.0 g, respectively. At higher glucose concentrations, the amount of gluconic acid increased during the cultivation period. The total amount of gluconic acid produced correspondes to the amount of glucose consumed in the period during which gluconic acid was increasing. This suggested that glucose not consumed by bacteria was metabolized to gluconic acid and other substances, with increase in glucose concentration the accumulation of gluconic acid also increased and no more glucose was available for the bacteria to grow [13]. Moreover, the accumulated gluconic acid also lowered the pH of the culture media and inhibited cellulase production by deactivating the bacteria.

Amount of extracellular protuberant structures on the fiber due to cellulase production by bacteria *Brevibacillus parabrevis* and *Streptomyces albaduncus* increased linearly with culture time upto 3rd day and 5th day, respectively and then decreased. This is due to the fact that after 3rd and 5th day most of the glucose was metabolized via gluconic acid to other substances and hence, no more glucose was available for the bacteria to grow. Moreover, the accumulated gluconic acid also lowered the pH of the culture media which deactivated the bacteria resulting in inhibition of bacterial cellulase [13].

Characterization of biologically modified sunn hemp fiber

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of original and biologically modified sunn hemp fibers are depicted in Figures 2.1- 2.3. IR spectrum of original sunn hemp fiber (Fig.1) showed a weak peak at 3427 cm-1, 2918 cm-1, 1644 cm-1 and 1192 cm-1 due to –OH, –CH2, C-C and C-O stretching, respectively. In case of bacteria *Brevibacillus parabrevis* treated sunn hemp fiber (Fig. 2) peaks at 3417 cm-1, 2990 cm-1, 1643 cm-1 and 1155 cm-1 has been observed, whereas in case of bacteria *Streptomyces albaduncus* treated sunn hemp fiber (Fig. 3) peaks at 3441 cm-1, 2918 cm-1, 1645 cm-1 and 1153 cm-1 has been observed due to –OH, -CH2, C-C and C-O stretching, respectively. In addition to this, IR spectrum of bacteria *Brevibacillus parabrevis* and *Streptomyces albaduncus* showed extra peak at 3501 cm-1 and 3510 cm-1, respectively, due to –OH stretching.

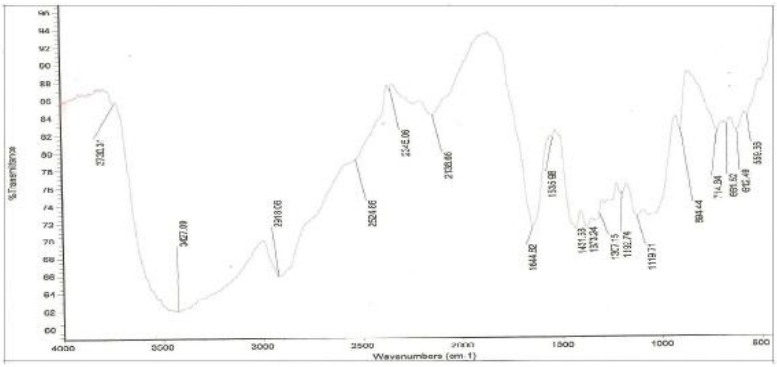

Fig. 1: FTIR spectra of original sunn hemp fiber

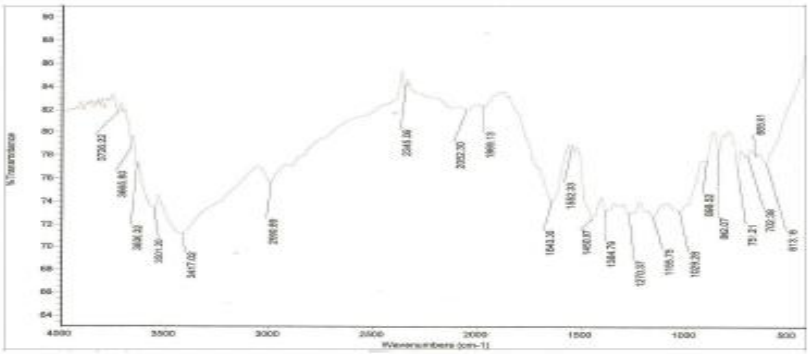

Fig.2: FTIR spectra of *Brevibacillus parabrevis* treated sunn hemp fiber

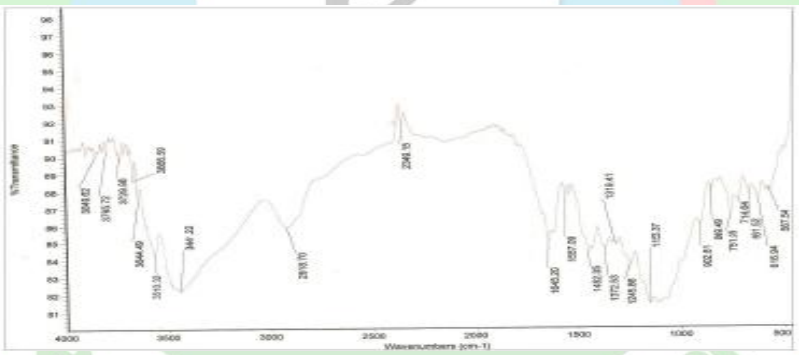

Fig.3: FTIR spctra of *Streptomyces albaduncus* treated sunn hemp fiber

Morphology of original and modified sunn hemp fiber

Figure 4-6 shows the morphology of original and biologically modified sunn hemp fiber. Comparison of the scanning electron micrographs reveals a clear cut distinction between the modified and unmodified sunn hemp fiber. Morphology of sunn hemp fiber was changed through biological treatment. Surface of original sunn hemp fiber (Fig. 4) was smooth but on biological treatment with bacteria *Brevibacillus parabrevis* (Fig. 5) and *Streptomyces albaduncus* (Fig. 6) the surface of sunn hemp fiber showed the enhanced softness and brightness due to presence of extracellular protuberant structures of cellulase, which results in removal of gum materials and small fibrils protruding from the fiber surface [11, 12, 14, 15].

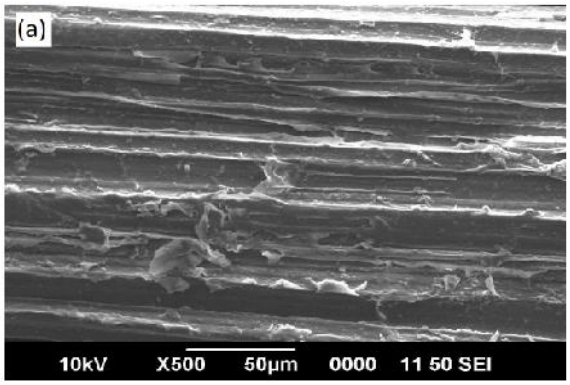

Fig.4: SEM of original sunn hemp fibers

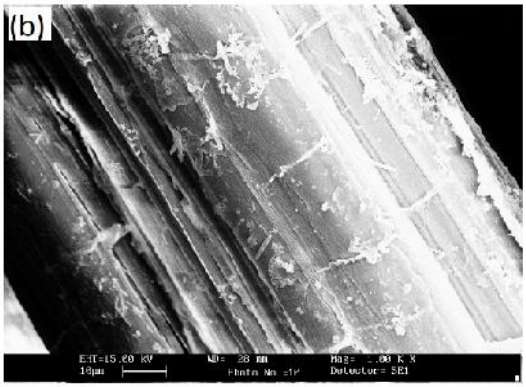

Fig.5: SEM of *Brevibacillus parabrevis* treated sunn hemp fibers

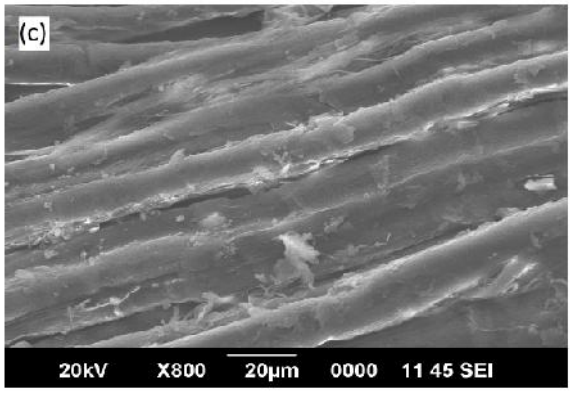

Fig.6: SEM of *Streptomyces albaduncus* treated sunn hemp fibers

Thermal properties of original and modified sunn hemp fiber

TGA of original sunn hemp fiber and biologically modified sunn hemp fiber were carried out at a heating rate of 10 °C/minute in nitrogen as a function of percentage weight loses versus temperature.

It is evident from Table 1 that initial decomposition temperature of original sunn hemp fibers, *Brevibacillus parabrevis* treated sunn hemp fiber and *Streptomyces albaduncus* treated sunn hemp fiber is around 285 °C, 280 °C and 282 °C, respectively. Whereas, final decomposition temperature of original sunn hemp fiber, *Brevibacillus parabrevis* treated sunn hemp fiber and *Streptomyces albaduncus* treated sunn hemp fiber is around 410 °C, 423 °C and 437 °C, respectively. Maximum thermal stability (437 °C) has been found in case of modified sunn hemp fibers in comparison to original sunn hemp fibers. In case of bacterial cellulase treatment, thermal stability is enhanced due to the removal of amorphous and gel like polysaccharide layer on the surface and small fibrils protruding from the surface.

Table I: Thermal analysis of original and biologically modified sunn hemp fiber

S.no	*Sample*	*TGA*		*DTA exothermic peak at temperature (°C)*
		IDT	*FDT*	
1	Original Sunn hemp fiber	285	410	315 °C and 400 °C
2	*Brevibacillus parabrevis* treated	280	423	312 °C
3	*Streptomyces albaduncus*	282	437	319 °C and 413 °C

Differential thermal analysis of original sunn hemp fiber and bacteria *Streptomyces albaduncus* treated sunn hemp fiber showed sharp exothermic peak at 315 °C and 319 °C, respectively. This peak showed disturbance in the Hbonded amorphous region. Another sharp exothermic peak was observed at 400 °C and 413 °C, respectively, which indicates the complete breakdown of C-C and C-O bonds of the crystalline region. However, in case of bacteria *Brevibacillus parabrevis* treated sunn hemp fiber a continuous exothermic rising temperature has been observed and a sharp exothermic peak at 312 °C has been observed.

Crystallinity of original and modified sunn hemp fiber

It is evident from Table 2 that percentage crystallinity of original sunn hemp fibers, *Brevibacillus parabrevis* treated sunn hemp fibers and *Streptomyces albaduncus* treated sunn hemp fibers has been found to be 73.3, 77.1, and 75.4 %, respectively. Therefore, %Cr of sunn hemp fibers has been found to enhance on biological modification. But the percentage crystallinity of bacteria *Brevibacillus parabrevis* treated sunn hemp fiber was found to be less as compared to sunn hemp fibers treated by bacteria *Streptomyces albaduncus*. So, sunn hemp fibers modified with bacterial cellulase showed enhanced crystallinity, which is due to the decrease in amount of amorphous and gel like polysaccharide layer on the surface of fibers and small fibrils protruding from the fiber surface. Crystallinity index of original sunn hemp fiber, *Brevibacillus parabrevis* treated sunn hemp fibers and *Streptomyces albaduncus* treated sunn hemp fibers was 0.63, 0.70 and 0.67 respectively. A high crystallinity index in case of biologically modified sunn hemp fiber means better order of cellulose crystals in the fibers. This clearly indicates that the cellulose crystals are better oriented in biologically modified sunn hemp fibers followed by original sunn hemp fibers. Results are further supported by thermal behaviour of original and biologically modified sunn hemp fibers.

Table II: Percentage crystallinity (%Cr) and crystallinity index (C.I.) of original and biologically modified Sunn hemp fiber

Sample	***At 2θ scale***		***%Cr***	***C.I.***
	I_{22}	I_{18}		
Original sunn hemp fiber	4230	1540	73.3	'0.63
Brevibacillus parabrevis treated sunnhemp fiber	1530	455	77.1	0.70
Streptomyces albaduncus treatedsunn hemp fiber	3450	1125	75.4	0.67

CONCLUSION

Surface of original sunn hemp fiber was smooth but on biological treatment with bacterial cellulase, the surface of sunn hemp fiber showed the enhanced softness and brightness due to removal of gum materials and small fibrils protruding from the fiber surface. It has been observed that initial decomposition temperature of original sunn hemp fibers, *Brevibacillus parabrevis* treated sunn hemp fiber and *Streptomyces albaduncus* treated sunn hemp fiber is around 285 °C, 280 °C and 282 °C, respectively. Whereas, final decomposition temperature of original sunn hemp fiber, *Brevibacillus parabrevis* treated sunn hemp fiber and *Streptomyces albaduncus* treated sunn hemp fiber is around 410 °C, 423 °C and 437 °C, respectively. It was found that percentage crystallinity of original sunn hemp fibers, *Brevibacillus parabrevis* treated sunn hemp fibers and *Streptomyces albaduncus* treated sunn hemp fibers has been found to be 73.3, 77.1, and 75.4 %, respectively.

Therefore, %Cr of sunn hemp fibers has been found to enhance on biological modification.

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