J Ind Microbiol Biotechnol (2009) 36:239–245
DOI 10.1007/s10295-008-0490-y

ORIGINAL PAPER

Large-scale degumming of ramie fibre using a newly isolated *Bacillus pumilus* DKS1 with high pectate lyase activity

Snehasish Basu · Manabendra N. Saha · Dhrubajyoti Chattopadhyay · Krishanu Chakrabarti

Received: 11 July 2008 / Accepted: 7 October 2008 / Published online: 28 October 2008

Abstract A combined (enzymatic and chemical) process using a *Bacillus pumilus* strain (DKS1), isolated from the soil, was used to degum ramie bast fibres. After 24 h of incubation with the isolated pectinolytic strain using a low-cost medium, the weight loss of the ramie fibre was found to be 25% under small scale. High activity of pectate lyase was detected in the culture supernatants; 400 kg of ramie fibres was degummed with 24% weight loss in large-scale degumming under field conditions. No cellulase activity was found. Microbial intervention followed by mild (0.1%) alkali treatment showed high percentage of weight loss from the ramie fibre. Bacterial degumming followed by chemical treatment resulted in an increase of single fibre tenacity (cN/tex) by more than 20.81% as compared to non-degummed (decorticated) fibre samples. Scanning electron micrographs (SEM) and fluorescence microscope showed that after *Bacillus pumilus* DKS1 treatment the surface of the decorticated ramie fibre becomes very smooth. These results indicate the process provides an economical and eco-friendly method for the small scale as well as large-scale degumming of decorticated ramie fibre. This study has great relevance to the textile as well as paper industry.

S. Basu · D. Chattopadhyay · K. Chakrabarti (✉)
Department of Biochemistry, University College of Science, Calcutta University, 35, Ballygunge Circular Road, Kolkata 700019, West Bengal, India
e-mail: kcbioc@gmail.com

M. N. Saha
Central Research Institute of Jute and Allied Fibres (CRIJAF), Kolkata, West Bengal, India

Keywords *Bacillus pumilus* DKS1 · Ramie fibre degumming · Pectate lyase · Fibre tenacity

Introduction

Alkalophilic bacilli play an important role in the degradation of plant residues. Biodegradation of pectin, a polysaccharide built mainly of α-(1–4)-linked D-galacturonic acid and its methylester, occurs as the result of a synergistic action of different extracellular enzymes [1, 2]. Two different classes of pectinolytic enzymes depolymerize pectin or its non-esterified form either by transeliminative cleavage (lyases) or by hydrolysis (hydrolases). In addition, the ester bonds in pectin are hydrolysed by a pectinesterase. Pectinolytic enzymes have been found in plants and microorganisms such as moulds, yeasts, and bacteria [3]. Pectate lyase enzymes assist in the maceration of plant tissues by degrading the pectin located in the middle lamella and in the primary cell wall of higher plants [4]. They are involved in the retting and degumming of jute, flax, hemp, and ramie bast fibres [5–8]. Cellulose fibres obtained from ramie are considered the longest, strongest, and silkiest plant fibers. The fibers are obtained by mechanical removal of the bast from the stem of the plant (decortication). Decorticated ramie fibres contain about 20–30% gummy material consisting mainly of pectin and hemicellulose. This material is removed in a chemical degumming process by treating the decorticated fibres with hot alkaline solutions with or without application of pressure [5, 9]. This process produces polluting effluents and can cause damage to the fibres. The gummy material could also be removed by polysaccharide-degrading microorganisms or their enzymes in a biochemical degumming process. A combination of microbial and chemical processes has been proposed to reduce the

consumption of chemicals and energy [10, 11]. In this work, we report the degumming of the ramie bast fibre by using a newly isolated pectinolytic *Bacillus pumilus* DKS1 strain and the characterisation of the degummed ramie fibre. Our microbial technique of degumming was more eco-friendly, energy-saving and cost-effective as compared to the chemical process. We initially standardised a process for the degumming under laboratory or small-scale conditions and scaled it for bulk-processing applications.

We used readily available ingredients for a cost-effective growth medium. Under small-scale as well as large-scale conditions the release of pectate lyase was high with no cellulase activity. The procedure could be utilised for running repeated batches with the same culture under field conditions. In addition the released pectate lyase was very stable under crude conditions with potential for use in subsequent downstream processes.

Materials and methods

Source of ramie fibre

Decorticated ramie fibres (*Boehmeria nivea*) used in this study were collected from CRIJAF, West Bengal, India.

Culture conditions for degumming on small scale

The *Bacillus pumilus* DKS1 strain (GenBank accession no. EF467045) was grown overnight in a 5 ml salt–papaya medium (0.5% NaCl, 0.4% NH_4Cl, 0.1% $MgCl_2$, 0.25% K_2HPO_4, 0.15% KH_2PO_4,1 mM $CaCl_2$ and 1.0% papaya pH 7.0). Of this culture 2 ml was subsequently inoculated into a 200 ml salt–papaya medium containing 2 g ramie fibres, in a 1,000-ml conical flask. Pieces of papaya were used as the source of pectin during all processes of the degumming. At the laboratory scale the salt–papaya medium was sterilised by a standard autoclaved method (at 121°C or 249°F for 15 min). The flask was incubated at 30°C on a rotary shaker (150 rev/min) for 24 h. The culture was centrifuged (7,000 rpm, 10 min, 4°C) and the cell-free supernatant was assayed for pectate lyase activity by the TBA method (A_{550}) according Basu et al. [12] based on earlier methods [13, 14]. A control system was run without adding the microbial culture.

Activity assay of pectate lyase

The pectate lyase activities were determined by the TBA (thiobarbituric acid) assay which measured absorbance at 550 nm. Suitable dilutions of the supernatant (1 ml) were added to 5 ml of PGA (polygalacturonic acid, sodium salt) solution (0.75%, w/v). The assay volumes were made up to 10.0 ml with Tris–HCl buffer (25 mM, pH 8.5) containing 1 mM $CaCl_2$ and incubated at 75°C for 2 h. About 0.6 ml zinc sulphate (9.0%, w/v) and 0.6 ml sodium hydroxide (0.5 M) were then added. The samples were centrifuged ($3{,}000 \times g$, 10 min) and 5.0 ml of the clear supernatant was added to a mixture of thiobarbituric acid (3.0 ml, 0.04 M) and HCl (1.5 ml, 0.1 M). The mixture was heated in a boiling water bath for 30 min, and the absorbance of the coloured solution was measured at 550 nm against a reference cuvette which contained the same reagents as that of the experimental cuvette but for which the zinc sulphate and sodium hydroxide were added before adding the enzyme and substrate. One unit of activity was defined as the amount of enzyme that caused a change in absorbance of 0.01 under the conditions of the assay.

Culture conditions for large-scale degumming of ramie fibre

In this process, we degummed a total of 400 kg ramie fibre using 4,000 l medium in two separate batches (200 kg each) at CRIJAF, Barrackpore. The process was carried out in cement-lined tanks (dimensions: 8.8 m × 1.62 m × 1.45 m). The medium contained salts (6 kg NaCl, 6 kg NH_4Cl, 3 kg $MgCl_2$, 2 kg K_2HPO_4, 6 kg KH_2PO_4, 1 mM $CaCl_2$) and papaya (400 kg). The pH of the medium was 7.0 and the temperature varied from 30 to 40°C in field conditions. The ratio of *Bacillus pumilus* DKS1 inoculum was 1:1,000 to total medium. The total system was non-sterilised. Before inoculation with *Bacillus pumilus* DKS1, the water was treated with sodium hypochlorite (five drops per litre) to eliminate the contaminant load. Pieces of papaya (400 kg) were washed with sodium hypochlorite (five drops per litre) solution. These pieces were added to the sodium hypochlorite (five drops per litre) treated water. The mixture was agitated and the fibre bundles were turned over at 12 h intervals using bamboo paddles. After harvesting the first batch of 200 kg, another 200 kg of ramie fibre was immersed as a second batch in same medium. The second batch of 200 kg was harvested after 240 h. An aliquot of the system was centrifuged and assayed for pectate lyase activity by the TBA method as described earlier. Fibre samples were collected (from marked bundles) at specified time points for treatment and assay of weight loss.

Alkali treatment of ramie fibres

The processing of ramie fibres was carried out in two steps. Microbial degumming (step I) followed by alkali degumming (step II). In the latter step the fibre was boiled in 0.1% NaOH for 15 min. The fibre was then washed under running water and put in a drier. Dried fibre samples

were weighed and the weight loss was compared to the raw fibre.

Bleaching of degummed ramie fibre

The degummed ramie fibres were immersed in an alkaline hydrogen peroxide solution for approximately 1 h or until the desired bleach or whiteness was obtained at room temperature. The bleaching solution contained sodium hydroxide (0.1%), EDTA (0.5%) and H_2O_2 (0.5%) [15]. The liquor to fibre ratio was 10:1 (w/v). The bleached degummed ramie bast fibre was rinsed under tap water and dried at 80°C.

Scanning electron microscopy of treated and untreated ramie fibre samples

Scanning electron microscopy (SEM) was used to observe the microstructure and the surface morphology of untreated and treated ramie fibres of small scale as well as large scale. The variation of surface structure of decorticated, controlled, enzyme followed by alkali treated and bleached ramie fibers has been reported here. Ramie fibre bundles were 50–100 µm in diameter. The samples were coated with gold to provide about 200 Å gold layer thickness using a vacuum sputter [16] and then samples were observed under SEM (HITACHI-S2360N, Japan) (Fig. 2).

Fluorescence microscopy of ramie fibre samples

Fluorescence microscopy (Model BX51/B52; Olympus, Japan) was used to observe the surface morphology of treated and untreated ramie fibres. As in the SEM experiment, the variation of surface structure of decorticated, control, enzyme and alkali treated and bleached ramie fibers were analyzed. To examine the surface structure of the ramie fibre, four different filters were used; bright field (band pass 400–700 nm) (Fig. 3a), green (band pass 530–550 nm, narrow band) (Fig. 3b), ultraviolet (band pass 360–370 nm, narrow band) (Fig. 3c) and blue (band pass 470–490 nm, narrow band) filter (Fig. 3d). To study the surface structure of ramie fibres under the microscope a single ramie fibre strand was fixed on a glass slide and a cover slip was kept on the sample fibre. The fibre samples were observed under 10× magnification in dark field.

Tenacity (cN/tex) of the single ramie fibre

The constant rate of elongation (CRE) method was applied to measure the tenacity [17] of the ramie fibre. The tenacity (cN/tex) of the fibre was measured using a single-fibre strength tester (Zwick/Roell, Z010) which determined the max load (N) of clean and dry fibre strands (5 cm length) collected from near the middle portion of the fibre reeds. The tenacity of the fibre samples was calculated from the ratio of max load (N) to its fineness (tex). Gauge length (test length) was 10 mm and each end of a single fibre was fastened with adhesive between two small pieces of paper for gripping. Test speed was 10 mm/min. A paper frame supported the fibres during sample loading to avoid bending or breaking, and the frame was cut prior to the stress-strain scan [17]. To obtain reliable results ten samples of each type were taken for single-fibre tenacity testing. The results are the mean and standard error of ten specimens of each sample.

Results and discussion

Enzyme activity in small-scale degumming

Our process used green papaya as the source of pectin. Green papaya is rich in pectin containing 10% of it on dry weight basis. Bacterial degumming of ramie bast fibres was optimal in the 200 g papaya content in the 200 ml salt–papaya medium. The maximum activity of pectate lyase (25 U/ml) in the medium was observed after 24 h of growth in the presence of ramie fibres (Table 1). The DKS1 pectate lyase has been purified and also characterised [12]. Neither pectin esterase (EC 3.1.1.11) nor polygalacturonase (EC 3.2.1.15) was detected in the cell-free culture fluid. These results are similar to that obtained with different strain of *B. pumilus* isolated by Dave and Vaughn [18]. The ready availability of green papaya throughout the year, its relatively low price and the extent of the degumming in the presence of this pectin source, indicate that the process is both cost-effective and efficient.

Percentage reduction in the dry weight of ramie fibres in small-scale degumming

Degumming of ramie fibres was done by *Bacillus pumilus* DKS1 followed by alkali (0.1%) treatment in a combined process. The percentage decrease in the weight of ramie fibres in presence of only bacterial enzyme treatment was up to 17% after 24 h (Table 2). However, the combined

Table 1 Pectate lyase activity in salts-papaya medium in small-scale

Medium (ml)	Papaya (g)	Pectate lyase activity (U/ml)
200	50	12
200	100	19
200	200	25
200	300	29
200	400	33

Table 2 Percent weight loss of ramie fibres in after degumming (small scale)

Papaya (g)	Wt. loss (%) Control	Wt. loss (%) after only 0.1% alkali treatment	Wt. loss (%) after step I	Wt. loss (%) after step II
100	6.75	8.8	12	23.8
200	7.8	9.2	17	25
300	8.2	10.1	18.5	25

treatment of enzymatic procedure followed by chemical process decreased the fibre weight by 25% after 24 h. Therefore, it might be concluded that to achieve maximal degumming of ramie fibres, the fibres should first be subjected to enzymatic treatment followed by addition of mild alkali treatment. Only 0.1% alkali treatment showed little amount of weight loss. These results indicate that microbial intervention loosens the pectin components in the fibre leading to the weight loss, possibly creating smaller fragments in the process. The subsequent treatment with mild alkali removes the fragments resulting in the final weight loss. This hypothesis is further supported by the fact that alkali treatment alone resulted in a weight loss of 8.8–10.1%, which was far lower than that achieved by the combined process. Reduction in the gum content of ramie fibre [19–21] using alkaline pectinase from *Bacillus* sp. has already been reported. However, degumming of ramie fibres by *Bacillus pumilus* DKS1 in presence of papaya is being reported for the first time as per our knowledge. The degumming of ramie fibres using *Bacillus pumilus* dcsr1 [21] has been also reported. The pectinase enzyme selectively degraded only the noncellulosic gummy material of fibres causing 10.96% fibre weight loss after 24 h. This weight loss was lower than that obtained using *Bacillus pumilus* DKS1. However, 0.04% NaOH was used during the degumming by using *Bacillus pumilus* dcsr1, which was lower than the alkali used in degumming made by *Bacillus pumilus* DKS1[12].

Again according to the procedure of Zheng et al. [19] using alkalophilic *Bacillus* sp. (NT-39, NT-53 and NT-76) a loss of only about 5% was obtained with ramie fibres after 48 h. As compared to these results our procedure gave a greater weight loss under similar or lesser incubation periods.

Further, during the degumming of ramie fibre by *Bacillus* sp. NT-39, NT-53 and NT-76 strains [19] both pectate lyase and xylanase were active. However, with DKS1 the secreted enzyme was pectate lyase with no trace of either cellulase or xylanase. We expect that this would result in better removal of the gum-like material with retention of fibre strength. Our suggestion was supported by the SEM (Fig. 2), fluorescence microscopy (Fig. 3), and physical studies (Table 4) where the fibre became progressively smoother with microbial intervention. The processed ramie fibre (degummed and bleached) did not show the presence of any spores under SEM (Fig. 2). As a further confirmation we added the processed fibre to a salt–papaya medium (described above) and incubated at 30°C on a rotary shaker (150 rev/min) for 24 h. Pectinolytic strains could not be detected when the culture medium was plated on YP-agar (NaCl 0.5%, Yeast extract 1.0%, Pectin 0.75%, 1.5% agar; pH 7.0) plates and stained with ruthenium red.

Pectate lyase activity in large-scale degumming

We collected samples (25 ml each) of the culture liquor from five different locations of the tank each day after incubation with DKS1 from large-scale degumming medium up to 10 days. The samples were analysed for pectate lyase activity by the method described by Basu et al. [12]. Detectable enzyme activity was observed only after 72 h. Thereafter enzyme activity increased almost seven times up to 10 days (Fig. 1). Large-scale degumming medium showed more than 6 U/ml pectate lyase activity at 10 days. The delay in the production of the enzyme in the large-scale preparation (as compared to the small scale) could be a consequence of bacterial adaptation to the conditions of the process. The initial pH of the degumming medium was 7.0, but after degumming pH became near to 5.0. The pectate lyase enzyme showed maximum activity even after 10 days of incubation and retained 50% of the total enzymatic activity after 16 days (data not shown in Fig. 1).

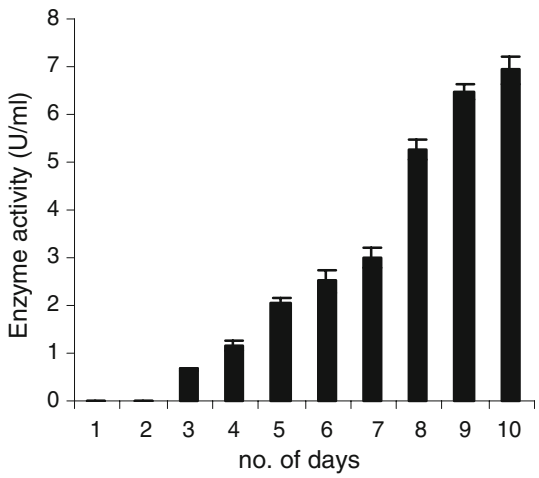

Fig. 1 Pectate lyase production in large scale degumming

Table 3 Percent weight loss after degumming of ramie fibre (large scale)

Time of harvest	Wt. loss (%) after Step I	After Wt. loss (%) after Step II
72 h (1st batch)	10	19
96 h (1st batch)	12	22
120 h (1st batch)	16	24
216 h (2nd batch)	22	25

Percentage reduction in the dry weight of ramie fibres in large scale

The water in the large-scale process was free of contaminating bacteria after zeoline treatment; 400 kg of ramie fibers was degummed by using 4,000 l salt–papaya medium in two separate batches of about 200 kg each. The results obtained are given in Table 3.

Less time was required to degum the ramie fibres in the second batch than in the first batch due to the presence of high pectate lyase activity after 5 days when another 200 kg of ramie fibres was introduced into the same culture medium. The percentage of weight loss in the second batch ramie fibre showed 25% after 96 h as compared to 24% after 120 h in the case of the first batch after bacterial treatment followed by alkali treatment. But, the percentage of weight loss of ramie fibre after step I was higher in the second batch (22%) than in the first batch (12%) after 96 h due to the presence of high enzyme concentration during 5–10 days. The percentage of weight loss (22%) after 96 h of step I in second batch showed better result even after 120 h of step I in first batch.

Study of surface structure of ramie fibres using scanning electron microscopy (SEM) and fluorescence microscopy

The reduction in the gummy material of the ramie fibres after treatment with bacterial enzyme followed by alkali treatment (0.1%) was also confirmed by observing the treated as well as untreated fibres under SEM. Decorticated (Fig. 2a) as well as control (Fig. 2b) fibres revealed plenty of gummy material on their surfaces, whereas bacterial enzyme followed by alkali-treated ramie fibres in large scale (Fig. 2c), bacterial enzyme followed by alkali-treated fibres in small scale (Fig. 2d), and bleached ramie fibres of small scale (Fig. 2e) exhibited less gummy material on their surfaces. In contrast, combined treatment of the ramie fibres in small scale resulted in significant removal of gummy material (Fig. 2d) than that obtained following large scale (Fig. 2c).

To further analyse the structure of fibre surface, fluorescence microscopy was used with four filters: bright field, green, UV and blue.

From the Fig. 3a–d, it was observed that by using four different filters, fibres (treated and untreated) showed different surface structures. Therefore, from the above observation it could be easily suggested that effect was not due to the fluorescence property of the ramie fibre, it may be due to the light scattering. Light scattering intensity was

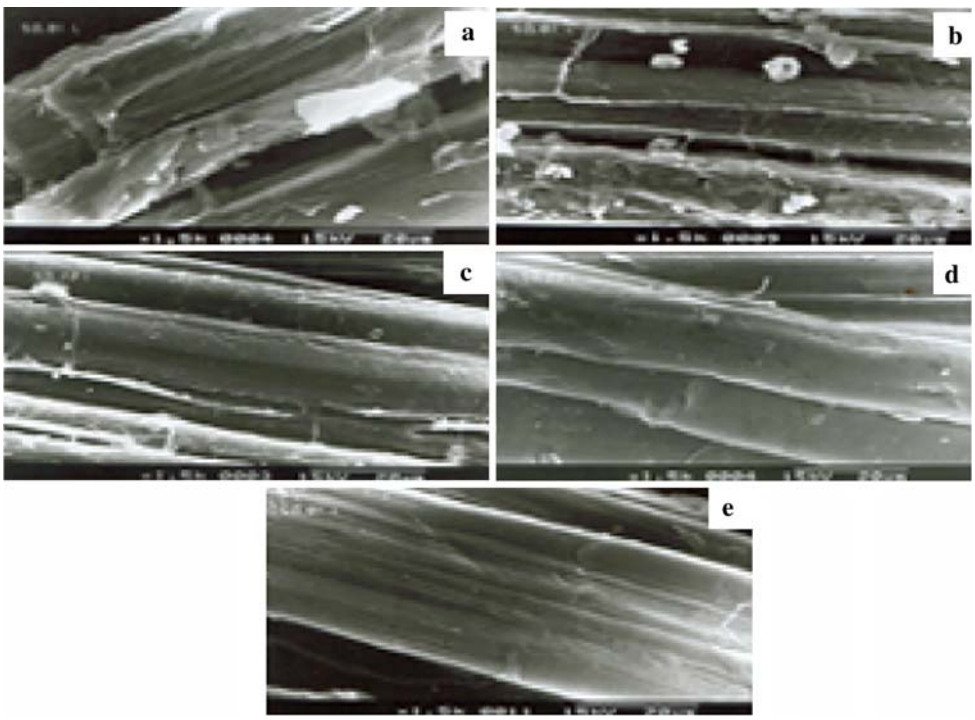

Fig. 2 Scanning electron micrograph of **a** decorticated; **b** control; **c** bacterial enzyme + 0.1% alkali (large scale); **d** bacterial enzyme + 0.1% alkali (small scale); **e** bleached ramie fibre (small scale). *Scale bars* = 20 μm in each micrograph

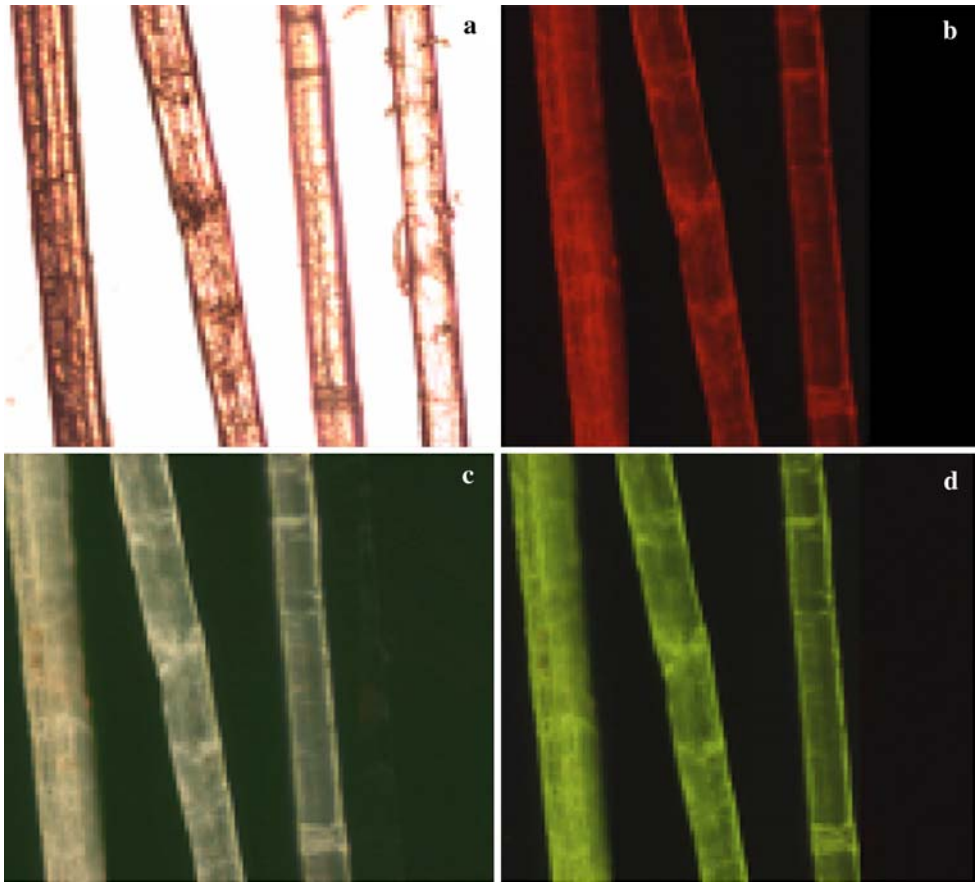

Fig. 3 Fluorescence microscopy of decorticated, control, degummed and bleached ramie fibre under Bright field (**a**), Green filter (**b**), UV filter (**c**) and Blue filter (**d**)

Table 4 Tenacity measurement of ramie fibre samples

Sample	Max load (N)	Elasticity (%)	Work of rupture (Nmm)	Fineness (tex)	Tenacity (cN/tex)
Decorticated	0.82 ± 0.29	2.9 ± 1.17	0.09 ± 0.07	2.40	34.16
Control	0.75 ± 0.24	3.1 ± 1.94	0.11 ± 0.09	2.28	32.89
Degummed (ss)	0.71 ± 0.21	4.1 ± 1.48	0.16 ± 0.08	1.72	41.27
Bleached (ss)	0.64 ± 0.19	3.83 ± 1.35	0.14 ± 0.06	1.66	38.78
Degummed (ls)	0.7 ± 0.28	3.7 ± 1.5	0.13 ± 0.08	1.88	37.23

N Newton, *Nmm* Newton millimeter, *cN* centiNewton, *ss* small scale, *ls* large scale

increased with increasing roughness on the ramie fibre. Therefore, untreated and controlled ramie fibres showed high colour intensity than degummed ramie fibres. After bleaching no light scattering or colour intensity was observed due to the maximum smoothness of the fibre. From the above observation it could be concluded that after processing by bacterial intervention and alkali treatment, followed by bleaching, gum content had been removed to a sufficient extent to make the ramie fibre smoother.

Tenacity measurement of single-ramie fibre

The single-ramie fibres of decorticated, control, degummed (bacterial enzyme + chemical) and bleached samples were tested for their tensile properties (Table 4).

For natural fibres, the fineness throughout each fibre was not constant and fibre-to-fibre variation was much higher. Due to the large variation in fineness in a single fibre, the single-fibre tenacity was considered as the tenacity per unit fineness of the fibre. The fineness of the treated single fibre was increased after microbial intervention followed by alkali. The fineness was increased after bleaching. The percentage increases in tenacity as a result of the processing indicates that the fibre could be used for commercial purposes.

Tenacity or tensile strength of the fibre is a very important parameter for grading for industrial purposes. In this context our observations were very significant. After degumming by combined (bacterial enzyme and chemical) treatment, single-ramie fibre tenacity (cN/tex)

was increased up to 20.81% in small scale and 8.98% in large scale compared to decorticated ramie fibre. The tenacity of fibre from large-scale processing (as compared to the small-scale processing) was lower. This was probably due to the 9.3% higher tex (or lower fineness) value of the fibre from large-scale processing. Tenacity was found to be decreased by 6% after bleaching with alkaline H_2O_2 as compared to degummed fibre in small scale. However, after bleaching, tenacity remained 13.52% higher as compared to decorticated fibre. On the basis of both SEM and fluorescence microscopy, bleaching (after microbial intervention) enhanced the smoothness or fineness of the fibre. Therefore, after degumming followed by bleaching the fibres retained enough tenacity to meet textile requirements due to their high intrinsic strength.

Acknowledgments We are very grateful to Prof. Anjan Kumar Dasgupta (Biochemistry Department, University of Calcutta, India) for developing the fluorescence microscopy image of the treated ramie fibre. We are also grateful to Dr. Asish Mukherjee (Institute of Jute Technology, Kolkata, India) for the fibre tenacity measurement. The authors also acknowledged the support from Dr. Himadri Sekhar Sen (former director, CRIJAF, West Bengal, India). This study was also supported by a grant from the Special Assistance Programme of the Department of Biochemistry, University Grants Commission, India. This work has been supported by Indian Council of Agricultural Research (ICAR), India.

References

1. Voragen FGJ, Heutink R, Pilnik W (1980) Solubilization of apple cell walls with polysaccharide degrading enzymes. J Appl Biochem 2:452–468
2. Osborne JM, Dehority BA (1989) Synergism in degradation and utilization of intact forage cellulose, hemicellulose, and pectin by three pure cultures of ruminal bacteria. Appl Environ Microbiol 55:2247–2250
3. Whitaker RJ (1989) Microbial pectolytic enzymes. In: Fogarty WM, Kelly CT (eds) Microbial enzymes and biotechnology. Elsevier, London, pp 133–175
4. Herron RS, Jacques AEB, Scavetta RD, Visser J, Jurnak F (2000) Structure and function of pectic enzymes: virulence factors of plant pathogens. Proc Natl Acad Sci USA 97(16):8762–8769. doi:10.1073/pnas.97.16.8762
5. Bhattacharyya SK, Paul NB (1976) Susceptibility of ramie with different gum contents to microbial damage. Curr Sci 45:417–418
6. Sharma HSS (1987) Screening of polysaccharide-degrading enzymes for retting flax stem. Int. Biodeterior Bull 23:181–186. doi:10.1016/0265-3036(87)90053-4
7. Baracat MC, Valentin C, Muchovej JJ, Silva DO (1989) Selection of pectinolytic fungi for degumming of natural fibers. Biotechnol Lett 11:899–902. doi:10.1007/BF01026849
8. Gillespie AM, Keane D, Griffin OT, Tuohy GM, Donaghy J, Haylock WR, Coughlan PM (1990) The application of fungal enzymes in flax retting and the properties of an extracellular polygalacturonase from *Penicillium capsulatumn*. In: Kirk TK, Chang HM (eds) Biotechnology in pulp and paper manufacture. Butterworth-Heinemann, Stoneham, pp 211–219
9. DasGupta PC, Sen K, Sen KS (1976) Degumming of decorticated ramie for textile purposes. Cell Chem Technol 10:285–291
10. Paul NB, Bhattacharyya SK (1979) The microbial degumming of raw ramie fibre. J Textile Inst 12:512–517
11. Deshpande KS, Gurucharanam K (1985) Degumming of ramie fibres: role of cell wall degrading enzymes of Aspergillus versicolor. Ind J Bot 8:79–81
12. Basu S, Ghosh A, Bera A, Saha MN, Chattopadhyay D, Chakrabarti K (2008) Thermodynamic characterization of a highly thermoactive extracellular pectate lyase from a new isolate *Bacillus pumilus* DKS1. Bioresour Technol 99(17):8088–8094. doi:10.1016/j.biortech.2008.03.032
13. Roberts PD, Phyllis MB, Caitilyn A, Yerlyn KS, George HL, Mark SM (1986) Requirement for two or more *Erwinia carotovora* subsp. Carotovora pectolytic gene products for maceration of potato tuber tissue by *Escherichia coli*. J Bacteriol 167:279–284
14. Bekri AM, Desair J, Keijers V, Proost P, Leeuwen SM, Vanderleyden J, Broek VA (1999) Azospirillium irakense produces a novel type of pectate lyase. J Bacteriol 181(8):2440–2447
15. Hebeish A, El-Bazza S (2003) Single stage process for desizing, scouring, and bleaching of cotton fabric. Angew Makromolekulare Chem 129(1):169–188. doi:10.1002/apmc.1985.051290114
16. Sirisart Q, Robert AS (2005) Morphology and structure of hemp fibre after bioscouring. Macromol Biosci 5(2):124–134. doi:10.1002/mabi.200400151
17. Booth JE (1968) Principles of textile testing, 3rd edn. pp 371–372
18. Dave BA, Vaughn RH (1971) Purification and properties of a polygalacturonic acid trans-eliminase produced by *Bacillus pumilus*. J Bacteriol 108:166–174
19. Zheng L, Du Y, Zhang J (2001) Degumming of ramie fibers by alkalophilic bacteria and their polysaccharide-degrading enzymes. Bioresour Technol 78:89–94. doi:10.1016/S0960-8524(00)00154-1
20. Kapoor M, Kuhad RC (2002) Improved polygalacturonase production from *Bacillus* sp. MG-cp-2 under submerged (SmF) and solid state fermentation (SSF). Lett Appl Microbiol 34:317–322. doi:10.1046/j.1472-765X.2002.01107.x
21. Sharma DC, Satyanarayana TA (2006) marked enhancement in the production of a highly alkaline and thermostable pectinase by *Bacillus pumilus* dcsr1 in submerged fermentation by using statistical methods. Bioresour Technol 97:727–733. doi:10.1016/j.biortech.2005.04.012