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Utilization of Pectinases for Fiber Extraction from Banana Plant's Waste

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Abstract

Today, biotechnology is perceived as a revolution throughout the world. With biotechnology, certain crops have been developed that can withstand the brutalities of weather changes, helping poor farmers of the developing countries to retain their yield and increase their output manifold. Biotechnology has also made agriculture more competitive and sustainable by creating new non-food markets for crops. To exploit the vast potential of biotechnology involved in non-food plant-products, the present study was taken up to explore the possibilities of improving the fiber extraction process of banana plant with the help of commercially available pectinase enzyme. Waste biomass of banana plant is widely available in many countries and the fiber extracted from its pseudo stem has utility for diversified range of applications including the manufacture of good quality handmade paper. The enzymatic treatment of green stem and trunk of banana plant before extracting fiber with the Raspador machine has resulted into an improvement in the yield as well as the quality of fiber obtained. This may not only result into a better utilization of the waste biomass of banana plant but may also increase profitability of the banana cultivators besides providing a source of good raw material for making handmade paper.

Keywords: Banana psuedo stem; Waste biomass utilization; Fiber; Handmade paper; Pectinase; Raspador machine

Introduction

Banana plant is a valuable bioresource which is distributed in more than 120 countries, over an area of 48 lakh hectares, with an annual production of 99.99 million tons (Indian Horticulture Database, 2011). The banana plant is highly valued for its fruit, but it also yields vast quantities of bio-mass residues from the trunk and fruit bunch (raquis), which are discarded on the field or – in the case of raquis – at the fruit processing sites (packing for exports). Thus banana farming generates huge quantities of biomass most of which goes as waste due to non-availability of suitable technology for its commercial utilization. From these residues, good quality of fibers can be extracted along with numerous other plant components (juice) with bioconversion potential. India has about 8.3 lakh ha under banana cultivation producing approximately 51.18 million tons of pseudo stem waste per year. This can be profitably used for extracting approximately 3.87 million tons of fiber [1]. Banana fibers are mainly placed in the superimposed leaves which form the pseudostem and provide resistance to the plant. These fibers run longitudinally along the leaves [2,3].

There are two ways to extract banana fiber i.e. either manually by hand or by mechanically through Raspador machine. Therefore, two qualities viz. hand-extracted and machine-extracted banana fiber are available in the market. Machine-extracted fiber is the low-grade fiber and cheaper in cost while the hand-extracted fiber is good in quality with higher price. Hand-extracted fiber has been found suitable for making high-grade paper due to its high purity while due to the presence of adherent pith; the machine-extracted fiber produces inferior quality of the product. Although appreciable efforts have been made by various institutions like Krishi Vigyan Kendra and Khadi & Village Industries Commission (KVIC), Mumbai, India to develop improved versions of machines for fiber extraction but even the best available machine in the market is not able to take out the fiber in its purest form and lot of pith remains attached to the extracted fiber. This pith creates problems during its utilization for making specialty handmade papers and behaves as a dead load on the fiber thereby consuming lot of chemicals, resulting into poor quality of the product and raising concerns about environmental pollution. Therefore, a suitable technology is the need of the hour for improving the quality of machine-extracted fiber so that it may become a good quality cellulosic fiber for making varieties of handmade paper as well as various other fibrous products, thereby promoting better utilization of this valuable bio resource.

There are several new technologies using enzymes able to modify fiber parameters, achieve desired properties, improve processing results and ecology in the area of bast fiber processing and fabric finishing. Enzymatic retting of flax, enzymatic cottonization of bast fiber, enzymatic hemp separation, enzymatic processing of flax rovings before wet spinning etc. create a new group of technologies supported by effective mechanical treatments [4]. A lot of work has been carried out on retting of bast fibers like flax, hemp etc. and similarly the enzyme treatment aspects of textile fibers has been studied extensively. However not many reports are available in the literature about using certain enzymes for extracting leaf fibers particularly from the banana plant.

Pectinases are believed to play a leading role in the processing of bast and leaf fibers, since 40% of the dry weight of plant cambium cells is comprised of pectin [5]. The enzymatic processing of plant fibers using pectinolytic enzymes result in no damage to the fibers and most importantly in addition to being energy conservative is environmentally friendly [6,7]. Pectinolytic enzymes from *Actinomycetes* are reported to be used for the degumming of ramie bast fibers [8]. Pectinase obtained from *Bacillus pumilus dcsr1* has been used for the treatment of dried and decorticated ramie fibers [9]. Jacob et al. [10] have reported scanning electron microscopic studies of the banana fibers treated with crude pectinase obtained from *Streptomyces lydicus* and showed that fiber cells were intact in the control while the cells were separated in

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the treated samples.

Keeping in view of above, the present study was taken up to explore the possibilities of improving the quality of machine-extracted banana fiber through pectinase treatment of green stem/trunk of banana plant and the waste banana leaves. Extraction of better quality banana fiber through enzymes may help in addressing the issues of global warming and other environmental concerns as well as generating an innovative outlet for sustainable development all over the world.

Experimental

Fiber extraction from waste banana leaves through enzymatic and non-enzymatic route

The waste banana leaves (broad having thick mid ribs) were procured from the vegetable market of Muhana Mandi Sanganer. In addition to this, small cut pieces of the branches of banana could also be obtained. For the fiber extraction studies, mid ribs were de-leaved and the small branches were cut vertically to divide into three layers.

Analysis of fiber yield

The yield percentage of extracted fiber was calculated using the formula:

$$\text{Yield, \%} = \frac{\text{OD Weight of the Extracted Fiber}}{\text{OD Weight of the Plant Part Used For Extraction}} \times 100$$

Pulping of the banana fiber extracted from waste banana leaves

The banana fiber extracted at KNHPI using the Banana Fiber Extractor with and without treatment was subjected to enzyme treatment (0.5% enzyme at 40°C for 4 hours) followed by "Open Hot Digestion", the pulping procedure commonly utilized in the handmade paper sector (NaOH-8%, time-3.5 hours, temperature-100°C, bath ratio: 1:8). Black liquor was collected before thoroughly washing the cooked fibers with tap water. Then the cooked material was subjected to the beating to a CSF (Canadian Standard Freeness) of 300-400 ml using the Standard Test Methods (IS 6213, T-227). Actual time to attain the desired degree of beating i.e. CSF was also noted in all the cases so as to evaluate the effect of enzyme treatment on beating energy.

Characterization of black liquor collected

Black liquor collected was characterized for the parameters of interest viz. pH, Total solids and Residual Active Alkali (RAA). All of these parameters were tested in duplicates and the average values have been reported here.

The pH was determined at 30°C using the standard TAPPI Test method number T-625 CM-85.

Total solids (% w/w) were estimated by drying 10-20 g of black liquor in pre-weighed glass petri dishes in the oven at 102 + 2°C for an overnight. Then the dried contents were weighed to the constant weight. Petri dishes were cooled in desiccators before weighing. Calculation was done as per the given formula:

$$\text{Total solids, \% w/w} = \frac{\text{Weight of dried contents}}{\text{Weight of black liquor originally taken}} \times 100$$

For determining the Residual Active Alkali in black liquor, 25 ml of it was taken in a small beaker and its pH was noted with pH meter. The liquor was then titrated with 0.1 N HCl to a pH of 7.0 and the RAA was calculated using the formula given below. (Standard TAPPI Test method no. TAPPI T-625 CM-85).

$$\text{R.A.A. (g/l as NaOH)} = \frac{\text{Milli equivalent of acid x 0.1x40}}{\text{Volume of black liquor taken (25 ml)}}$$

Evaluation of physical strength properties of pulps obtained

All the pulps thus obtained from the extracted banana fibers were used for making hand sheets of 60 GSM as per the TAPPI Standard procedures using the British Sheet Former. These hand sheets were evaluated for the physical strength properties using standard TAPPI TEST Methods and/or ISO methods (Tensile Strength: T-411, Tear Strength: ISO-1924, Grammage (GSM): T-423) so as to ascertain the effect of enzyme treatment on the quality of extracted banana fiber. A total of three hand sheets were evaluated for each of the test parameters and values reported here is an average of them.

Processing of the waste obtained from fiber extraction

The left over waste of banana fiber extraction through the enzymatic and non-enzymatic route were cooked separately with 4% NaOH at boiling temperature for a period of 3-4 hours, the pulps thus obtained were washed thoroughly and subjected to the beating to a CSF (Canadian Standard Freeness) of 300-400 ml. Hand sheets of 60 GSM were prepared as mentioned in 3.5 above.

Fiber extraction from green stem and trunk of banana plant

To study the effect of enzyme treatments on fiber extraction from the green stem of banana and banana trunk, experiments were conducted at one of the fiber extraction facilities at Guntur, Hyderabad, Andhra Pradesh, India so as to utilize the opportunity of the availability of both the Raspador machine and the green stem/trunk together at a single location. For this, besides observing and understanding the routine process of fiber extraction using the Raspador machine, enzyme soaking of the banana stem and trunk was carried out under the optimized conditions separately in two different drums. Further, the enzyme liquors and the pieces of banana trunk and stem each were collected at an interval of 12 hours, 24 hours, 36 hours and 48 hrs. Then the fibers were extracted from those pieces with the help of Raspador for their further evaluation at KNHPI, Jaipur.

Analysis of enzyme liquors collected from fiber extraction

The enzyme liquors collected before fiber extraction from the treated banana stem and trunks at the intervals of 12, 24, 36 and 48 hours were analyzed for color and total solids. Color of the filtrates was determined by measuring absorbance at 465nm and converting it into the Platinum Cobalt Units (PCU) using the conversion factor (500 PCU=0.41 Absorbance). Total solids were determined by drying a measured amount of liquor in a petri dish kept inside the Hot Air Oven at 100$\pm$2°C for an overnight. lignin content in the enzyme filtrate was quantified according to TAPPI method T-222 by measuring absorbance at a wavelength of 280 nm using 20.2 l/g/cm as the extinction coefficient [11].

Analysis of fiber extracted from green stem and trunk of banana plant through CIRCOT Mumbai

Since the quantity of banana fiber extracted from enzyme treated stem and trunk were less enough to process them for handmade papermaking, the fiber strength was needed to be evaluated as such. Due to the non-availability of such facilities at KNHPI, Jaipur, all the extracted fiber samples were sent to the Central Institute for Research on Cotton Technology (CIRCOT), Matunga, Mumbai, India for their

Figure 1: Fiber extraction from waste banana leaves

Parameters	De-leaved Ribs of Banana Plant		Banana Branches
	Natural/Untreated Control	Enzyme treated	Natural/untreated
Fiber yield,%	8.94%	23%	3.6%

Table 1: Fiber Extraction from Waste Banana Leaves

analysis.

Results and Discussion

Fiber extraction from waste banana leaves

For extracting fiber from the waste banana leaves, the mid rib portion was utilized. So, mid ribs were deleaved before processing them further as shown in Figure 1. The fiber yield obtained with and without enzyme treatment is given in Table 1 which indicates a great difference in the case of enzyme treated leaves as compared to the control case (23% vs. 8.94%). Table 2 shows the various parameters analyzed while processing the extracted banana fiber for making handmade paper. Actually, banana fiber obtained from banana ribs without enzyme treatment was less in quantity and having lot of dust, pith and waste along. So, the waste obtained during fiber extraction from untreated banana ribs was also cooked through open digestion using 4% NaOH for 3-4 hours to mix with it so as to enable its beating. Strength properties of the pulps prepared from waste left out of enzyme treated fiber extraction was found to be much poorer than that prepared from the waste left out of control fiber extraction. This implied that fiber extraction through enzymatic route resulted into lesser fibrous waste than through the untreated route (i.e. control) wherein lot of fiber was also lost into the waste generated down the equipment (Table 3).

Fiber extraction from green stem and trunk of banana plant

The fiber extraction process from banana trunk and banana stem has been shown in Figure 2 and 3 respectively. A pre-treatment of the green stem and trunk of banana plant with pectinases was found to be useful for fiber extraction process because in the fiber quality and yield obtained from banana trunk as well as banana stem was better with enzyme soaking than at the zero hours' stage which was equivalent to the conventional fiber extraction process. The quality of fiber extracted from banana trunk was better than the banana stem. The fiber yield varied with different periods of retention. In the case of banana stem, retention of 12 and 36 hours was found to be useful from the viewpoint of fiber yield (Table 4) but 36 hours retention could produce best quality fiber from the banana stem. Whereas in the case of banana trunk, a period of 24 hours was found to be the best for soaking it in the enzyme solution because it resulted not only in the maximum yield of fiber but also in the best quality of fiber (Table 4 and 5). Analysis of colour and lignin in the collected effluents is presented in Table 6. Chauhan et al. [12] have also reported an increase in fiber yield and fiber quality through a prior treatment of *Calotropis procera* (Ankra twigs) with pectinase enzyme.

Analysis of strength properties of the extracted fiber through CIRCOT Mumbai

Physical strength properties of the extracted banana fiber were evaluated for the four important parameters of fiber quality viz. fiber strength, tenacity, fiber fineness and elongation through Central Institute of Research for Cotton Technology (CIRCOT), Mumbai.

Elongation percentage: Percent elongation at break is an important parameter of judging the quality of any fiber since it is the maximum possible extension of the fiber until it breaks. From the Table 6, it can be seen that the banana fiber extracted from the trunk always had better elongation % than that extracted from the banana stem. However, on comparing the fiber extracted from enzyme treated and untreated banana trunk, it was found to be higher in the treated stuff which may be due to more efficient removal of pectin by the pectinase treatment and increased proportion of cellulose during fiber extraction process through Raspodar [13]. But maximum elongation % could be achieved in the fiber extracted from the banana trunk after 24 hours of enzyme treatment while it took 36 hours of incubation for getting maximum value of elongation percentage in the case of banana stem. Further incubation till 48 hours resulted into its reduction from 2.4 to 1.8 (Table 6). Thus the retention period of enzyme treatment had a significant effect on the % of breaking elongation of banana fiber. Similar effect of retting period on elongation % has been reported by Resmina et al. [14] while treating banana pseudo stem with pectin decomposing bacteria and /or MgO.

Parameters	Fiber Extracted Without Enzyme Treatment Of Ribs	Fiber Extracted With Enzyme Treatment Of Ribs
Fiber yield, %	84.7%	93.2%
T.S in enzyme liquor, %	0.70%	0.44%
Pulping Of The Treated Fibers Through Open Digestion		
Pulp yield, %	18.24%	50%
T.S in the black liquor, %	1.25%	1.14%
pH RAA in the black liquor (gpl)	10.33 0.176gpl	10.37 0.224 gpl
Beating time and CSF	5.06 360 ml	3.20 370ml.
Physical Strength Properties of the pulps obtained		
Tensile index (Nm/gm)	41.8	48.85
Tear index (mN.m2/gm)	9.07	7.6
Folding Endurance, no.	448	244

Table 2: Enzyme Treatment of the Extracted Fibers

Parameters	Waste from untreated banana leaves	Waste from treated banana leaves
Pulp yield	78%	98%
T.S in the liquor	1.57%	2.03%
pH RAA in the liquor (gpl)	8.61 0.224	7.84 0.064
Beating time and CSF	7.22 410ml.	17.00 350ml
Physical Strength Properties of the pulps obtained		
Tensile index	13.55	1.05
Tear index	6.46	6.66
Double Fold	108	32

Table 3: Pulping Of Waste Obtained On Fiber Extraction through Open Digestion

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Figure 2: Fiber extraction from banana trunk before & after treatment

Figure 3: Fiber extraction from banana stem before and after treatment

Fiber strength: Fiber strength which is reported in the form of breaking strength, tensile strength and tenacity/intrinsic strength was also evaluated in all the extracted banana fibers. A significant increase in the value of this parameter in both the cases of banana trunk and banana stem as compared to the respective controls indicates a positive effect of pectinase enzyme treatment. However, the highest strength (432.4 gf Vs. 239.0 gf of control) was obtained after 36 hours of enzyme incubation in the case of banana stem while after even 24 hours of enzyme treatment in the case of banana trunk (401.7 gf Vs. 258.4). This also reflects about the efficiency of pectinase enzyme for improving the quality of extracted banana fibers because pectinases contribute to the breakdown of non-cellulosic substances like pectin materials and thereby separate the fibers from the core. Resmina et al. [14] have also reported the effectiveness of retting process on banana fiber by the quality of tensile strength and breaking elongation of the fiber but they had used pectin decomposing bacteria and MgO instead of the enzymes.

Fiber fineness: Fiber Fineness is also one of the most important fiber characteristics. Fibers exhibit a variety of cross sectional shapes and they also vary in section along their length and vary from fiber to fiber. Fiber fineness denotes the size of the cross sectional dimensions of the fibers. As the cross sectional features are irregular, direct determination of the area of cross section is difficult and often laborious. Some dimensional features such as swollen diameter, ribbon width etc. can be determined directly and sometimes used to specify the fineness of cotton fiber. The linear density or weight per unit length of the fiber is the more commonly used index of fineness. The linear density is called either the fiber weight per centimeter or hair weight per centimeter and is usually expressed in units of 10^{-8} g/cm or 10^{-5}mg/cm. In Tex system, the linear density of cotton fibers is expressed in terms of milli Tex which is the weight in milligrams of one kilometer length of fiber. It should be noted that it is quite possible to have fibers with identical linear densities but different cross sectional areas. For example, a fiber with a high density will have a smaller cross sectional area than a fiber of low density. Fineness of fibers (μg/inch) to a larger extent depends on the maturity of the fibers and to some extent is also influenced by the amount of the moisture present in the material [15]. Maximum value of fineness could be obtained after 36 hours of enzyme incubation in the case of banana stem while in the case of banana trunk the value of fiber fineness was almost equal in all the periods of enzyme incubation evaluated but it was of course more than the untreated/conventional case (Table 6).

Tenacity: Tenacity is the customary measure of strength of a fiber. It is usually defined as the ultimate (breaking) force of the fiber (in gram-force units) divided by the denier. Because denier is a measure of the linear density, the tenacity works out to be not a measure of force per unit area, but rather a quasi-dimensionless measure analogous to specific strength. In order to compare strength of two fibers differing in fineness, it is necessary to eliminate the effect of the difference in cross-sectional area by dividing the observed fiber strength by the fiber weight per unit length. The value so obtained is known as "Intrinsic Strength or Tenacity". Tenacity is found to be better related to spinning than the breaking strength. The strength characteristics can be determined either on individual fibers or on bundle of fibers. Mean single fiber strength determined is expressed in units of "grams/tex". As it is seen the unit for tenacity has the dimension of length only, and hence this property is also expressed as the "Breaking Length", which can be considered as the length of the specimen equivalent in weight to the breaking load. Since tex is the mass in grams of one kilometer of the specimen, the tenacity values expressed in grams/tex will correspond to the breaking length in kilometers. Tenacity was found to be maximum at an enzyme incubation of 24 hours in the case of the fiber extracted from both the banana stem and the trunk (Table 6).

The results obtained in the present study can be further co-related with some of earlier reports available in literature. Brahamkumar and Manilal [16] have studied SEM of bioextracted and physically extracted banana fibers and have reported cleaner and smoother surfaces in bioextracted fibers. Mooney et al. [17] have reported that the enzymatic hydrolysis of all the pectic and hemicellulosic materials results in a high yield, environmentally friendly produced pure cellulose and the production of individual fibers without the generation of Kink bands (resulting from the breaking and scotching process) generates fibers with much higher intrinsic fiber strength. Saleem et al. [18] have also reported positive effect of pectinase treatment on the mechanical properties of hemp fibers and the fiber reinforced polypropylene. The positive effect was told to be accomplished by enzymatic decomposition of middle lamella which caused separation of technical fibers into smaller bundles and single fiber cells. This in

Banana Stem					Banana Trunk				
Time interval	pH	Total solids (%)	Fiber extracted from 4 pieces of 700 gms each (i.e. nearly (2800gm)	Yield %	Time interval (hours)	pH	Total solids	Fiber extracted from 5 pieces of trunk (i.e. nearly 3000gm)	Yield %
Zero hours/ conven-tional process	5.53	0.016	20.370	0.73	**Zero hours / conventional process**	5.33	0.205	46.319	1.5
12 hrs.	6.16	0.014	24.358	0.87	**12**	6.44	0.263	55.902	1.9
24 hrs.	4.90	0.077	17.691	0.63	**24**	5.46	0.20	69.292	2.3
36 hrs.	4.64	0.089	23.015	0.82	**36**	5.76	0.29	52.062	1.73
48 hrs.	5.08	0.020	18.505	0.66	**48**	6.35	0.21	53.599	1.8

Table 4: Extraction of Banana Fiber from Banana Trunk and Stem

Parameters	Banana Stem					Banana Trunk				
	0 hrs	12 hrs	24 hrs	36 hrs	48 hrs	0 hrs	12 hrs	24 hrs	36 hrs	48 hrs
Fiber Strength (gf)	239.0	367.1	286.1	432.4	381.8	258.4	279.6	401.7	253.0	357.7
Elongation, %	1.4	1.6	1.2	2.4	1.8	2.6	3.2	4.0	3.8	4.0
Tenacity (gf/tex)	19.3	17.6	23.8	20.4	24.5	14.4	13.7	22.3	13.5	17.2
Fiber Fineness (Tex)	12.4	20.8	12.0	21.2	15.6	17.9	20.4	18.0	18.8	20.8

Table 5: Strength Properties of Banana Fiber Extracted From Banana Stem and Trunk through Enzymatic Routes at Guntur (Analyzed Through CIRCOT, Mumbai)

Banana Stem			Banana Trunk		
Sample	Lignin (gpl)	Color (PCU)	Sample	Lignin (gpl)	Color (PCU)
Zero hours/ conventional process	0.031	41.50	**Zero hours/ conventional process**	0.034	48.80
12 hrs.	0.015	31.71	**12 hrs**	0.02	53.60
24 hrs.	0.024	28.05	**24 hrs**	0.035	50.0
36 hrs.	0.036	90.24	**36 hrs**	0.051	142.7
48 hrs.	0.043	101.24	**48 hrs**	0.028	81.7

Table 6: Analysis of Color and Lignin of the Effluents Collected

turn resulted into improved tensile and flexural characteristics of the thermoplastic composites. Nalankilli et al. [19] have also reported efficacy of pectinase on removal of non-cellulosics but they have used cotton fibers. In comparison to the solvent and alkali scouring, they had reported lowest reduction in strength and elongation values of the enzyme scoured cotton fibers against the control fibers. Azzaz et al. [20] have reported that treatment of hand stripped and dried banana fibers with crude pectinase results into destruction of middle lamella which in turn effects the separation of fibers. Similarly, Chauhan and Sharma [21] have reported a positive role of enzyme treatment in improving the quality of pseudo stem fiber of banana plant but they have used the commercially available machine-extracted fiber of banana instead of the green plant parts.

Thus the present study/investigation has shed some light on the influence of pectinase enzyme treatment on fiber extraction from the banana plant's waste (leaves, pseudo stem and trunk). However, the time period of enzyme treatment must be judged carefully to get the best quality of fiber because an under-treatment makes the separation difficult while an over-treatment may weaken the fibers.

Conclusion

Thus, the overall investigation has been quite useful for solving out the basic problem of improving the quality of machine extracted banana fiber. KNHPI has also experienced good response during the field trial studies conducted in Southern India. During fiber extraction from the enzyme treated banana stem and trunk and evaluation of strength of the extracted fiber, it was found that an incubation period of 24 hours is sufficient for banana trunk but it requires an incubation of 36 hours for getting best quality fiber from banana stem. Adoption of the enzymatic route shall help in enabling the handmade paper manufacturers to utilize the machine extracted banana fiber that is available at cheaper price than the hand-extracted banana fiber to produce a good quality handmade paper thereby improving their profitability. Further studies may be required to make the process of fiber extraction more cost-effective through enzymatic route. This may open great opportunity for employment generation for the economically backward youth and women particularly belonging to the rural areas of all the banana cultivating countries throughout the world besides addressing the problem of solid waste disposal generated from banana farming.

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