

## Pine Apple Leaf Wastes-Boon for Low Input Sustainable Technology (LISA)

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### Abstract

Agricultural byproducts are a copious and cheap source for lignin and cellulose fibers. Agro-based biofibers have the composition, properties and structure that make them suitable for uses such as composite, textile, pulp and paper manufacture. In the present investigation pineapple leaf wastes had been selected as a major sources of agro-based biofibers. This research paper focused on the eco-friendly production processes, comparison of properties of processed and unprocessed pineapple leaf fiber and suitability of these biofibers for various industrial applications. In the present study a modified method of cellulose extraction and the yield in terms of fiber and pulp is about 75% which is on par with the control in T1 (*Trichoderma harzianum*) and up to 81% in T5 which has the co cultures of *Trichoderma harzianum* + *Pleurotus sajor-caju*.

**Keywords:** Low input sustainable technology; Pineapple leaf fiber; Biocomposites

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### Introduction

Ignocelluloses are available plenty in nature derived from agricultural residues, forestry wastes and municipal solid wastes. Several biological methods for lignocelluloses recycling based on the enzymology of cellulose, hemicellulose and lignin degradation have been suggested. Among them, recycling and their use as raw material for the production of various biocomposites as an alternative combustible seem to be the most economically feasible. The three "R" (recycle, reuse, reduce) concept could be followed in the alternative eco-friendly technologies. In the present study we introduce lignocelluloses enzymes at different stages of textile, pulp and paper manufacture as a pretreatment to pulping (biopulping), bleaching (biobleaching), or wastewater treatment. This kind of approach has allowed considerable electrical power savings and a reduction of pollutants in the waste water from these industries. In addition, pretreatment of agricultural wastes with lignocellulolytic fungi enable their use as raw material for paper manufacturing. The use of microorganisms or their enzymes to enhance the de-inking of recycled fibers.

Agro industries and food processing industries practice eco-friendly processes under different agro-ecological zones in different countries. These experiences should be documented to learn more about the alternative and viable strategies to develop sustainable technological systems. One such important technology is Low Input Sustainable Technology (LISA). It involves minimizing the costs involved in preparation of manures, expenses for buying pesticides/insecticides through the organic resources. In the present study textile, paper and

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food processing industries were focused to follow Low Input Sustainable Technology (LISA). This study also helps the food processing and agro- industries to insist on LISA for the eco-friendly production through waste management and prevention of pollution. Inter-disciplinary approach in the present investigation is highlighted with reference to textile industry, paper industry and food processing industry.

Pineapple (*Ananas comosus* - a perennial plant), the pineapple plant grows about two to four feet tall. The reddish yellow fruit has a scale like surface surmounted by a crown of stiff, spiky leaves. The leaves are sword-shaped arising from a stem about 3 to 5 ft (0.9 to 1.5 meters) long and 1 to 2 inches (2.54 to 5.1 cm) wide tapering to a point. The leaves, particularly of native variety, produce excellent fibers. Commercially available technique is a time consuming and non-eco-friendly process. Hence the present investigation is focused to produce various valuable products from the renewable agro-wastes based on bioprocesses to curtail the pollution threats due to heavy usage of chemicals in the textile and paper industry.

## Materials and Methods

Pine apple leaves from the nearby farms and the crown leaves of fruits and fruit peels were collected from pine apple juice and jam factories in and around Salem, Hosur, Kolli Hills of Tamilnadu, India. Pine apple leaves were chopped into small pieces, dried and stored in chambers with good aeration.

To the 100 mL Erlenmeyer flask, 0.5g of pine apple leaves in 50 mM sodium citrate buffer pH 4.8 containing 0.02% sodium azide (antibacterial) and incubated at 50°C for 26 hrs with agitation at 150 rpm in an orbital shaker. Ligno-cellulolytic activity of the fungi on pine apple leaves was experimented with monoculture and co-cultures in various treatments as follows:

Control-Hydrogen peroxide & Carboxy methyl cellulose (1:1 proportion)

T1- *Trichoderma harzianum*

T2- *Phanerochaete chrysosporium*

T3- *Pleurotus sajor-caju*

T4- *Trichoderma harzianum*+ *Phanerochaete chrysosporium*

T5- *Trichoderma harzianum* + *Pleurotus sajor-caju*

T6- *Pleurotus sajor-caju* + *Phanerochaete chrysosporium*

Samples were taken periodically analyzed for the amount of reducing sugars by DNS method (Miller, 1969) against standard curve of glucose. Pine apple waste/leaf was incubated with hydrogen peroxide and carboxymethyl cellulose (1% w/v) at 50°C for 25 hrs and the pH 4.8 was maintained in different treatments. Amount of reducing-sugar was determined by DNS method (Miller, 1969) against standard curves of glucose. One International Unit (IU) of enzyme was defined as the amount of enzyme that releases one micromole of reducing-sugar per minute under standard assay conditions.

Enzyme assay was determined by the modified method of Sohail, *et al.* 2009. The cellulase producing fungi selected by qualitative screening were screened for cellulase production in liquid medium. They were inoculated into 250 mL Erlenmeyer flasks containing 50 mL of Mandels and Reese medium along with 1% carbon source carboxymethyl cellulose (CMC). The inoculation of the medium was done with 1.0 mL spore suspension and incubated at 30°C and 180 rpm for 3 days. The contents of the flasks were filtered through Whatmann No. 1 filter paper and cell-free culture filtrates were used in enzyme assays.

Carboxymethyl Cellulase (CMCase) Assay: The endoglucanase/carboxymethyl cellulase (CMCase) activity was measured according to IUPAC (Ghose, 1987). In the test tube, 0.5 mL carboxymethyl cellulose (1%), 4.8 pH, and 0.05M citrate buffer) was added with 0.5 mL appropriately diluted enzyme and incubated at 50°C for 30 minutes. The reducing sugar concentration was estimated by DNSA method (Miller, 1969). At the end of incubation period, the reaction was stopped by adding 3.0mL DNSA reagent. The tubes were incubated

for 5 minutes in boiling water bath for colour development and the optical densities (OD) were taken at 540 nm. The CMCase activity were calculated following the concept that 0.185 units of enzymes will liberate 0.5 mg of glucose under the assay conditions and was expressed as U/mL.

Cellulolytic activity was assessed separately in the retting process for the monoculture and co-cultures by the method of Hodge and Hofreiter, 1962. Ligninolytic activity was assessed by the method suggested by Kaar and Brink 1991.

Triplicates were maintained for all the experiments. The results were analysed statistically for the standard error and critical difference.

## Results and Discussion

There are lot of regular industrial processes involved in the textile and paper industries which follow time consuming methods and pose pollution threats due to heavy usage of chemicals. Alternative solution for these menaces is to involve and implement Low Input Sustainable Technology (LISA) to improve and sustain the availability of natural resources for the present and future.

In the present study agro-wastes from pine apple farms, industrial wastes from juice and jam industries were considered as the (inputs) raw materials for the production of fiber, paper and other non-woven products. Based on various textile and paper industrial processes in the present study bioprocesses like retting with reference to lignocelluloses degradation from the pineapple wastes had been assessed and the results are predicted in the table-1 and 2.

S. No	Properties	Unprocessed PALF	Bioprocess	Chemical process
1	Tensile Strength	17.14 kg-m/g	26 kg-m/g	23.06 kg-m/g
2	Fineness	13.4 Denier	14.7 Denier	15.7 Denier
3	Moisture Content	9.5%	11.8%	10.2%
4	Total Cellulose	75.2%	55.5%	69.9%
5	Alpha Cellulose	57.20%	35.4%	55.4%
6	Residual Gum	31.70%	20%	30%
7	Lignin	5.00%	3.3%	5.3%

**Table 1:** Quality Profile of Pine apple Leaf fiber (PALF).

Treatments	Lignocellulose degradation in pineapple fibers (% of dry matter)			
	12 hrs	24 hrs	36 hrs	48 hrs
T1	55.8	49.0	48.5	39.2
T2	55.1	50.4	45.0	44.1
T3	56.2	50.7	45.2	43.5
T4	51.3	41.1	40.5	39.8
T5	49.0	38.1	35.5	34.0
T6	52.4	49.7	44.0	42.5
Control	56.6	52.9	50.8	43.5
S.E	±2.01	± 1.05	± 1.18	± 1.29

**Table 2:** Ligno- cellulolytic potentiality of mono and co-cultures of Fungal isolates.

- T1- *Trichoderma harzianum*
- T2- *Phanerochaete chrysosporium*
- T3- *Pleurotus sajor-caju*
- T4- *Trichoderma harzianum*+ *Phanerochaete chrysosporium*
- T5- *Trichoderma harzianum* + *Pleurotus sajor-caju*
- T6- *Pleurotus sajor-caju* + *Phanerochaete chrysosporium*
- Control-Hydrogen peroxide & Carboxy methyl cellulose

Treatments	Reducing sugar content of PALF (mg/ml)			
	12 hrs	24 hrs	36 hrs	48 hrs
T1	3.18	6.30	7.01	7.92
T2	3.05	6.06	7.02	7.69
T3	3.13	5.91	7.11	7.70
T4	3.17	6.47	7.24	7.61
T5	3.52	6.99	7.92	8.25
T6	3.15	6.48	7.29	8.09
Control	2.99	4.17	5.50	6.33
S.E	± 1.25	± 1.08	± 1.15	± 1.07

**Table 3:** Reducing sugar production by mono and co-cultures of Fungal isolates.

- T1- *Trichoderma harzianum*
- T2- *Phanerochaete chrysosporium*
- T3- *Pleurotus sajor-caju*
- T4- *Trichoderma harzianum*+ *Phanerochaete chrysosporium*
- T5- *Trichoderma harzianum* + *Pleurotus sajor-caju*
- T6- *Pleurotus sajor-caju* + *Phanerochaete chrysosporium*
- Control-Hydrogen peroxide & Carboxy methyl cellulose

Retting involves the immersion of the pineapple leaves in water for some time to soften the plant gums. The total cellulose content in the pineapple leaf is about 46-70% (Leao., *et al.* 2010). Thus the present task is the removal of lignocelluloses from pine apple leaves and other pine apple wastes to obtain fibers and pulp respectively. The microbes produce cellulase enzymes (Bastioli, 2005) that break down cellulose content. By which the leaves get soft and loosen segregate into thin fibers. In the present study a modified method of cellulose extraction and the yield in terms of fiber and pulp is about 75% which is on par with the control in T1(*Trichoderma harzianum*) and up to 81% in T5 which has the co cultures of *Trichoderma harzianum* + *Pleurotus sajor-caju*.

As a result of bioprocess strong white and fine fiber was obtained. Sun/air drying is mostly preferable. Fiber strands are from 7.5 to 10 cm long (Guimarães., *et al.* 2010).In order to increase the grade of fiber, application enzymes and mechanical processing could be an essential stage where we get still more refined and lustrous fiber ( Zadeh., *et al.* 2017).

Properties of unprocessed and bioprocessed pineapple leaf wastes were recorded, the results are predicted in the table -1 and discussed as follows:

The commercially available technique involves various chemicals in the following stages- Scraping, Decortication and retting takes about 14 days. Experiments of the present study had been done on the pine apple leaves treated by microbial consortia of fungi *Trichoderma harzianum* + *Pleurotus sajor-caju* revealed that in which fiber can be extracted within 24-48 hrs. In the present investigation

75% fiber yield was obtained compared to existing chemical technology (Shenai, 1990; Booth 1996; Yasodha 2013; Dune., *et al.* 2016) in which yield is only 46%.

With reference to the lignocelluloses degradation a consortia of *Trichoderma harzianum* + *Pleurotus sajor-caju* were found to higher fold of 20 to 37% within 36 hrs than the control in which hydrogen peroxide and carboxy methyl cellulose were added as degrading agents. Fiber properties were tested at Textile technology department, Bannari Amman Institute of Technology for their tensile strength and finness. This kind of approach was experimented with Verweris., *et al.* 2004; Shibata, 2008; Huda., *et al.*, 2008 and Leao., *et al.* 2010. This eco-friendly technology can be followed and scale – up by the small scale agro-industries and can be adopted for the rural economy. The Low Input Sustainable Technology will minimize the wastes and that can be recycled by rural women and farmers. Similar experiments had been done on various cellulose wastes by Kato., *et al.* 2004; Merve Akpinar and Raziye Ozturk Urek 2012.

Treatments T1, T2, T3 were presenting similarities during 12 hrs of incubation in relation to reducing sugars, showing higher content compared to the other treatments, with the exception of T6, which showed lower content. The enzyme assay in terms of reducing sugar was ranging from 29 to 34% in monocultures of fungi (T1, T2, T3) showed the efficacy of *Trichoderma harzianum*, *Phanerochaete chrysosporium* and *Pleurotus sajor-caju* on pine apple leaves. An enhancing result increased from 35% to 68% in the T4, T5, T6 co-culture treatments.

## Conclusion

From the present investigation it was confirmed that the bioprocessed composites can be prepared from the waste leaves of pineapple which supports Low Input Sustainable Technology (LISA). This valuable agro-industrial waste is used as raw material for the production of handkerchief, table napkins, table cloth, fans, gowns, non-woven cloths in textile industry. Fiber based biocomposites could be highly useful in preparing bricks, cardboards and handicrafts.

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