PRODUCTION OF ECO FRIENDLY PAPER FROM COCONUT AND LEAVES AND NUT SHELLS OF ARECA PALM

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ABSTRACT

Paper is a most elemental segment of most aspects of the society. Approximately 300 Million tons of paper is produced of which 90% of the paper is produced from mature pulp wood. The history of paper making goes back to over 2000 years while the first official report on the manufacture of paper has been reported in China in 105 AD. Paper manufacturing knowledge which spread towards west, along with the silk and trade routes, reaching India is round 605 AD. Pulp and paper production industry have an increased in the global market and also expected to increase in the near future and it is one among the largest manufacturing sectors in the world. Per capita consumption of paper is sometimes a scale for measurement of industrialization. There are about 6.5 billion people living on planet Earth. Worldwide paper consumption in this century has been increased 4 times faster than population. Worldwide the Paper and paperboard will reach 640 million tonnes in 2020 with world population of 8000 million people and per capita consumption of paper and paperboard of 80 kg. There are about 500 Kraft mills and thousands of many other types of pulp and paper mills in the world. India is one among the fastest growing country in pulp and paper in the world market with growth rate of 10% over one year in per capita consumption, which is expected to grow in future. The Indian paper industry is among the top 15 global players today, with an output of more than six millions tones annually with an estimated turnover of Rs 150,000 million. The Projected demand in the global market is of 13 million tonnes by 2020. This paper focuses on the production of Eco-friendly Paper from Coconut and Leaves and Nut shells of Areca Palms.

KEYWORDS: *Paper, Industrialization, Eco-friendly, Coconut and Areca Palms.*

INTRODUCTION

The need of sustainable non wood resources as the raw material for the pulp and paper industry is in extreme need. The fibrous plant parts or wastes may be utilized for making pulp and paper alone or in blends with other agricultural residues (**Arafat*, et.al.*, 2016)**. However, in developing nations, such as India, the lack of technology and investments has led to a fall back in this industrial sector. For a nation, it is best to identify and make available the non woody plant source as raw material for paper production and obtain paper in a completely environmental friendly manner (**Thukkaramsudhakari *et.al.*, 2015)**.

COCONUT

The coconut palm (*Cocos nucifera*) is one among the most cultivated palms in the world and one which is used for many purpose by the human kind. It is mainly appreciated for its fruits. *Cocos nucifera* is a monoecious palm, which measures between 25 and 30 meter in height. Its trunk is marked by the scars of the leaves, it is 30 to 40 cm in diameter and has a heavy base formed by the roots. The trunk is pointed into a bunch with twenty pinnateleaves that reach up to six meters long approximately. Every year 10 to 20 new leaves are formed, according to the variety and other growth conditions (**Al-Sulaimani, Khalsa and Dwivedi, Priya, 2017)**. The extremely developed root system is composed of thousands of thin and long roots. The inflorescences have number of flowers of both sexes. These inflorescences or spadices are contained in a great bract called spathe. The female flowers are produced in the base of the ramification,also the masculine flowers are produced above the same. After their fertilization the flowers are transformed into ovoid drupes, each one composed of a seed. This seed is the coconut, whose fibrous covering dries up and hardens when ripening. The coconut is formed with very hard endocarp, 5 mm

thick, inside the endosperm consists of a whitish colour opaline and sweetened fluid, commonly known as "the coconut milk', filling three quarters of its central cavity. The fruits are gathered in a cluster to form a unit consisting of 10 to 15 coconuts (**Alam *et.al.*, 2018)**.

ARECA PALM

Areca palm (*Areca catechu*) is an erect, unbranched palm reaching heights of 12-30 m, depending upon the environmental conditions. .Areca nut palm is an monoecious plant with male and female flowers occurring on the same spadix. The stem is marked with scars of the fallen leaves in a regular annulated form, which becomes visible only when the palm is about 3 years old. Girth depends on genetic variation and soil conditions. Root system adventitious, typical of monocots (**Laurijssen, *et.al.*, 2010)**. The adult palm has 7-12 open leaves, each with a sheath, a rachis and leaflets. The leaf stalk extends as the midrib until the end of the leaf and ends as leaflets. Male flowers very numerous, sessile, without bracts, calyx 1-leaved, small, 3-cornered, 3-parted, petals, oblong, rigid striated, stamens, anthers. Female flowers are solitary or 2 or more near the base of each ramification of the spadix, sessile, without bracts, sepals permanent, staminodes, connate stigmas, short, triangular. Fruits are monolocular, one-seeded berry, 3.8-5 cm long, smooth orange or scarlet when ripe, with a dense fibrous outer layer. The generic name is derived from the common name used by the people of the Malabar Coast in the south western part of India. Every year 3-4 inflorescences are produced. The first inflorescence on the young palms is produced only by male flowers. Areca nut almost always exists in cultivation therefore, the conditions of its natural habitats are difficult to predict. It thrives in the areas of high rainfall. Although tolerant to moderate elevations on mountains, it generally do not grow best in low altitudes. Being a shade-loving species, it grows well in a a mixed cultivation with fruit trees. Soil should be deep to ensure a well-developed root system with high organic carbon content and a pH range from acidic to neutral (**Sunita Chauhan and A.K.Sharma, 2014)**.

BIOPULPINGAND BIO BLEACHING

Bio pulping is the treatment of wood chips and other lignocellulosic materials with naturally occurring wood decay fungi prior to thermomechanical pulping. The technical and economic feasibility of bio pulping was established through improvement in technology. The fungal treatment process applies well into an industrialwood yard operations. Wood source is debarked, chipped and screened according to normal industrial operations. Then chips are initially steamed to reduce microorganisms in the raw material, cooled down with forced air, and inoculated with the fungus which does bio pulping process. The inoculated chips are piled and ventilated with filtered and humidified air for 1 to 4 weeks prior to processing (**Amit Ramdhonee and PratimaJeet, 2017)**. While engineering analysis indicates that the bio pulping process is technologically feasible, economic analysis indicates that the bio pulping process is also economically beneficial. The use of bio pulping as a pre-treatment for the Kraft process is still an open research issue. Finally, the use of this technology for other substrate – non woody plants such as kenaf, straw, and corn stalks will have to be investigated. It reduces electrical energy consumption (at least 30%) during mechanical pulpingwith an30% increase potentially in industrial through put for the mechanical pulping process, with improved paper strength properties and reduced pitch content as well as reduced environmental impact (**Vishtal, A. and Retulainen, E, 2018***).*

The bio bleaching of Kraft with mediator continues to receive strong interest, in part due to the discovery of new mediators for laccase. Therefore, new environmentally begin elemental chlorine free (ECF) and totally chlorine free (TCF) bleaching technologies are necessary for minimizing the hemi-cellulose content in dissolving pulp, adjusting the brightness at a high level and improving simultaneously, the quality of the effluents in terms of toxicity and absorbable organic halogen (AOX). Biological methods of pulp prebleaching by using xylanases provide the possibility of selectively removing up to 20% of xylan from pulp and saving up to 25% of the chlorine containing bleaching chemicals. Alternatively, the pulp can also be bleached with white-rot fungi and other lignolytic enzymes, enabling chemical savings to be achieved and a chlorine free bleaching process to be performed which eliminates the set free of the chemicals (**Milala *et.al.*, 2005)**. Reduced consumption of bleaching chemical, reduced absorbable organic halogen, improved pulp and paper quality, improved brightness, reduced effluent toxicity and pollution load (**Jiménez, Luis *et.al.*, 2008)**.

CELLULASE

Cellulose is the most abundant renewable biological resource and a low-cost energy source. The production of bio-based products and bioenergy from low cost renewable lignocellulosic materials bind up the benefits to the local economy, environment, and national energy security. Cellulase are enzymes that hydrolyse β -1,4 linkages in cellulose chains. They are produced by fungi, bacteria, protozoans, plants, and animals. The catalytic modules of cellulase have been classified into numerous families based on their amino acid sequences and crystal structures. Cellulase contain noncatalytic carbohydrate-binding module functionally known or unknown modules, which may be located at the N- or C-terminus of a catalytic module. The complete cellulose hydrolysis is been mediated by a combination of three main types of cellulases: (1) endoglucanases (EC 3.2.1.4), (2) exoglucanases, including cellobiohydrolases-(CBHs) (EC 3.2.1.91), and (3) β -glucosidase (BG) (EC 3.2.1.21). To hydrolyse and metabolize insoluble cellulose, the microorganisms must secrete the cellulase) which are either free or cell-surface-bound. Cellulasebeing used in a large amount for a large variety of industrial purposes such as textile industry, pulp &paper industry, and food industry, as well as an additive in detergents which increasingly improves the digestibility of animal feeds (**Narasimha *et.al.*, 2005, Xiao-Zhou Zhang and Yi-Heng Percival Zhang, 2013, Sathitsuksanoh *et.al.*, 2010, KruusK *et.al.*, 1995 and Chauhan *et.al.*, 2013)** (**Figure 1**).

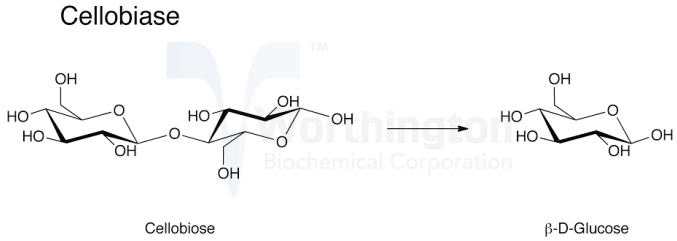


Figure 1 : Cellobiase Structure

MATERIALS AND METHODOLOGY

Samples were collected near the plantation area. After collecting the samples, the isolation of organism was carried out by applying the standard protocols in the laboratory. Plating was carried out using the Spread Plate Technique. Colony morphology and Microscopic Examination were also carried out. Liquid State Fermentation was carried out using Potato Dextrose Broth Media and the product obtained was purified by Dialysis. This was followed by SDS PAGE Analysis, Enzyme Immobilization by Calcium Alginate, Cellulase Plate Assay and Estimation of Cellulase.

BIOPULPING

The enzymes act on the fiber and digest the cellulose along with the bonds and sets free of cellulosic fibers from lignocellulosic complex. This helps in the softening of the fiber.1 kg of plant samples were submerged in distilled water overnight in a large container. 20 grams of immobilized enzyme as beads were added to each tray and stirred to ensure uniform distribution. The Process was allowed to carry on for a period of 15 days.

BIOBLEACHING

500 gms of pulped samples were strained and steamed at 121°C, 15lbs pressure for 1 hour. It was then allowed to cool, collected in containers and suspended with distilled water. 20grams of Immobilized enzyme was added to each container and left for bleaching for a period of 5 days.

BINDING AGENT

Starch was added to the paper to enhance or to modify the bonding and coherence between the fibres. Also to increase the dry strength of the paper material.

LAYING

The pulped samples were mixed with starch. The sample was then poured onto the Mould and deckle. Then it was manually pressed for the removal of water. It was sun dried and sized accordingly.

RESULTS AND DISCUSSION

COLLECTION OF SAMPLES

The samples used were Raw materials such as Coconut flower cover **(Figure 2A)**, leaves **(Figure 2B)** shells of Areca Palm **(Figure 2C)** and Soil samples **(Figure 2D)** which was collected from the Plantation Area of Mallapuram, Kerala.




2A **2B** **2C** **2D**

Figure 2A: COCONUT FLOWER COVER
Figure 2B: LEAF OF ARECA PALM
Figure 2C: SHELL OF ARECA PALM
Figure 2D: SOIL SAMPLE

ISOLATION OF THE ORGANISM

1ml of 10^{-3}diluted soil sample was inoculated on the potato dextrose agar (PDA). The plates were incubated at 72 hours at room temperature. Mixed fungal colonies were observed after incubation period **(Figure 3).**

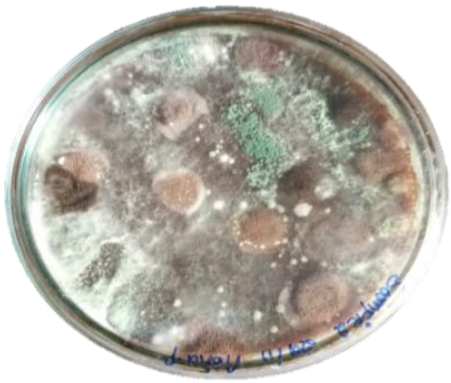
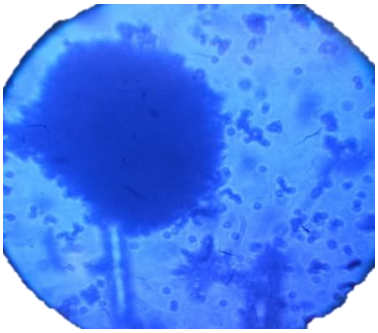
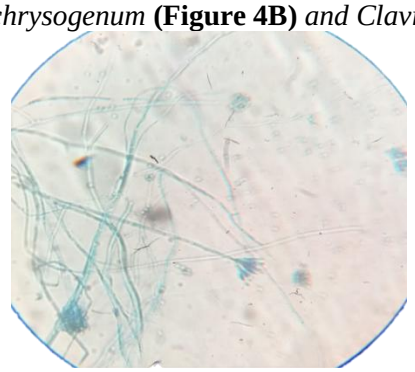


Figure 3: PDA plates with fungal growth

IDENTIFICATION OF ORGANISM

Lacto phenol cotton blue was used for the staining the fungal mycelium. They were found to be *Aspergillus niger* **(Figure 4A)**, *Penicillium chrysogenum* **(Figure 4B)** *and Claviceps spp* **(Figure 4C)**.

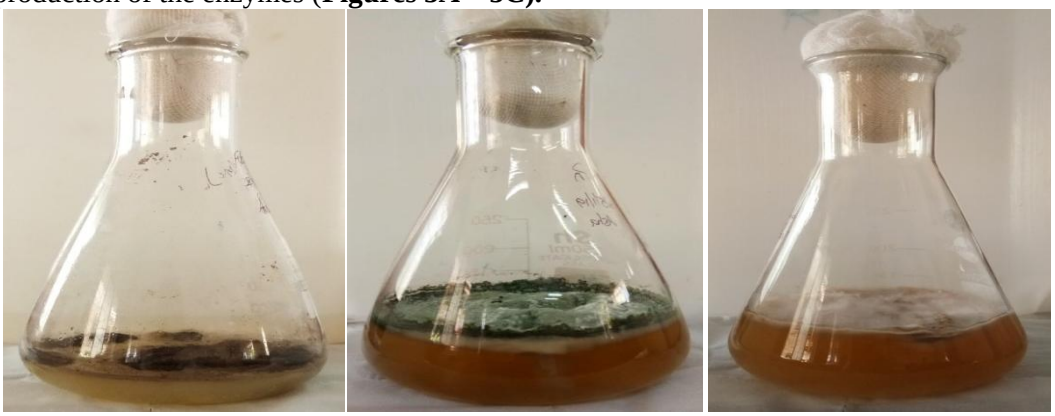




4A **4B** **4C**

Figure 4A : *Aspergillu*
Figure 4B : *Penicillium chrysogenum*
Figure 4C : *Claviceps spp*

LIQUID STATE FERMENTATION

The fungi were inoculated into potato dextrose broth with cellulose as carbon source. They are involved in the mass production of the enzymes (**Figures 5A – 5C).**



5A **5B** **5C**

Figure 5A : *Aspergillus niger*
Figure 5B : *Penicillium chrysogenum*
Figure 5C : *Claviceps spp*

CRUDE ENZYME PREPARATION

The resultant filtrate was precipitated by ice cold ethanol and stored in -20^{o}C (**Figure 6).**

Figure 6: Ice Cold Ethanol Precipitation

PURIFICATION BY DIALYSIS

The dialysis was performed for the suspended pellet obtained by centrifugation of the ethanol precipitation filtrate **(Figure 7).**

Figure 7: Purification by Dialysis

SDS PAGE ANALYSIS

The samples were subjected to SDS PAGE Analysis for the determination of the molecular weight of the enzyme **(Figure 8).**

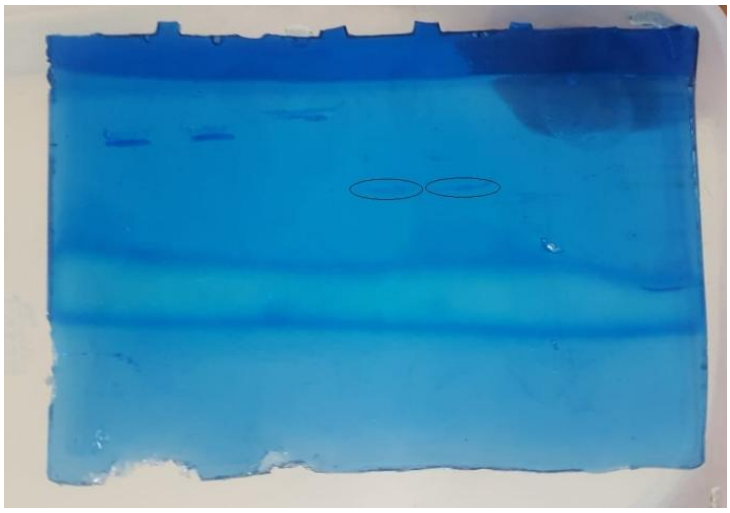


Figure 8: Determination of Molecular Weight

ENZYME IMMOBILIZATION BY CALCIUM ALGINATE

The cross reaction between Sodium Alginate and Calcium Chloride leads to the formation of beads. The enzymes were added to sodium alginate to immobilize efficiently **(Figure 9).**

Figure 9: Immobilization of enzyme

CELLULASE PLATE ASSAY

Cellulase plate assay was performed to detect the enzyme activity of the purified enzyme solution **(Figure 10A)** and immobilized enzymes **(Figure 10B)**. A zone of clearance was observed which was stained with Congo red and de-stained with sodium chloride.

Figure 10A: Cellulase Plate Assay of Free Enzyme solution

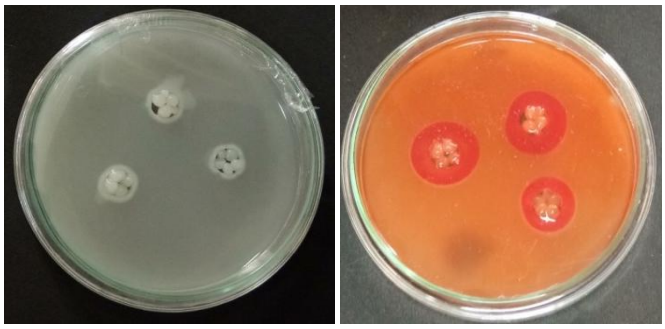


Figure 10B: Cellulase Plate Assay of Immobilized Enzyme

ESTIMATION OF CELLULOSE

Estimation was determined by DNSA method by using glucose as Standard **(Figure 11)**. *Penicillium chrysogenum* produced the highest concentration of Cellulose in the potato dextrose broth Medium **(Table 1).**

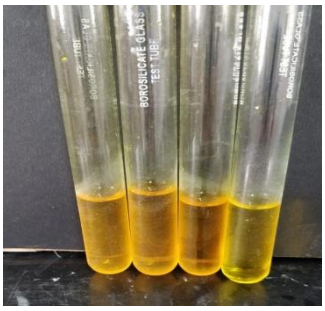


Figure 11: Estimation of Cellulose by DNSA Method

	Aspergillus niger	***Penicillium chrysogenum***	***Claviceps spp***
Unknown Absorbance at 540nm	**0.02**	**0.04**	**0.03**
Unknown concentration (µg/ml)	**40**	**80**	**60**

Table 1: Unknown absorbance and concentration of Cellulose

ACTIVITY OF CELLULASE

The activity of cellulase was found using the following formula:

$$\textbf{Cellulase Activity} = \frac{\textbf{µg/ml of glucose}}{\textbf{Molecular weight of glucose}} \times \frac{\textbf{1}}{\textbf{Incubation time}} \times \textbf{1000}$$

Where,

µg/ml: concentration of unknown solution

Incubation time: Time of incubation of enzyme with substrate.

ENZYME ACTIVITY		
Aspergillus niger	***Penicillium chrysogenum***	***Claviceps spp***
22.22	44.44	33.33

Table 2: Enzyme activity exhibited by different fungal organisms.

The highest enzymes activity was exhibited by ***Penicillium chrysogenum*** **- 44 IU.**

BIOPULPING AND BIOBLEACHING

The samples were soaked prior to Biological processing **(Figure 12A – 12C)**. The immobilized enzymes were incorporated into the soaked plant material collected in a container. Pulping was carried out for a period of 15 days and bleaching for 5 day.

12A **12B** **12C**

Figure 12A: LEAF OF ARECA PALM
Figure 12B: SHELL OF ARECA PALM
Figure 12C: COCONUT FLOWER COVER

PAPER LAYING

The pulp was poured into the mould and deckle **(Figure 13A & B)** which was diluted according to the thickness required along with the binding material **(Figures 14 - 16).**

13A **13B**

Figure 13A: Mould and Deckle used for Paper Laying
Figure 13B: Sample Laid on Mould and Deckle

FINAL PRODUCT

Figure 14 : Coconut Bract

Figure 15 : Areca Palm Leaf

Figure 16 : Areca Plam Nut

CONCLUSION

Paper is one of the utmost habitual and important materials used in everyday life. It's purpose is in the field of work, education and packaging. In India, paper industries are stumbling under twin pressure of scarcity of raw material and improvement of technologies. The 3 non woody sources like Coconut flower cover and leaves, shells of Areca palm and soil samples were collected from the plantation area. The 3 different Non woody plant sources were bio pulped and bio bleached using the isolated enzyme from the fungal organism using soil sample to produce eco-friendly paper. The soil sample was serial diluted and inoculated on the agar plates. After 5 days of incubation Fungal colonies were observed. The fungal colonies were microscopically observed to be *Aspergillus niger, Penicillium chrysogenum, Claviceps spp.* The observed colonies were sub cultured to obtain pure culture. Enzyme production was carried out in potato dextrose broth where the fungal culture was mass cultivated. The resultant filterate was precipitated and purified by Dialysis method. The enzyme was determined by plate assay to detect the activity. The purified enzymes were immobilized. The non woody samples were priorly soaked to which the immobilized enzymes was added for Bio bleaching and Bio pulping process.The pulped samples were then paper layed by using mould and deckle to obtain the appropriate size and shape. It was manually pressed and sun dried to obtain the final product. The resultant papers produced are completely biodegradable and safe to the environment because no chemical were used for the treatment of the plant sources for pulping and bleaching processes. The use of enzymes in the production process helps in the elimination of the use of harmful chemicals as well as in the effluent discharged from the industry in creating water pollution problem which is of serious issue to look upon in the future. Thus this technique targets to gear up handmade papers technology for the rural sectors by creating employment opportunity and this process is techno economically more acceptable.

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