

POTENTIAL APPLICATIONS OF PINE WASTE

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CERTIFICATE

This is to certify that thesis entitled “**Potential applications of Pine waste**”, being submitted by **Akansha Gupta** to the ‘**Indian Institute of Technology-Delhi**’ for the award of “**Doctor of Philosophy**” is a record of bonafide research work carried out by her. She is worked under our guidance and supervision, and has also fulfilled the requirements for the submission of this thesis. To the best of our knowledge the results obtained in this thesis has not been submitted in part or full to any other institute and university for the award of any degree or diploma.

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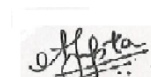
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Akansha Gupta

ABSTRACT

Paddy straw (PS) and pine needles (PN) are one of the challenging biomasses in terms of disposal and compost making due to their high silica and tannin contents. Particulate air pollution, loss of biodiversity and respiratory impairments are some of the disastrous outcomes of burning. However, high percentage of cellulose and hemicellulose makes them potential substrate for paper and pulp industries. The main aim of work was to study and utilize a combinatorial approach of weak chemical treatment and lignin degrading fungal species as agents of effective production of lignin modifying enzymes (LME's) for lignin depolymerisation from the biomasses. Three fungal agents were experimented in the study which included *Trichorma reesei*, *Pleurotus ostreatus* and *Phanerochaete chrysosporium*. *Phanerochaete chrysosporium* was found to be the best degrader of lignin (47.11% in PS+PN in 28 days) with maximum LME's production between 10th-17th days. Efficient lignin degradation in the PS and PN biomass will aid further application in pulp production supporting the transition to a circular economy in a greener way.

The study further aims at providing a cleaner technology by employing a combinatorial approach of mild chemical treatment of 1% Ca(OH)₂ as optimized followed by *Phanerochaete chrysosporium* (laccase producer) on PS+ PN in a novel combinatorial ratio of 1:1, for getting cellulose-rich pulp for making biodegradable paper sheets. Response surface methodology was adopted by choosing two response parameters – cellulose content and residual lignin content after pulping to optimize the cooking conditions. The pulp derived after optimizing the parameters i.e., solid: liquid ratio (1:7), temperature (160°C), time of digestion (60 mins), and NaOH concentration (4%) was characterized. SEM and FTIR analysis showed a more condensed cellulose network and -OH bonds when compared with alpha-cellulose.

Best pulp (kappa no. 24 and SR 59) was casted to produce biodegradable paper sheets of different GSM types (40, 50, 75, 100 GSM), and their different mechanical properties were tested. The maximum tensile strength (110 Nm/g) and Cobb index (345.77 g/m²) were seen in 100 GSM sheets which is comparable with regular corrugated sheets. Such an alternative application could serve as a perfect replacement to cardboard or wood-based paper.

Plastic cutlery for food packaging and serving has long been in trend due to its versatile properties. However, accumulation of the single used plastic cutlery creates disposal problem, and their degradability is also a major concern. Binding agents such as carboxymethyl cellulose, gum acacia and potato starch along with cross-linking polymer were blended with pulp in binding matrix to cast durable and non-brittle biodegradable cups. PLA (bio-plastic) polymer was applied in the form of a thin film over the developed tableware to avoid fibrous material of tableware to enter food. The developed tableware was further verified for anti-microbial properties by testing it with common food-poisoning pathogens.

Mechanical stiffness and rigidity were estimated along with thermal stability examined over a wide temperature range by dynamic scanning calorimetric (DSC). Tableware was also checked for water and oil solubility and absorptivity along with studying its functional group by FTIR spectroscopy. Lattice structure and morphology was studied by SEM and XRD analyses. Based on the analytical analyses, the two biomasses selected are prospective biomasses for development of biodegradable tableware and can be a suitable alternative to combat plastic cutlery menace. Three binders that were used to optimize the binder ratio in the pulp for developing biodegradable cups were carboxy-methylcellulose (CMC), gum acacia and potato starch. Among the three, CMC showed the best binding properties with pulp in the ratio of 2:3. Similarly, for bowls, 75 GSM sheets casted the best bowls among all the combinations experiment with different GSM types.

Fungal infestations are a major problem in the pulp and paper production industries. The lignocellulosic nature of pulp attracts fungal growth, thereby causing huge losses to this vital sector. Essential oils and plant extracts act as potent inhibitors of various fungal pathogens. To overcome this problem, the present study aimed to test the emulsifiable concentrates (EC) formulation of botanicals to control severe fungal infestations in paper and pulp industry. Purposely, EC formulations were prepared using *Curcuma longa* (Turmeric) and *Pongamia pinnata* (Karanja) oils which were mixed with solvents (polar, non-polar) and surfactants in varying ratios (1.5, 1.25, 1.0, and 0.75%). The formulations were screened and the shelf-life of formulations was evaluated under hot and cold conditions.

Two EC formulations were finally obtained (particle size of 550–660 nm) and bio-efficacy trials (growth inhibition studies) were performed against selected paper pulp-infesting fungal pathogens (*Aspergillus flavus*, *Rhizoctonia solani*, and *Fusarium oxysporum*). The formulations TEC-3 (Turmeric oil-based EC) and TKEC-29 (Turmeric and Karanja oil-based EC) exhibited the best efficacy showing 100% inhibition against *R. solani* with 1.5% (10.5 g/L) concentration. The presence of curcuminoids and terpenoids, along with other bioactive molecules in Turmeric oil and karanjin in Karanja oil might have been responsible for the antifungal activity of the bio-formulations.

सारांश

धान का भूसा (पीएस) और पाइन सुई (पीएन) अपनी उच्च सिलिका और टैनिन सामग्री के कारण निपटान और खाद बनाने के मामले में चुनौतीपूर्ण बायोमास में से एक हैं। कणीय वायु प्रदूषण, जैव विविधता की हानि और श्वसन संबंधी हानियाँ जलने के कुछ विनाशकारी परिणाम हैं। हालाँकि, सेलूलोज और हेमिकेलुलोज का उच्च प्रतिशत उन्हें कागज और लुगदी उद्योगों के लिए संभावित सबस्ट्रेट बनाता है। काम का मुख्य उद्देश्य बायोमास से लिग्निन डीपोलाइमराइजेशन के लिए लिग्निन संशोधित एंजाइमों (एलएमई) के प्रभावी उत्पादन के एजेंट के रूप में कमजोर रासायनिक उपचार और लिग्निन को नष्ट करने वाली फंगल प्रजातियों के संयोजन दृष्टिकोण का अध्ययन और उपयोग करना था। अध्ययन में तीन फंगल एजेंटों का प्रयोग किया गया जिसमें ट्राइकोर्मा रीसी, प्लुरोटस ओस्ट्रीटस और फेनेरोचेटे क्राइसोस्पोरियम शामिल थे। फेनेरोचेटे क्राइसोस्पोरियम को लिग्निन का सबसे अच्छा डिग्रेडर (28 दिनों में पीएस+पीएन में 47.11%) पाया गया, जिसमें 10वें-17वें दिनों के बीच अधिकतम एलएमई उत्पादन होता है। पीएस और पीएन बायोमास में कुशल लिग्निन क्षरण से लुगदी उत्पादन में आगे के अनुप्रयोग में मदद मिलेगी, जिससे हरित तरीके से एक परिपत्र अर्थव्यवस्था में संक्रमण का समर्थन होगा।

अध्ययन का उद्देश्य 1% Ca(OH)_2 के हल्के रासायनिक उपचार के संयोजनात्मक दृष्टिकोण को नियोजित करके एक स्वच्छ तकनीक प्रदान करना है, जिसके बाद 1:1 के नवीन संयोजन अनुपात में PS+ PN पर फेनेरोचेटे क्राइसोस्पोरियम (लैकेस उत्पादक) का उपयोग किया जाता है। बायोडिग्रेडेबल पेपर शीट बनाने के लिए सेलूलोज-समृद्ध लुगदी प्राप्त करना। खाना पकाने की स्थितियों को अनुकूलित करने के लिए पल्पिंग के बाद दो प्रतिक्रिया मापदंडों - सेल्यूलोज सामग्री और अवशिष्ट लिग्निन सामग्री को चुनकर प्रतिक्रिया सतह पद्धति को अपनाया गया था। मापदंडों को अनुकूलित करने के बाद प्राप्त गूदा, यानी ठोस: तरल अनुपात (1: 7), तापमान (160°C), पाचन का समय (60 मिनट), और NaOH एकाग्रता (4%) की विशेषता थी। एसईएम और एफटीआईआर विश्लेषण ने अल्फा-सेल्यूलोज की तुलना में अधिक संघनित सेल्यूलोज नेटवर्क और -ओएच बांड दिखाया।

विभिन्न जीएसएम प्रकार (40, 50, 75, 100 जीएसएम) की बायोडिग्रेडेबल पेपर शीट बनाने के लिए सर्वश्रेष्ठ लुगदी (कप्पा नंबर 24 और एसआर 59) का उपयोग किया गया और उनके विभिन्न यांत्रिक गुणों का परीक्षण किया गया। अधिकतम तन्यता ताकत (110 एनएम/जी) और कॉब इंडेक्स (345.77 ग्राम/एम²) 100 जीएसएम शीट में देखी गई जो नियमित नालीदार शीट के साथ तुलनीय है। ऐसा वैकल्पिक अनुप्रयोग कार्डबोर्ड या लकड़ी-आधारित कागज के लिए एक आदर्श प्रतिस्थापन के रूप में काम कर सकता है।

खाद्य पैकेजिंग और परोसने के लिए प्लास्टिक कटलरी अपने बहुमुखी गुणों के कारण लंबे समय से चलन में है। हालाँकि, एकल उपयोग वाली प्लास्टिक कटलरी का संचय निपटान की समस्या पैदा करता है, और उनकी गिरावट भी एक बड़ी चिंता का विषय है। टिकाऊ और गैर-भंगुर बायोडिग्रेडेबल कप बनाने के लिए क्रॉस-लिंकिंग पॉलिमर के साथ कार्बोक्सिमिथाइल सेलूलोज, गोंद बबूल और आलू स्टार्च जैसे बाइंडिंग एजेंटों को बाइंडिंग मैट्रिक्स में लुगदी के साथ मिश्रित किया गया था। टेबलवेयर की रेशेदार सामग्री को भोजन में प्रवेश करने से रोकने के लिए विकसित टेबलवेयर पर एक पतली फिल्म के रूप में पीएलए (बायो-प्लास्टिक) पॉलिमर लगाया गया था। विकसित टेबलवेयर को आम खाद्य-विषाक्तता रोगजनकों के साथ परीक्षण करके एंटी-माइक्रोबियल गुणों के लिए और अधिक सत्यापित किया गया था।

डायनेमिक स्कैनिंग कलरिमेट्रिक (डीएससी) द्वारा व्यापक तापमान सीमा पर जांच की गई थर्मल स्थिरता के साथ-साथ यांत्रिक कठोरता और कठोरता का अनुमान लगाया गया था। एफटीआईआर स्पेक्ट्रोस्कोपी द्वारा इसके कार्यात्मक समूह का अध्ययन करने के साथ-साथ पानी और तेल की घुलनशीलता और अवशोषण के लिए टेबलवेयर की भी जांच की गई। जाली संरचना और आकारिकी का अध्ययन एसईएम और एक्सआरडी विश्लेषण द्वारा किया गया था। विश्लेषणात्मक विश्लेषणों के आधार पर, चयनित दो बायोमास बायोडिग्रेडेबल टेबलवेयर के विकास के लिए संभावित बायोमास हैं और प्लास्टिक कटलरी खतरे से निपटने के लिए एक उपयुक्त विकल्प हो सकते हैं। बायोडिग्रेडेबल कप विकसित करने के लिए गूदे में बाइंडर अनुपात को अनुकूलित करने के लिए जिन तीन बाइंडरों का उपयोग किया गया था, वे थे कार्बोक्सी-मिथाइलसेलुलोज (सीएमसी), गोंद बबूल और आलू स्टार्च। तीनों में से, सीएमसी ने 2:3 के अनुपात में लुगदी के साथ सबसे अच्छा बंधन गुण दिखाया। इसी तरह, कटोरे के लिए, 75 जीएसएम शीट ने विभिन्न जीएसएम प्रकारों के साथ सभी संयोजन प्रयोगों के बीच सर्वश्रेष्ठ कटोरे का चयन किया।

लुगदी और कागज उत्पादन उद्योगों में फंगल संक्रमण एक बड़ी समस्या है। गूदे की लिग्रोसेल्यूलोसिक प्रकृति फंगल विकास को आकर्षित करती है, जिससे इस महत्वपूर्ण क्षेत्र को भारी नुकसान होता है। आवश्यक तेल और पौधों के अर्क विभिन्न फंगल रोगजनकों के शक्तिशाली अवरोधक के रूप में कार्य करते हैं। इस समस्या को दूर करने के लिए, वर्तमान अध्ययन का उद्देश्य कागज और लुगदी उद्योग में गंभीर फंगल संक्रमण को नियंत्रित करने के लिए वनस्पति के पायसीकारी सांद्रता (ईसी) फॉर्मूलेशन का परीक्षण करना है। जानबूझकर, ईसी फॉर्मूलेशन कर्कुमा लोंगा (हल्दी) और पोंगामिया पिनाटा (करंजा) तेलों का उपयोग करके तैयार किया गया था, जिन्हें अलग-अलग अनुपात (1.5, 1.25, 1.0 और 0.75%) में सॉल्वेंट्स (ध्रुवीय, गैर-ध्रुवीय) और सर्फैक्टेंट के साथ मिलाया गया था। फॉर्मूलेशन की जांच की गई और गर्म और ठंडी परिस्थितियों में फॉर्मूलेशन की शेल्फ-लाइफ का मूल्यांकन किया गया।

अंततः दो ईसी फॉर्मूलेशन प्राप्त किए गए (कण आकार 550-660 एनएम) और चयनित पेपर पल्प-इन्फेक्टिंग फंगल रोगजनकों (एस्परगिलस फ्लेवस, राइजोक्टोनिया सोलानी और फ्यूसेरियम ऑक्सीस्पोरम) के खिलाफ जैव-प्रभावकारिता परीक्षण (विकास अवरोध अध्ययन) किए गए। फॉर्मूलेशन TEC-3 (हल्दी तेल-आधारित EC) और TKEC-29 (हल्दी और करंजा तेल-आधारित EC) ने 1.5% (10.5 ग्राम/लीटर) सांद्रता के साथ आर सोलानी के खिलाफ 100% निषेध दिखाते हुए सर्वोत्तम प्रभावकारिता प्रदर्शित की। हल्दी तेल में अन्य बायोएक्टिव अणुओं और करंज तेल में करंजिन के साथ-साथ करक्यूमिनोइड्स और टेरपेनोइड्स की उपस्थिति, जैव-सूत्रों की एंटीफंगल गतिविधि के लिए जिम्मेदार हो सकती है।

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ABBREVIATIONS

PS- Paddy Straw

PN – Pine Needle (s)

TR- *Trichoderma reesei*

PO- *Pleurotus ostreatus*

PC- *Phanerochaete chrysosporium*

IARI- Indian Agriculture Research Institute

PDB/A - Potato Dextrose Agar/ Broth

ADF- Acid Detergent Fiber

NDF- Neutral Detergent Fiber

LME- Lignin Modifying Enzymes

SSF- Solid State Fermentation

Lac- Laccase

LiP- Lignin Peroxidase

MnP- Manganese Peroxidase

SR – Schoppler Reigler

GSM- Gram per meter Square

RSM- Response Surface Methodology

CCD- Central Composite Design

PFT- Poison Food Agar Test

GCMS- Gas Chromatography Mass Spectrometry

FTIR- Fourier Transform Infrared Spectroscopy

DSC- Differential Scanning Calorimeter

XRD- X-Ray Diffraction

CMC- Carboxy Methyl Cellulose

EC- Emulsifiable Concentrate

PLA- Poly Lactic Acid

WVPT- Water Vapour Permeability Test

DLS- Dynamic Light Scattering

TEC- Turmeric oil based Emulsifiable Concentrate

TKEC- Turmeric Karanjin oil based Emulsifiable Concentrate

TLC- Thin Layer Chromatography

ANOVA- Analysis of Variance

CFU- Colony Forming Units

Fig.- Figure

DMRT- Duncan's new Multiple Range Test

EC- Electrical Conductivity

PDI- Poly Dispersity Index

M- Molar

d- Days

h- Hour

s- Seconds

g- Gram

mg- Milligram

cm- Centimeter

mm- Millimeter

rpm- Rotations Per Minute

gL⁻¹ - Gram per litre

mL- Milliliter

μL- Microlitre

°C- Degree centigrade

mV – milli volts