BIOPACKAGING APPLICATIONS OF PINE NEEDLES: MICROFIBRILLATED CELLULOSE ISOLATION AND HANDMADE PAPERMAKING

Dissertation submitted in partial fulfillment of the requirement for the degree of

BACHELOR OF TECHNOLOGY
IN
BIOTECHNOLOGY

By

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UNDER THE GUIDANCE OF

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<u>Contents</u>

Title	**Page No.**

List of Abbreviations

- MCC – Microcrystalline Cellulose
- MFC – Microfibrillated Cellulose
- NFC – Nanofibrillated Cellulose

List of Tables

List of Figures

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

DECLARATION

I hereby declare that the work reported in the M-Tech thesis entitled **"Biopackaging applications of Pine Needles: Microfibrillated Cellulose isolation and handmade papermaking"** submitted at **Jaypee University of Information Technology,Waknaghat India,** is an authentic record of my work carried out under the supervision of **Dr Rahul Shrivastava**. I have not submitted this work elsewhere for any other degree or diploma.

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Date:

CERTIFICATE

This is to certify that the work reported in the M-Tech. thesis entitled "**Biopackaging applications of Pine Needles: Microfibrillated Cellulose isolation and handmade papermaking**", submitted by **Vedika Kayasth (141851)** at **Jaypee University of Information Technology, Waknaghat**, **India,** is a bonafide record of her original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

Dr. RAHUL SHRIVASTAVA

Associate Professor

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Date:

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Sincerely

Vedika Kayasth

ABSTRACT

Biopackaging is a new age technology that focuses on development of environment friendly, biodegradable packaging material derived from biological sources. Packaging is one of the most important aspects in preservation of quality and increasing the shelf life of a food product. It is a barrier between the food material and environmental as well as biological fouling agents such as water vapor, oxygen, solute migration and microorganisms. Majority of the packaging available is non-biodegradable and poses a great threat to the ecosystem. Packaging materials derived from biodegradable natural sources and edible films are the major parts of biopackaging techniques. There has been an upsurge in the use of bio based polymers as promising packaging material for food and medicine.

Pine trees are found in abundance in the Himalayan states of India and contribute to the deposition of pine needles in the forests in large quantities; as these are shed annually; and cause a menace. Pine needles as such do not have much of an industrial use and are an irritant to the local people as the form dense mats and do not allow the growth of fodder for the cattle. The aim of this project is to use pine needles for the extraction of Microfibrillated Cellulose (MFC) and utilization of the same for making Biocomposite Sodium Alginate and Biocomposite Agar films that can serve as candidates for food packaging and edible films. Another aspect of the project focuses on the utilization of pine needles for production of handmade paper so as to establish scientific proof of concept that can open arena for further research in the field.

MFC was successfully isolated from the pine needle sample giving an average yield of 42% and Biocomposites were also successfully prepared. It was also established that pine needles can also be successfully utilized for making paper and therefore further optimizations can be carried out for the industrial production of the same.

OBJECTIVES

- To isolate Microfibrillated Cellulose from pine needles
- To use isolated Microfibrillated Cellulose for making Biocomposite films
- To establish pine needles as a source for biodegradable tableware
- To make handmade paper from pine needles

CHAPTER I: INTRODUCTION

"Environmental pollution is an incurable disease. It can only be prevented." These words by famous biologist and an eminent environmentalist Barry Commoner point towards the seriousness and extent of a grave issue that is pollution. Environmental pollution has existed for centuries but only started to be significant following the industrial revolution in the 19th century. It is no longer a distant phenomenon but is now and here and is affecting the quality of our day to day life. It is high time that we realize our responsibility and start using eco-friendly processes so as to secure the survival of our very planet which is in danger.

Science is often looked at to find the solution to all our problems and so latest technologies are more focused on using environment friendly processes. Packaging waste forms a noteworthy part of urban solid waste and has been the cause of increasing environmental concerns, therefore in current years a spurge has been observed in the form of concentration in the use of biodegradable polymers for packaging materials so as to lessen the environmental pollution that is caused due to plastic wastes. Newer and novel bio-based materials have been subjugated to develop biodegradable and edible films as a huge effort to lengthen shelf life and improve quality of food at the same time reducing packaging waste. Nonetheless, the use of biodegradable and edible polymers has been inadequate because of issues related to performance (such as poor gas and moisture barrier or brittleness), processing (such as low heat distortion temperature), and cost.

Microfibrillated cellulose is one such bio-based bio-degradable material that has tremendous potential in the packaging industry. The cellulose fibers are separated into a three dimensional fibril network via the process of fibrillation, which have a large surface area. These separated entangled fibrils are termed as Microfibrillated cellulose (MFC).

The Himalayan Chir Pine is one of the most abundant tree species found in the forest cover in the area of Himachal Pradesh and other states of the Himachal as well as the Shivalik ranges of the Himalayas. It is a hard pine species that grows at an altitude of 500 - 2500m. These pine trees have a huge contribution in the deposition of the needles from these trees which are shed annually and form a dense mat over the forest floor. These shed needles are not much of use and are often a source of menace and forest fire due to the highly flammable nature. The deposition of pine needles also inhibits the growth of other plants by cutting off essential nutrient supply; therefore the lack of other plants is a common observation in the pine forests. Somen uses of pine needles have been reported, such as in fiber board, packing box, extracting essential oil, producing ligno-sulfonates and making pine wool, pine needles have not been utilized fully for any industrial purpose. [32]

Figure.1.1 – Chir Pine found in Himachal Pradesh

Pine needles are rich in cellulose and therefore can be used for the isolation of the same. The aim of this project is to use pine needles for the extraction of Microfibrillated Cellulose (MFC) and Nanofibrillated Cellulose (NFC) from pine needles and utilization of the same for making Biocomposite Sodium Alginate and Biocomposite Agar films that can serve as candidates for food packaging and edible films. If satisfactory results are obtained, models can be devised for large scale production of these biocomposite films for food and other packaging applications.

Paper is a flat and thin object, produced by pressing moist fibres of cellulose pulp which is generally isolated from wood, rags or grasses and then dried into flexible sheets. Paper is a handy material with numerous uses, for example writing and printing, cleaning, packaging, and a very large number of industrial as well as construction processes. The pulp and papermaking procedure is believed to have been developed in China at some point in the early 2nd century CE, possibly as early as the year 105 CE by Cai Lun.

The science, art and technology of paper-making deals with the methodologies, tools, and materials which are utilized to make paper and cardboard. These are used widely for writing, printing, and packaging, amongst numerous other usage and useful products. In the current times approximately the entire paper is contrived in the industries, but handmade paper still remains a particular craft and a means of artistic expression.

The utilization of pine needles for making paper at even small scale can be very helpful for putting the wasted biomass to use and could help in generation of employment opportunities for the villagers in the Hilly states of North India where pine needles are available in bulk and free of cost. This can also help in limiting fire hazards in these regions as dried pine needles are a major contributor to them annually. The production of paper can help in making way for further studies in the field of pulp & paper and to check the possibility of production of other packaging materials from pine needles like paperboard, cardboard, wrapping sheets etc. It will help in the promotion of environmentally friendly practices and support eco tourism in the Himalayan States of India.

CHAPTER II: REVIEW OF LITERATURE

2.1 BIOPACKAGING

Food packaging is the most essential agent in increasing the shelf life of food material and prevents spoilage by acting as a barrier between the environment and the food material. It is important to maintain food quality in the long run. It acts as obstacle to water vapor, aroma, gas, and solute migration between the food product and its environment as well as a variety of spoilage organisms present in the atmosphere. The organoleptic and nutritional characteristics of food in storage mainly depend upon the packaging and its barrier properties as it is able to ensure efficient protection against many kinds of degradation.[9] A large variety of materials are used for packaging applications which include metal, wood, glass, paper or pulp-based materials, plastics or combination of more than one type of material as composites.

But in the last few decades, majority of the food packaging is based on plastic and its derivatives. These materials pose a great threat to the environment as well as to the food packed in them. The fundamental problem of using plastics as packaging is the post-consumption waste. Packaging is the principal contributor (63%) of plastic waste. In addition to that, some materials are difficult to be reused. [9] It is anticipated that less than 14% of plastic packaging materials are recyclable.

Figure.2.1 – Biodegradable Packaging poster [24]

Many studies have revealed the passing on of harmful and carcinogenic compounds (for example plasticizers, additives, monomers, solvent residues from polymerization, by-products from polymer degradation, etc.) to the food with which they come in contact can occur from the plastic based packaging. [9] Moreover this type of packaging is non biodegradable and comprises a massive amount of every day wastes from across the globe. They pose a risk to environment and contribute to global pollution and other environmental problems. Owing to the need of alternate packaging, researches have been focused on production of biodegradable and nontoxic packaging material.

Bio packaging is a new age technology that focuses on development of environment friendly, biodegradable packaging material derived from biological sources. Packaging materials derived from biodegradable natural sources and edible films are the major parts of biopackaging techniques.

Biopackaging is often limited to edible films and bioplastics, but what is needed to be kept in consideration is that it is a bigger umbrella term which holistically includes biocomposites, bio-nanocomposites, biodegradable tableware and other such environmentally friendly packaging procedures. It basically involves all green packing technologies used throughout the world throughout the industries and is not limited to the food sector only. More weightage is given to the food sector because of its status of basic necessity and consumption of food items daily, which accounts for a huge chunk of the packaging pollution share throughout the world. Another reason for the same is because of the high costs of biopackaging, it has more potential to be absorbed in the food sector.

2.2 BIO PACKAGING CLASSIFICATIONS

2.2.A. CLASSIFICATION BASED ON RAW MATERIALS

2.2A.1 BIOPOLYMER BASED PACKAGING:

Biopolymers are naturally occurring biologically derived polymers which are obtained from different living systems and can or cannot be produced in vitro. They can broadly be classified into different categories based on the origin or manufacturing processes. These include natural biopolymers like plant or animal carbohydrates like cellulose, starch, alginate, agar, chitosan, carrageenan, etc., and proteins like corn zein, soy protein, wheat gluten, collagen, gelatin, casein, whey protein, etc. Another class of biopolymer based packaging includes synthetic biodegradable polymers such as poly(glycolic acid)(PGA), poly(l-lactide)(PLA), poly(-caprolactone)(PCL), poly(butylenesuccinate)(PBS), poly(vinyl alcohol) (PVA), etc. [13]

Apart from plant and animal origin, biopolymers arise from microorganisms and are produced by microbial fermentation for example microbial polyesters, such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), poly(hydroxyalkanoates) (PHAs) which also include poly(β-hydroxybutyrate) (PHB), etc., and microbial polysaccharides, like pullulan and curdlan. [13]

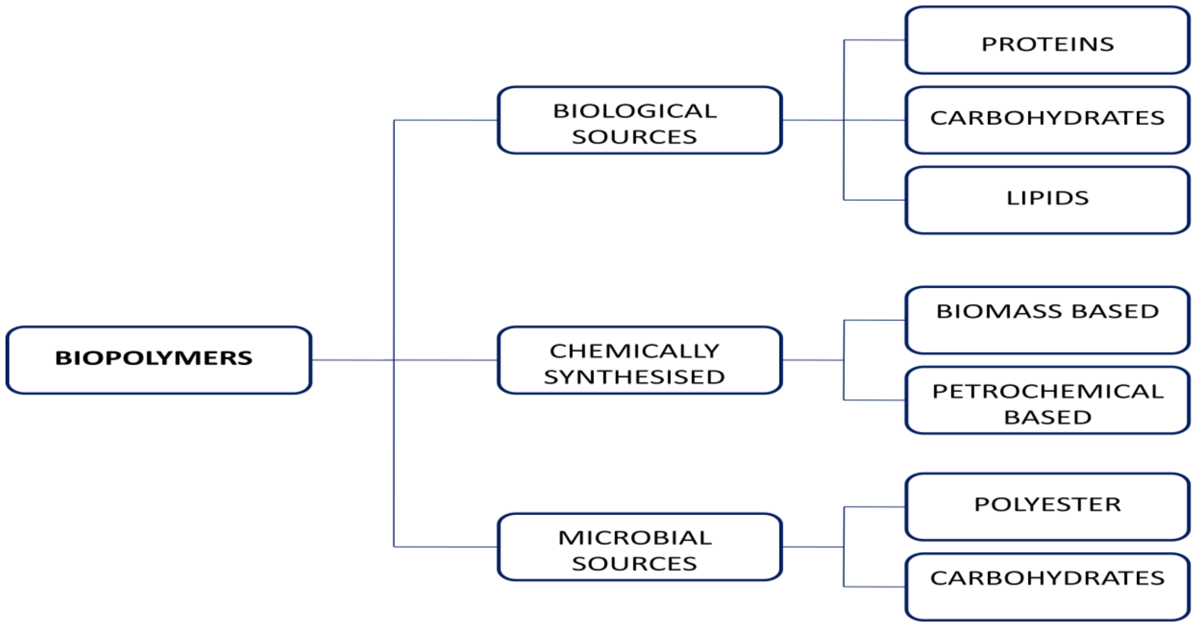


Figure.2.2 – Biopolymers used in packaging (Adapted from [13])

But biopolymers have relatively poor barrier and mechanical properties, which limit their industrial use. These issuess related with bio-based polymers are of three main types including performance, processing, and cost. Though these issues are rather interrelated but problems because of “performance and processing” are common to all biodegradable polymers irrespective of their origin. Certain particulars are low heat distortion temperature, brittleness, high gas and vapour permeability, poor resistance to extended processing operations. These shortcomings have strappingly limited their applications.

However researches in the last decades have focused on overcoming these shortcomings of biopolymers because even with the associated problems, biopolymers are far less harmful and toxic for the environment and the food items packaged in them and have applications much more than food in the near future.

2.2A.2 Nanocomposites:

As mentioned above, the use of biopolymers has been restricted due to problems related to performance, processing and cost. [45] But with the advancement of nanotechnology and its application to these polymers, new possibilities for enhancement have been generated. Biopolymer based composites have been developed by adding certain reinforcement compounds to polymers to enhance their mechanical, thermal and barrier properties. This is generally carried out by using nano-sized reinforcements including cellulose based nano-reinforcements, clays & silicates, carbon nanotubes and silica.

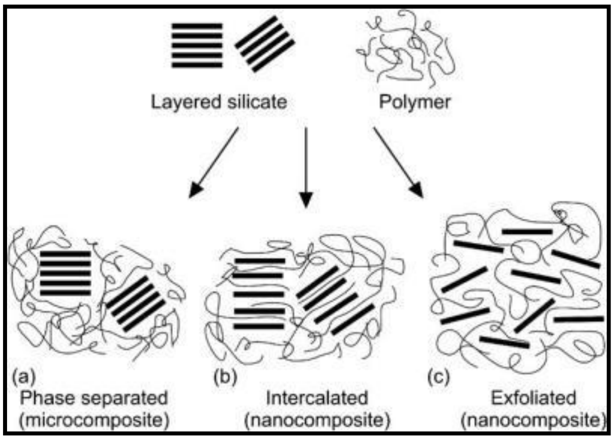


Figure.2.3 – Composites formed from interaction amongst clays and polymers [45]

Clays and silicates are inorganic solids which are one of the most widely used and considered forms of nano-reinforcements due to their easy availability, low cost and lesser processing requirements and considerable enhancement of barrier properties of the otherwise inefficient biopolymers. The most commonly used form is montmorillonite (MMT), which is a clay filler comprising of layered hydrated alumina-silicate.

Cellulose is a strong naturally occurring biopolymer and its microfibrils also have similar inherent properties. These are eco-friendly, low cost, widely available nano-materials with lower processing cost and therefore serve as ideal nano-reinforcement substrates. Two main types of nano-reinforcements can be derived from cellulose which are, fibrils and whiskers.[4]

Carbon nanotubes are a relatively newer technology in which, single nanotubes which are one or several atoms thick, are used as reinforcements. Another similar technology is Silica (SiO_2) nanoparticles which are used to improve barrier properties of several polymer matrices.

2.2A.3 Lipid based packaging:

The most widespread application of lipids in biopackaging is in the production of edible films due to their non toxic nature and the ability to be digested by humans without causing any harm to the system. Since the films are also biodegradable, they are efficient replacements for traditional plastic and wax coatings, specifically on fresh fruits and vegetables. Lipids are hydrophobic in nature and are therefore efficient water vapour barriers. The lipids which are used the most in edible films are fatty acids containing carbon atoms ranging from 14 to 18, stearyl alcohol, mono-, di- and tristearin, hydrogenated and non-hydrogenated vegetable oils, and waxes (beeswax, paraffin wax etc.).[8]

2.2B. CLASSIFICATION BASED ON END PRODUCT

Biopackaging for food industry has been vastly changing with time due to advancement in technology, from passive packaging to innovative packaging so as to cope up with global trends and consumer preferences. Active research is taking place in food industry and other scientific fields to develop innovative packaging materials including smart, intelligent and active food packaging which could be utilised for more competent and proficient packaging materials without a large amount of environmental issues.

Bio inspiration and mimicking the laws of the nature have been few of the ways through which nanotechnology has been applied to develop innovative food packaging. The vital cell machinery is mainly formed with the help of various biopolymers like carbohydrates, proteins or even nucleic acids and lipids, and these are involved in the regulation and control of biological functions. This is the reason why novel food packaging is based on biological systems so as to maintain the package integrity and food integrity in the food chains. The novel packaging technology is categorized majorly as intelligent packaging, smart packaging and active packaging systems. The details are further discussed below.

2.2B.1 INTELLIGENT FOOD PACKAGING:

Intelligent packaging means the packing material which keeps a check on the condition of the food or its environment packaged inside it and therefore reveals information like the temperature, pH etc. inside the package. This type of packaging system is able to sense, record and communicate the information to the user and hence has an additional function to the traditional packaging. These systems are developed with the objective of food safety, food quality management and to observe any problems during processing and delivery.

Intelligent packaging systems can be further characterized as data carriers (like radiofrequency identification (RFID) or barcode), sensors, indicators (like freshness or time-temperature indicators) and others such as hologram and organic light emitting diodes (OLED).[26]

2.2B.2 ACTIVE PACKAGING:

The active packaging systems are those in which the product, package and its environment interact in a positive manner contributing towards preservation of the quality of the packaged material. The main purpose of such packaging is to improve the shelf life of the food materials, maintain quality and safety of the food.[19] Active food packaging system can be categorized as scavengers (such as CO_2 or O_2 scavengers), blockers (like ethylene blocking), releasing (like antimicrobial or antioxidant packaging) and regulating or buffering packaging.[30]

2.2B.3 SMART FOOD PACKAGING:

Smart packaging systems are those which monitor and manage micro and nanostructural level changes that take place as the micro-environment of the packaged food changes. Smart packages have the ability to keep a check and modulate their interface properties based on the inherent bio-inspired surface motions, ionic channels, adsorption, wettability and adhesion characteristics.[30] the science of smart packaging development deals with shape memory polymers also called SMPs which are self healing, self heating and cooling materials and self cleansing materials out of which, self cleaning and self healing materials have a major share.[30]

A careful redesign of the packaging is essential to limit the packaging while ensuring that the packaging meets its functional and regulatory requirements. This redesign will also make primary and secondary packaging more recyclable.

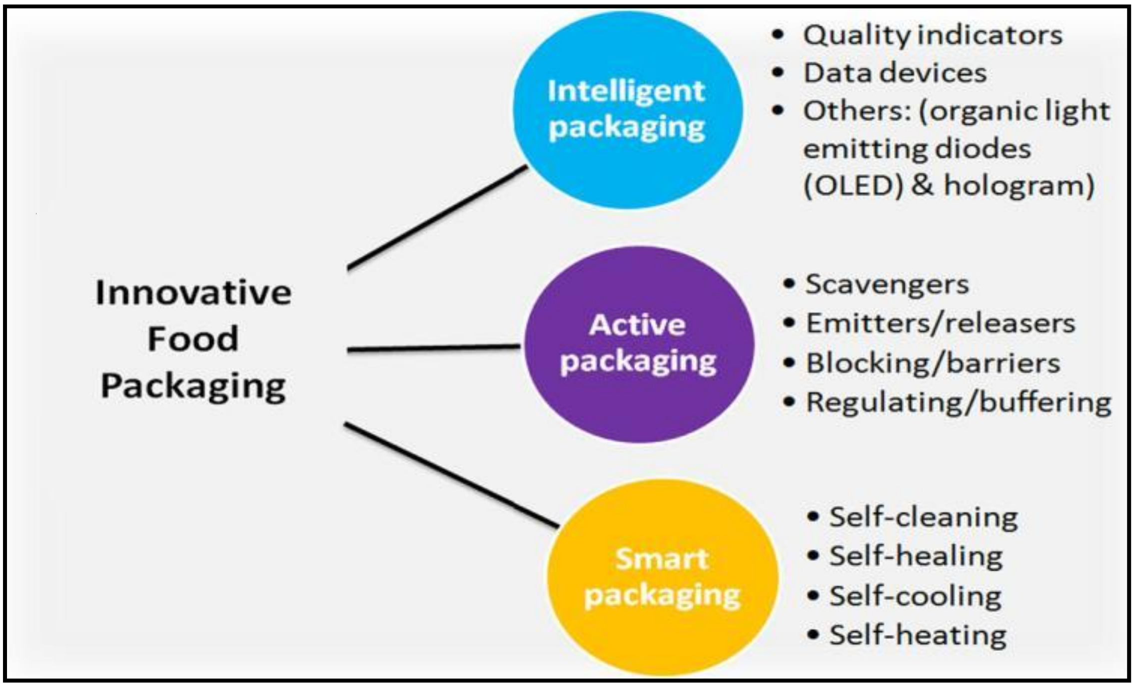


Figure.2.4 – Features and types of innovative food packaging[30]

2.3 CELLULOSE IN PACKAGING

Cellulose is the most abundant natural biopolymer occurring on earth which is the key component of the cell walls of woody as well as terrestrial plants. It is a linear homopolymer of glucose $(C_6H_6O_5)n$ with recurring units of D-glucose present in a 4C1 conformation. Cellulose is not soluble in water and is degradable by fungal and microbial enzymes.[5] Significant advancement in cellulose modifications by different modes like chemical, enzymatic or mechanical had led to an increase in the production of cellulose derivatives that have properties like physical, chemical or physiological, unique as compared to cellulose, and this has in turn resulted in an increase in cellulose related research.[5] Cellulose and derived fibers have been used as conventional packaging material in the form of paper and paperboard to pack various types of food products like frozen foods, beverages, liquid food products, dry and fresh foods etc. Another form of cellulose called cellophane, which is refurbished cellulose derived from natural sources like wood pulp via a physic-chemical method, is also used in food packaging, extensively as coating material.

Microfibrillated Cellulose (MFC) developed by Turbak et al. in 1983 is another interesting form of cellulose which has recently emerged as a promising packaging material because it displays plenty of mechanical as well as barrier properties which are essential for a good packaging material. Few other remarkable properties of MFCs are renewability, biodegradability and its application as an abundant fiber precursor. [43] The width of MFCs varies between 5 – 30 nm and these are highly crystalline in nature. MFCs are formed by lateral packing of long cellulose molecules containing H-Bonds in between. This results in a steady structure having very good mechanical properties like a high value of Young's Modulus: 138 GPa (in the crystal) and a very low value of coefficient of Thermal expansion 7 – 10 K^{-1} (along longitude). [43]

Researches in the past have revealed that MFC based packaging films can achieve the required strength at 32 g/m^2 base weight, which is a necessity for packaging applications. MFC based films also have desirable mechanical properties like 146nm/g tensile index, 8.6% elongation, 17.5 elastic modulus and 17 mL/m^2 oxygen transmission rates. These properties are comparable to oriented polyester ethylene vinyl alcohol based synthetic materials. [43]

2.4 PINE NEEDLES

Himachal Pradesh is one of the hilliest states in India. The state is naturally blessed with different kinds of natural fibers and amongst these fibers, pine needles are a renewable natural bioresource which is plentiful in many parts of this hilly region. These have several advantages which include swift growth, renewability, fairly high strength, and fine flexibility. But not much information is available in the literature about this bioresource.

Pine needles are shed annually which leads to their deposition on the forest floor as thick layers. These layers of dried needles are a serious fire hazard as they decompose at lower rates and also prevent any other vegetation growth due to blocking of seeds from reaching the soil. Some uses of pine needles like manufacture of fiber boards and packing boxes, essential oil extraction, manufacture of pine wool, lingo-sulfonates production etc. but these do not suffice for the industrial scale use of pine needles.

Figure.2.5 – Dried Pine needles

There is variation in composition of pine needle based on the geographical location. According to Rey et al. composition of pine needle is hollocellulose: 68.5%, lignin: 31.0% and extractives: 4.56% but studies carried out by Jan Asadullah show cellulose content of pine needles (41%) whereas the lignin content (35.1%) and ash content of pine needles (3.2%) The average fiber length of the pine needle is 13mm and the average diameter is 32ìm. The composition of pine needles according to Ghosh et al. is reported to be 64.12 % hollocellulose, 3.24% extractives and 27.79% lignin.

Parameters		Pine needle
Ash	%	4.45
Cold water solubility	%	2.98
Hot water solubility	%	7.53
N/10 alkali solubility	%	47.42
Alcohol benzene solubility	%	5.81
Lignin content	%	43.24
Holocellulose	%	51.62
Alpha cellulose	%	30.84
Beta cellulose	%	12.39
Gama cellulose	%	8.37

Table.2.1 – Contents of pine needle [33]

2.5 AGAR & AGAR FILMS

Agar is a natural hydrophilic colloid which is made up polysaccharides having the special property to form reversible gels that is done by cooling a hot aqueous solution. Agar has agarose and agaropectin subunits, and the overall polymer is made up of galactose as monosaccharide units. These units are generally 1,3-linked-D-galactose and 1,4-linked 3,6-anhydro-L-galactose arranged alternatively. Other subunits found in agar are sulfate esters, pyruvic acid residues or methoxyl residues. [15] It is the numbers and arrangement of the subunits that affect the gar gel physical properties and its functionality. The melting and gelling of agar on heating and cooling respectively can be repeated for unlimited times and so without any change in the mechanical properties of the same. Agar is naturally present in some red algae which are combined called as agarophytes. The class of algae which possess agar is Rhodophyta or red algae. It is present as an unbranced polysaccharide in the cell walls of algae genera *Gelidium* and *Gracilaria* functioning as a gelling agent. For commercial purposes the major source of agar is *Gelidium amansii*.

The subunits agarose and agaropectin are made up of galactose units with the former being a linear polymer of D-galactose and 3,6-anhydro-L-galactopyranose also called agarobiose and the latter having a heterogeneous alternate mixture of D-galactose and L-galactose sometimes with special side chains. Agar is soluble in hot water and it can be utilized to make a film if an appropriate plasticizer is added. This property is an indicative of the possible use of agar in packaging industry.

Molecular Formula	$C_{14}H_{24}O_9$
Molecular Weight	336.337 g/mol
Appearance	Transparent strips or pale powder

Table.2.2 – Properties of Agar

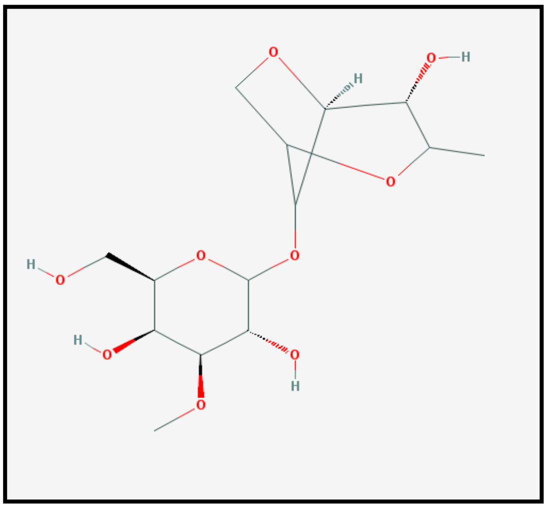


Figure.2.6 – Agar 2D Structure [25]

Agar is a natural plant/algae based gelatin equivalent. It is semi-translucent and white, and is sold in packages in powdered form or as washed and dried strips. It is used to make jellies, puddings, and custards in many of the cuisines world-wide. Agar has extensively used in biology as the growth material base for various microbial cultures and has a strong presence in the life sciences laboratories throughout the world. [44] In the life sciences laboratories, agar is used expansively in plant biology and microbiology and is supplemented with a vitamin and nutrient mixture which allows seedling germination or colony formation and growth (microbial) in Petri dishes under sterile conditions. The strong presence of this biopolymer projects it as a strong contender as a packaging candidate for food applications. In fact in the past researches agar has been utilized to make nanocomposite based on nanoclays with the resultant composite having improved mechanical properties and water vapor permeability.

2.6 ALGINATE & ALGINATE FILMS

Alginate is used to improve texture of food e.g. in ice cream or pie fillings, as an emulsifier, stabilizer and thickener. Alginic acid or algin or alginate, is present largely acoss the brown algae cell walls. It binds with water and forms a viscous gum like solution. [26] After extraction alginate can absorb water very quickly and it can absorb water upto 200-300 times its own weight. The color of alginate is variable, ranging from white to yellowish-brown. It is available in granular, filamentous or powdered form.

Molecular Formula	$C_{12}H_{20}O_{12}P_2$
Molecular Weight	418.228 g/mol
Density	1.601 g/cm3
Appearance	white to yellow, fibrous powder
Acidity (pKa)	1.5–3.5

Table.2.3 – Properties of alginate

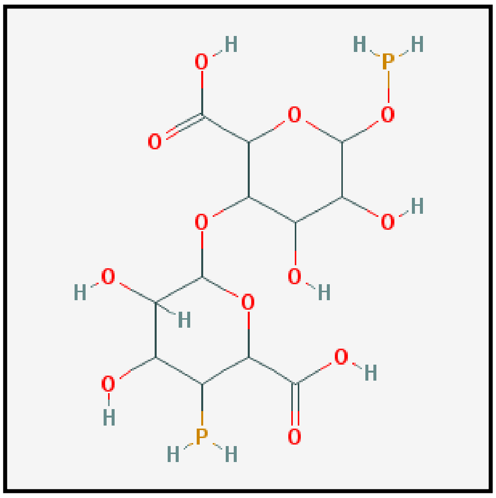


Figure.2.7 – Alginate (2D Structure) [26]

Alginate is a natural linear carbohydrate which is synthesized by brown algae *Ascophyllum nodosum* and *Laminaria digitata* and also by several types of soil bacteria. [16] Alginate is made up of M ang G block residues, where (1-4)-linked β-d-mannuronic acid is M block and α-l-guluronic acid is G block, present in variable proportions. In long chains, monomeric units are set alternately in the form of GG or MM blocks combined together with MG blocks. [18] It is anionic in nature and is soluble in water. Alginate is the only naturally occurring polysaccharide, which is naturally inclusive of carboxyl groups in each constituent residue, and possesses various abilities for functional materials. It is a potential biodegradable film candidate due to its colloidal properties like thickening, gel formation, stabilization, suspension, film formation etc. It is generally extracted with the help of alkali from brown algae. Another unique property of alginate is its ability to react with polyvalent metal ions specifically Calcium because of which it is utilized largely in the food industry. [41] It is also used in biotechnology for immobilization beads of enzymes or cells. Since alginate is edible, it is also a strong candidate for production of edible films.

The cross-linking of edible films with calcium is a property that is utilized in making biodegradable and edible films. It can also be used as a separating layer in heterogeneous foods so as to prevent moisture migration among elements which have different water activities. Alginate films can also be utilized as coating material for one-use cups or soup bowls made out of paper.

Alginate and alginate-based biocomposites have been used as adsorbents for ionic dyes, antibacterial films [39], wound healing materials and stimulus response drug releasing materials.[18] Though alginate based biocomposites have strong prospective as packaging material but there is a lack of required mechanical properties for example high strength in the polymer. To overcome this and increase the mechanical properties, alginate based biocomposites containing nanocrystals of cellulose as reinforcement agents have been studied in the past. Even then the information regarding the mechanical properties of alginate biocomposites is lacking and further investigation is required.

2.7 PAPER & PAPER MAKING

Paper is a very thin flat material, which is made by pressing together, moist fibres present in a cellulosic pulp generally procured from wood or grasses or rags, which is then dried to produce flexible sheets with a plain surface. Paper is a multipurpose material with major applications in writing and printing, packaging or cleaning and in numerous other industries as well as in construction processes. [46] The method and process of pulp and paper making is said to have originated in early 2nd century China, which was developed probably in the year 105 by Cai Lun. Even in the global modern pulp and paper industry China is the leading producer of paper and it is followed by the United States.

The technology of papermaking is the exact science and the subtle art dealing with the methods, materials and the apparatus that is used to make paper and paperboard. Their applications vary widely, ranging for writing and printing to packaging and in making of innumerable other useful products. Nowadays nearly all the paper and related material is manufactured in the industry, using automated heavy machinery, but even then handmade paper-making has the status of a craft and is considered a means of artistic expression.[9]

The overall procedure of paper making whether manual or industrial involves certain fixed steps from wood or raw material to paper which are:

- Pulping: conversion of raw material into a slurry/pulp
- Beating: evening out of the pulp
- Pressing: flattening of the beaten pulp and
- Finishing: smoothening and compacting and formation of paper reels

Half the fibre which is used for manufacturing of paper is obtained from wood that is specifically cultivated for the purpose of papermaking. The other half of the raw material is procured in the form of recycled paper, waste from sawmills, vegetable and vegetable related wastes and waste cloth. Earlier, conifers like fir and spruce were the principle sources of raw material for paper making because of the abundance in cellulose in the fibre content of the pulp and long fibers of the same. But in the past few decades, the demand of paper increased multi-fold and therefore the pulp processing technology improved which resulted in the utilization of any plant for papermaking. Some other plant species used to make paper are bamboo, sugarcane, flax, hemp, jute and straw. Flax, hemp and jute were earlier used for textile and ropes. Some high-quality cigarette paper is prepared from flax. [9] Cloth rags that are used for making good-quality paper like resume or letterhead paper, currency notes and security certificates etc. are generally derived from cotton or linen fabric. These rags are in general, waste cuttings coming from textile mills, to be utilized in manufacturing paper. The obtained rags are cut, cleaned and boiled and then beaten into pulp, so as to be used in the paper mill. Other resources used in paper manufacture include fillers such as chalk, clay, or titanium oxide, bleaches and dyes, and sizing for example rosin, gum, and starch.

The method of manual or handmade papermaking has changed very little with the passage of time, regardless of advances in technology. Handmade paper is also prepared in laboratories to study papermaking and in paper mills to check the quality of the production process. The procedure of manufacturing of handmade paper can be divided into five steps: [3]

- Separation: this involves separating the useable fibers from the remaining of raw material (for e.g. cellulose from wood, cotton, etc.). Screening of fibers includes with the help of a mesh generally made from non-corroding and inert material like aluminum stretched in a wooden frame (resembling a window).
- Beating of the separated fiber into pulp
- Adjustment of various properties (like color, chemical, biological, mechanical etc.) of the paper by addition of special chemical premixes
- Screening of the resultant solution
- Pressing and drying of this solution to get the sheet of paper

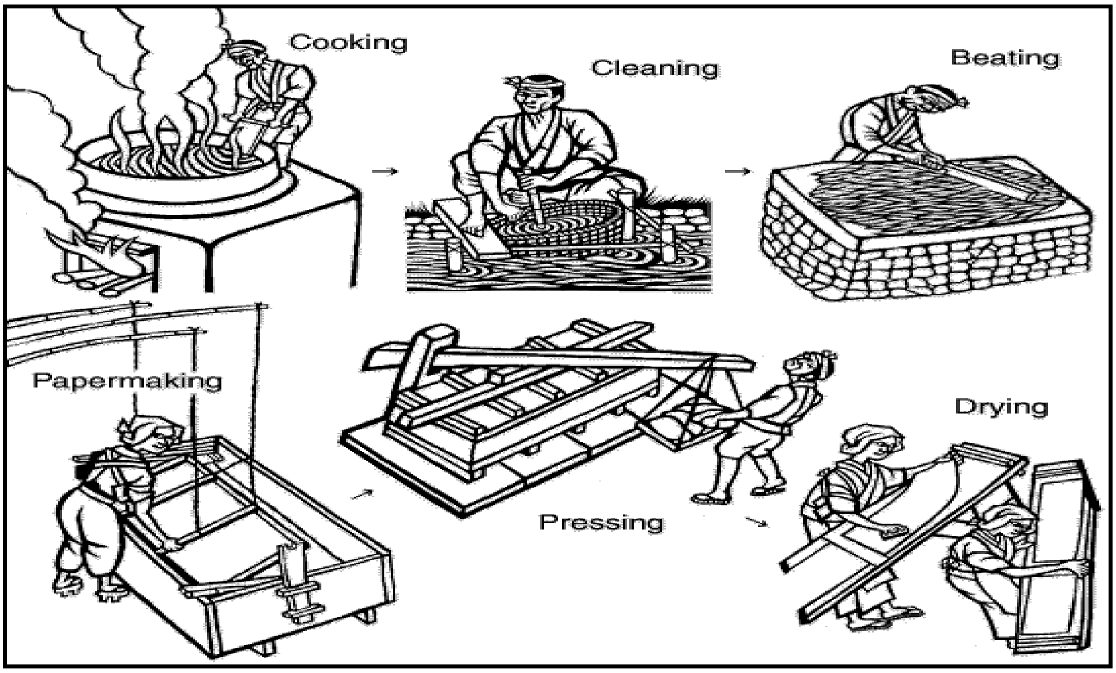


Figure.2.8 – Ancient procedure of papermaking [3]

The industrial production of paper is carried out in mills having huge machines which are generally automated and do not require human labor at any stage. Present day paper-making machines are based upon the principles of the Fourdrinier Machine, which utilized a moving woven mesh so as to create a continuous paper web, by filtration of the fibers held in a paper stock and production of a continuously moving wet mat of fiber. [46] The mat is then dried in the machine to fabricate a strong paper web. The overall process is the industrialized version of the ancient process of hand paper-making, which was not able to satisfy the demands of emergent present society for large quantities of printing and writing substrate.

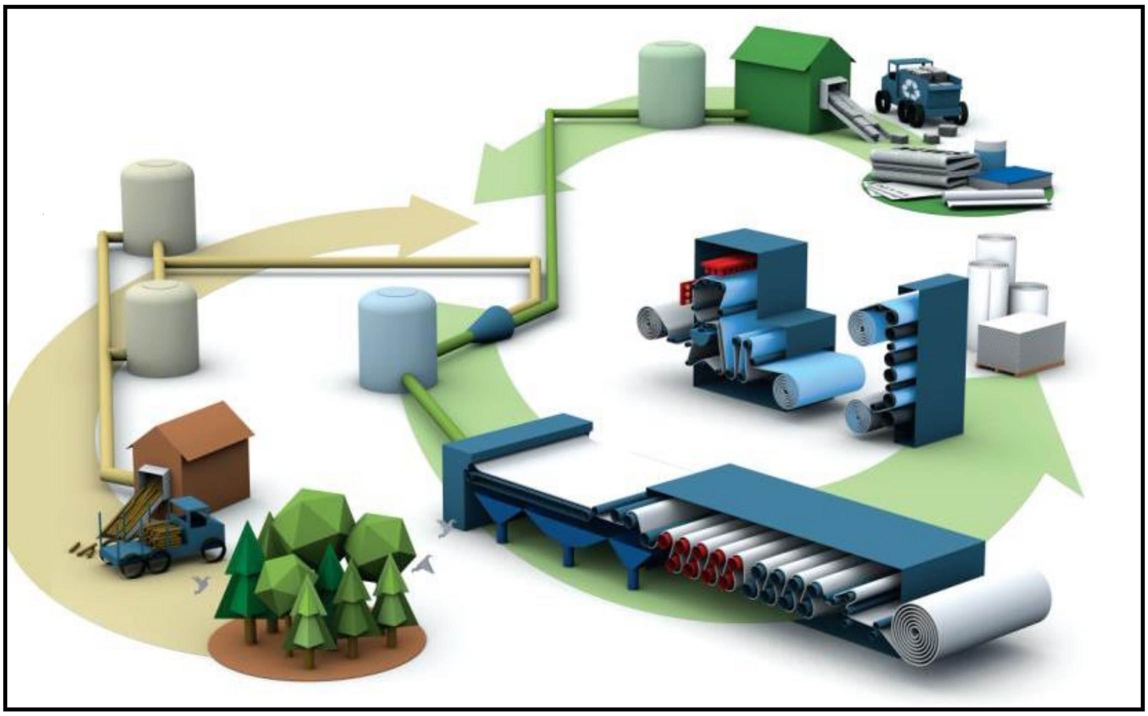


Figure.2.9 – Industrial papermaking process [9]

Paper machines generally have four distinct operational sections:

- Forming section- the slurry of fiber filters out fluid and forms a wet fiber web
- Press section- squeeze out maximum water from the web
- Drying section- pressed sheets are dried
- Calender section- dried paper is smoothened under high loading and pressure

CHAPTER III: MATERIALS & METHODS

3.1 PINE NEEDLE COLLECTION & PREPARATION

Shed dried pine needles were collected from JUIT, Waknaghat campus for isolation of MFC & NFC and fabrication of biodegradable tableware from the powdered form of the same.

Preparation of pine needles:

- Collected needles were washed with tap water until the effluent became free from mud and other particulate contaminants.

- These were then washed with distilled water and strained properly.

- Washed pine needles were then kept for drying in hot oven at 100ºC for 36 hours.

- Dried pine needles were grinded into a powder using a mixer grinder.

- Pine needle powder was filtered using a 60 mesh sieve.

- This powder was used for MFC isolation.

3.2 MICROFIBRILLATED CELLULOSE ISOLATION FROM DRIED GROUND PINE NEEDLES

Microfibrillated Cellulose was isolated from pine needle powder by chemical extraction using the method of Zuluaga et.al (2009) which they used on Banana rachis. The main objective of these treatments was to eliminate non-cellulosic components such as pectic substances, hemicelluloses and lignin. For this 2M NaOH stock solution was prepared in distilled water.

The chemical treatment steps are as follows:

- 5g of pine needle sample was treated with 300 mL 0.5M NaOH solution under mechanical stirring at 30ºC for 18 hours.
- The insoluble residue was extensively washed with distilled water for several hours, until pH was neutral.
- Then, the insoluble residue was treated with 200 mL 0.5M NaOH and 3 wt% H_2O_2 solution at 45ºC for 14 hours.
- The insoluble residue was again extensively washed with distilled water for several hours, until pH was neutral.
- Next treatment with 200 mL 2M NaOH solution at 55ºC for 2 hours was given in order to remove mineral traces.
- The insoluble residue was again extensively washed with distilled water for several hours, until pH was neutral.
- The insoluble residue was dried at 30ºC for 24 hours and its dry weight was taken.
- The isolated MFC was suspended in distilled water until further use and stored at 4 ºC.

3.3 BIOCOMPOSITE SODIUM ALGINATE AND AGAR FILM FORMULATION

Pure alginate and agar films as well as biocomposite alginate and agar films were formulated using the methods of Benavides et.al (2015) and Rhim et.al (2014) for alginate and agar respectively. The steps followed were:

3.3A) For Sodium Alginate films:

- Food grade sodium alginate was weighed to prepare the films. To make 1.5% w/v film of sodium alginate, 3g Sodium alginate was dissolved in 200mL heated distilled water.
- The solution was heated and stirred until all the lumps dissolved.
- To this, 0.243g Glycerol /g of Sodium alginate was added and mixed.
- The solution was casted in a 9cm Petri-plates.
- To make biocomposite films, 5% MFC by wt of alginate was added to the solution and mixed.
- This was also casted in Petri-plates and allowed.
- The films were dried at 30 ºC for 48 hours.

3.3B) For Agar films:

- To make 2% w/v film of agar, 3g agar was dissolved in 150mL heated distilled water.
- The solution was heated and stirred until all the lumps dissolved.
- To this, 0.9g Glycerol was added and mixed.
- The solution was casted in 9 cm Petri-plates.
- To make biocomposite films, 5% MFC by wt of alginate was added to the solution and mixed.
- This was also casted in Petri-plates and allowed.
- The films were dried at 30 ºC for 48 hours.

After the films got dried completely, they were peeled off from the plated and analyzed further.

3.4 BINDING WITH NATURAL GUM

In another experiment to check for the binding of Pine needle powder so as to establish its use in making biodegradable tableware, following steps were carried out:

- 2g Pine needle powder was mixed with 1g Acacia gum (2:1) in a beaker and 1mL of Glycerin was added as a plasticizer.
- Enough water was added to make a thick & even slurry
- Slurry was poured evenly on a Petri plate.
- This plate was kept at 40°C for 72 hours for drying.
- Dried product was scrapped to observe.

3.5 HANDMADE PAPER FROM PINE NEEDLES

For making handmade paper from pine needles the following procedure was completed at Department of Rural Management, Dev Sanskriti Vishwavidyalaya, Shantikunj, Haridwar.

- About 3kg Pine needle was digested with Caustic soda in excess of water inside the digester to make pulp for paper making. (Figure.13(a) – (1,2))

- The digested material was allowed to cool until it attained room temperature.
- Cooled pulp was then fed into the beater along with water where it underwent the process of cutting and softening to make smooth slurry. (Figure.13(a) – (3, 4))

- The pulp remained in the beater for 5-6 hours to improve water holding capacity.

- The slurry was passed onto a vessel in which a steel mesh was dipped and a sheet of pulp was taken out and excess water drained. (Figure.13(b) – (1,2))

- A piece of cloth was kept upon the pulp such that it sticks to it, to get an even surface.
- The obtained sheet was passed through a screw press to remove excess water. (Figure.13(b) – (3); Figure.13(c) – (1))

- After pressing, sheet stuck to the cloth was passed through roller to smoothen the surface. (Figure.13(c) – (2))

- Pressed sheet was sun-dried till the piece of cloth started peeling off from the paper. (Figure.13(c) – (3,4))

- Dried paper was passed through rollers to get a smooth surface of the sheet. (Figure.13(d) – (1,2,3))

- After this, paper was passed from callender machine to get sheets of a fixed size. (Figure.13(d) – (4))

Figure.3.1 (a) – PAPERMAKING PROCESS

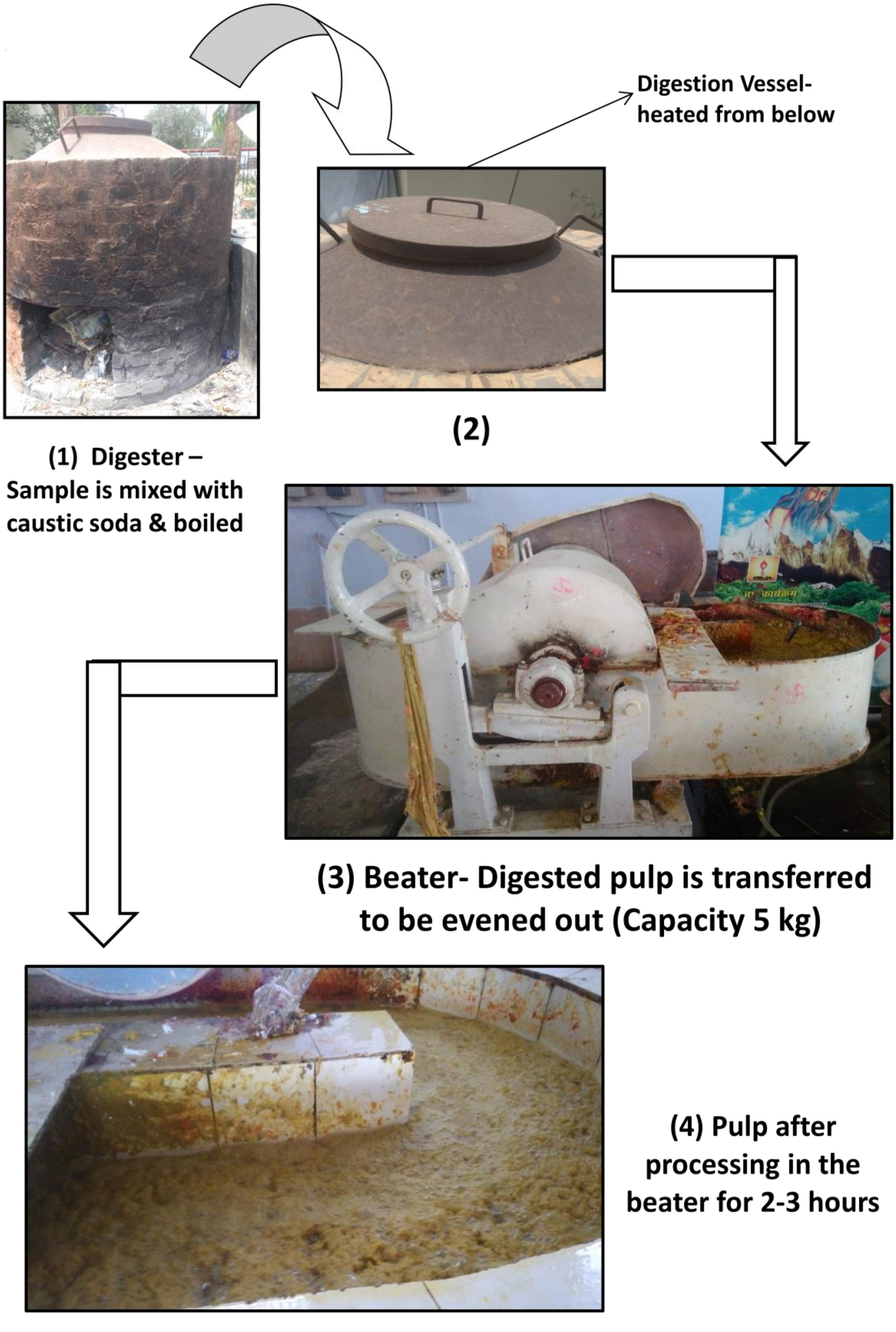


Figure.3.1 (b) – PAPERMAKING PROCESS

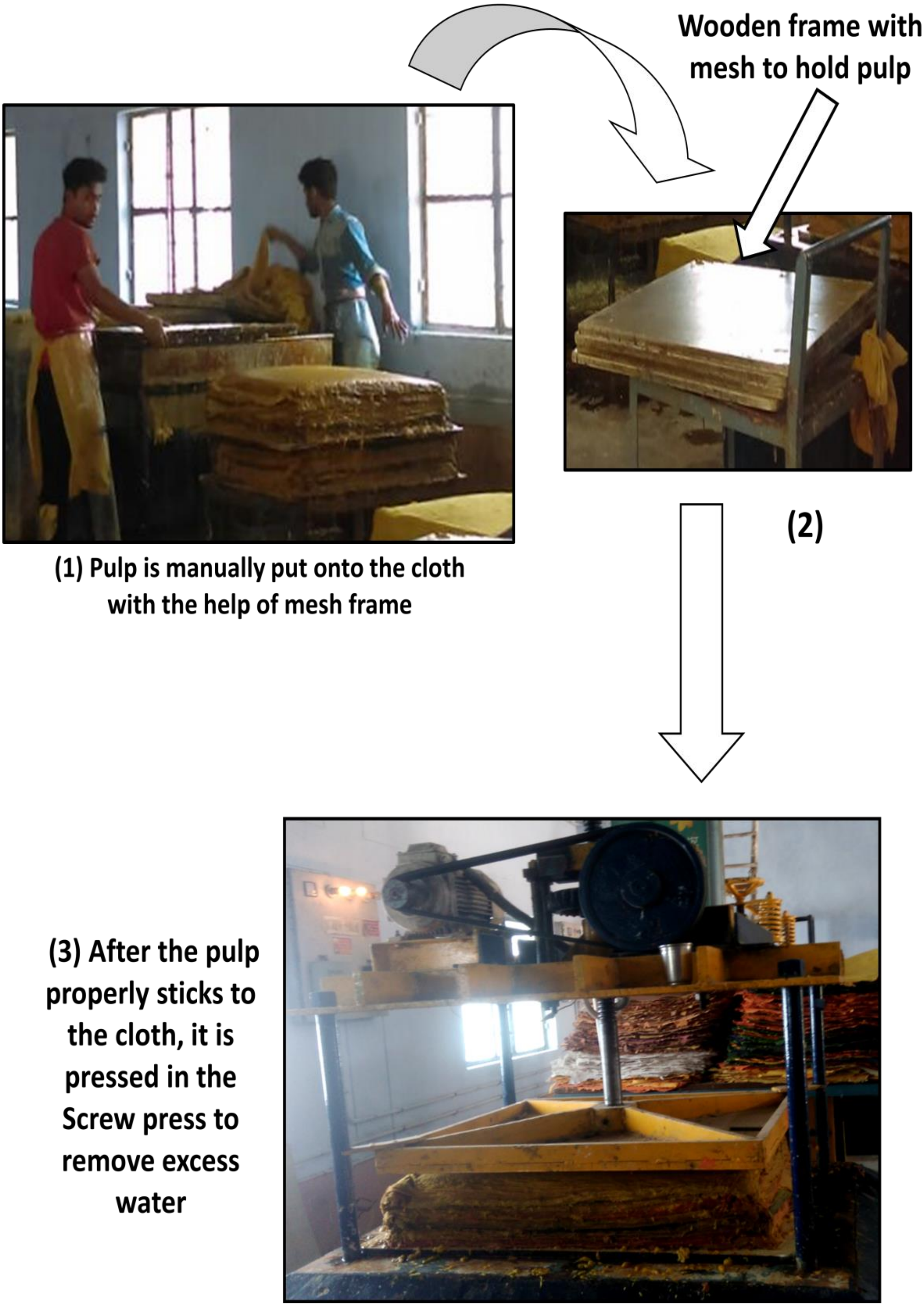
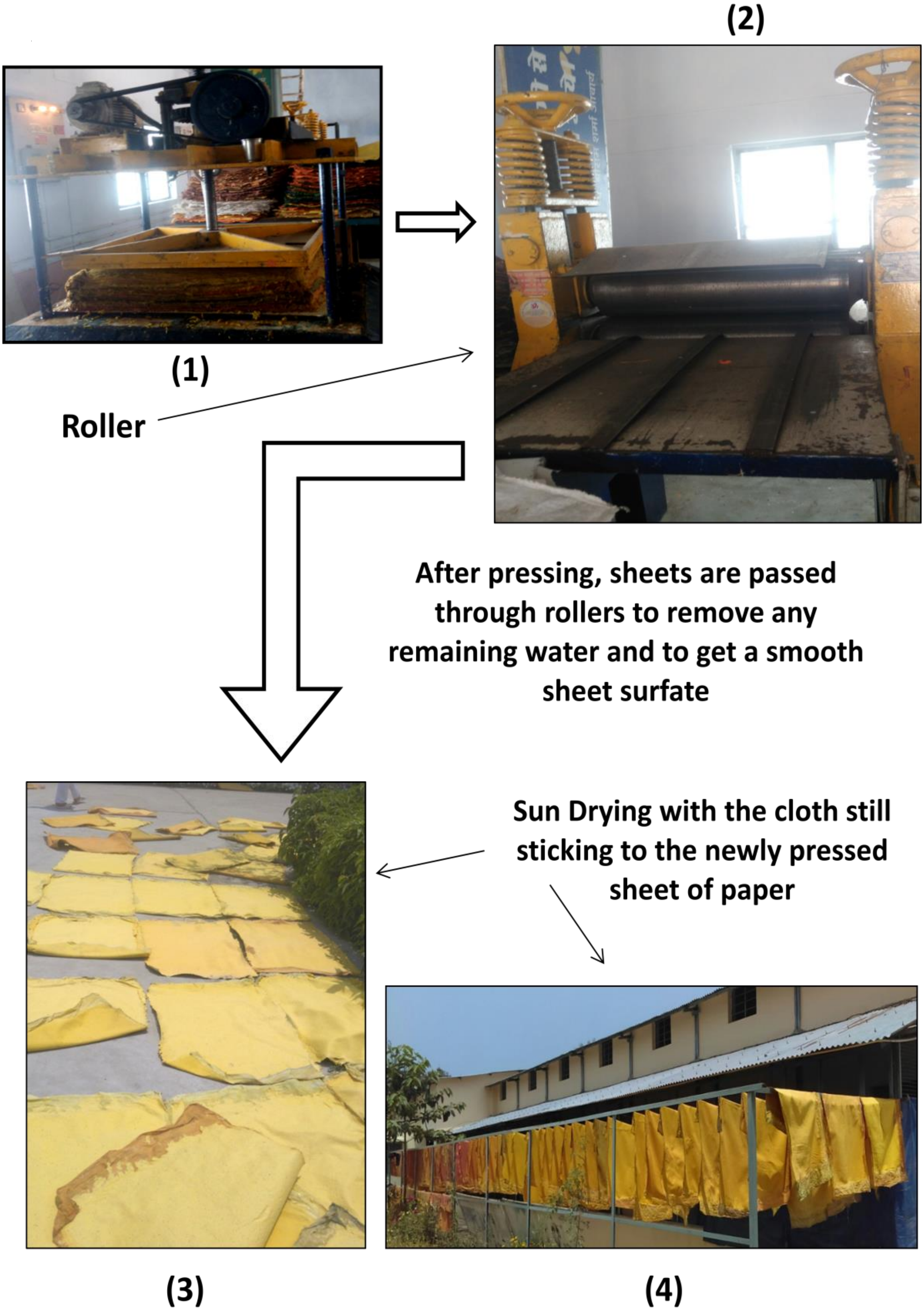


Figure.3.1 (c) – PAPERMAKING PROCESS



igure.3.1 (d) – PAPERMAKING PROCESS

(1) Paper sheets after separation of the support cloth

(2)

(3) Callendering Machine to smoothen the edges and surface of paper

(4) Final Product

CHAPTER IV: RESULTS

4.1 PINE NEEDLE COLLECTION & PREPARATION

Pine needles were collected from the campus of Jaypee University of information technology and were processed for further use. A fine powder of pine needles was obtained after the preparation steps.

Figure.4.1 - Dried collected pine needles

Figure.4.2- Pine needle powder after preparation

4.2 MICROFIBRILLATED CELLULOSE ISOLATION FROM DRIED GROUND PINE NEEDLES

The obtained fine powder of pine needles was given chemical treatments according to the method of Zuluaga et al. (2009) and it was found that the method is applicatble and effective for the substrate (pine needles.) Microfibrillated Cellulose (MFC) was obtained successfully and the average yield was 41.87%. The physical properties of isolated MFC are illustrated in the table1.

Figure.4.3- Pine needle powder after first NaOH treatment

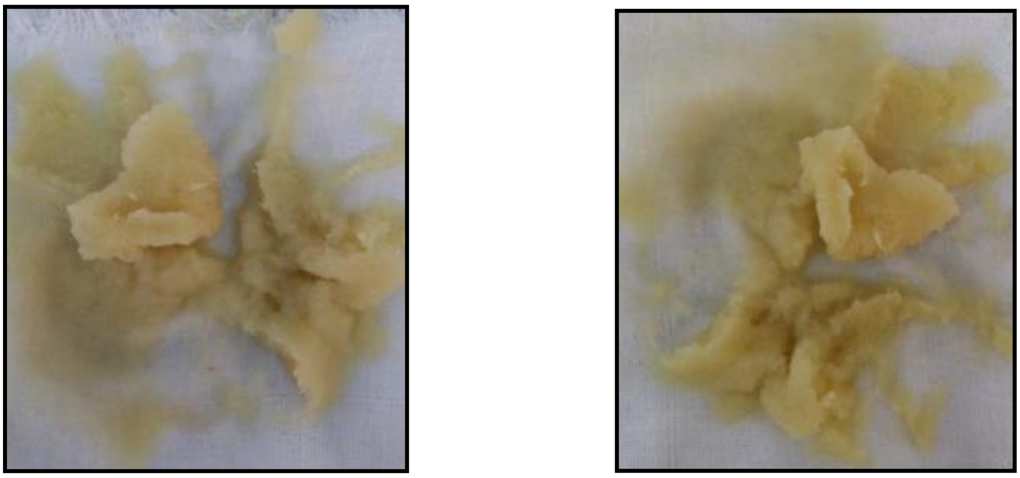


Figure.4.4- Freshly isolated MFC from pine needles after last NaOH treatment

MFC ISOLATED FROM PINE NEEDLES		
S No.	**PROPERTY**	**DESCRIPTION**
1	NATURE	Solid
2	COLOR	Yellowish white
3	TEXTURE	Very light and powdery when completely dried
4	YIELD	0.436g/g (2.18g from 5g powder)
5	SOLUBILITY IN WATER	Not soluble in water

Table.4.1 - Physical properties of MFC from pine needles

The product obtained was non sticky white fibrillated powder which is not soluble in water and complies with the pre-established characteristics of MFC. The yield of cellulose is also comparable to the cellulose content in Pine needles from earlier research. Therefore it can be said that the chemical isolation method of Zuluaga et al. is optimum for pine needles.

Figure.4.5 – Dried MFC isolated from Pine needles

Another part of this experiment included setting up two batches of needle samples including five samples each of 5g sample size and one batch of five samples of 2.5g sample size (Table7). The results of this part of experiment are shown in tables 5-6. The average yield of MFC was 41.87% which complies with previous data and hence establishes pine needles as a substrate for MFC isolation.

Batch 1				
S.No.	Sample (In g)	Product (In g)	Yield (g/g)	Percent Yield
1	5	2.01	0.402	40.20%
2	5	2.08	0.416	41.60%
3	5	2.12	0.424	42.40%
4	5	2.14	0.428	42.80%
5	5	2.17	0.434	43.40%

Table.4.2 – Yield properties of Batch 1

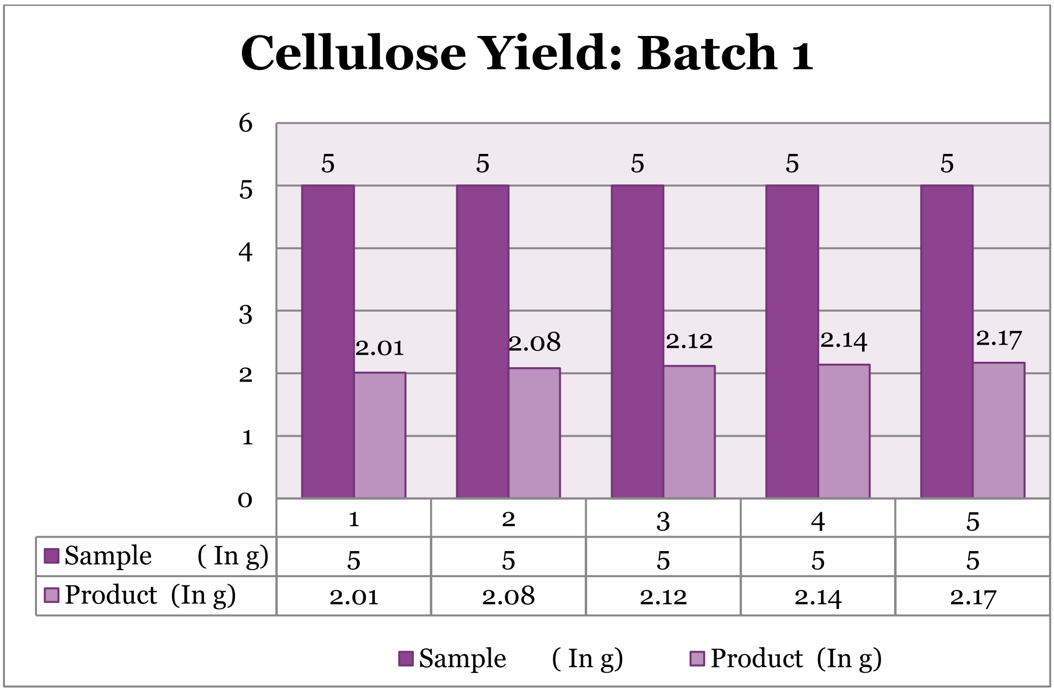


Figure.4.6 – Yield (Batch 1)

Batch 2				
S.No.	Sample (In g)	Product (In g)	Yield (g/g)	Percent Yield
1	5	2.18	0.436	43.60%
2	5	2.08	0.416	41.60%
3	5	2.01	0.402	40.20%
4	5	1.99	0.398	39.80%
5	5	2.15	0.430	43.00%

Table.4.3 – Yield Properties of Batch 2

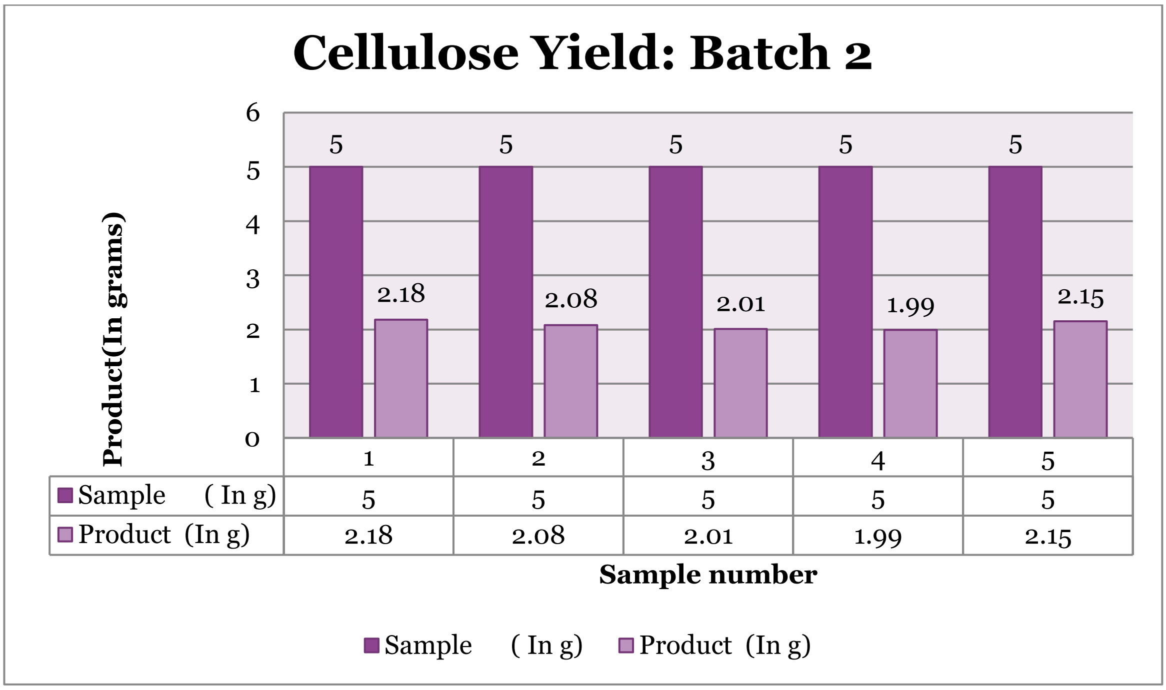
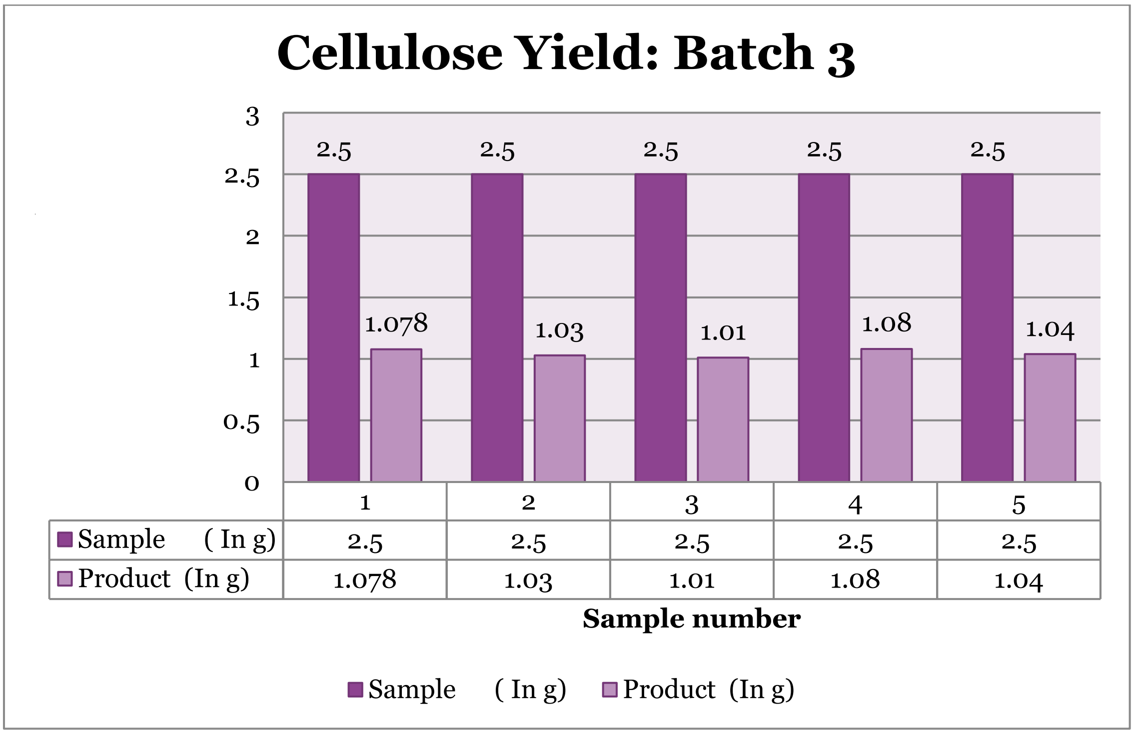


Figure.4.7 – Yield (Batch 2)

Batch 3				
S No.	Sample (In g)	Product (In g)	Yield (g/g)	Percent Yield
1	2.5	1.078	0.431	43.12%
2	2.5	1.03	0.412	41.20%
3	2.5	1.01	0.404	40.40%
4	2.5	1.08	0.432	43.20%
5	2.5	1.04	0.416	41.60%

Table.4.4 – Yield properties of Batch 3



	1	2	3	4	5
Sample (In g)	2.5	2.5	2.5	2.5	2.5
Product (In g)	1.078	1.03	1.01	1.08	1.04

Figure.4.8 – Yield (Batch 3)

4.3 BIOCOMPOSITE SODIUM ALGINATE AND AGAR FILM FORMULATION

MFC isolated from Pine needles (in 4.2) was utilized to make biocomposite films with agar and sodium alginate. The films casted demonstrate the properties illustrated in Table8. The biocomposite agar films had average grammage of 23.57 and that of sodium alginate was 5.9. Out of these, pure sodium alginate films got stuck to the Petri-plate surface and could not be isolated. The rest were comparatively satisfactory. If both types of films are compared to each other it can be observed that biocomposite from sodium alginate and MFC is lighter than that of agar and MFC.

S.No.	Sample Name	PROPERTIES			
		Film Weight (in g)	**Volume Poured (in ml)**	**Surface Area (in cm^2)**	**Grammage (in g/ m^2)**
Agar Films					
1	AR1	0.19	10	254.47	7.46
2	AR2	0.20	10	254.47	7.86
3	ARC1	0.56	10	254.47	22.0
4	ARC2	0.64	10	254.47	25.15
Sodium Alginate Films					
5	AG1	**NA**	**10**	**NA**	**NA**
6	AG2	**NA**	**10**	**NA**	**NA**
7	AGC1	0.14	10	254.47	5.50
8	AGC2	0.16	10	254.47	6.28

Table.4.5 – Properties of Biocomposite films

Figure.4.9 – Biocomposite agar film

Figure.4.10 – Biocomposite Alginate Film

4.4 BINDING WITH NATURAL GUM

The result obtained in this experiment was not so satisfactory probably because external pressure was not applied or the ratio of the binding agent (Acacia gum) to the substrate (Pine needle powder) was not good enough. Another plausible explanation is the improper mixing of the binding agent and the substrate.

Figure.4.11 – Some level of binding in Pine needle powder with Acacia gum

4.5 HANDMADE PAPER FROM PINE NEEDLES

An experiment was performed to check whether pine needles can be used to make handmade paper. The experiment was successful and therefore implied that it can serve as a substrate for papermaking at small and large scale with some optimizations and further research. The paper made from pine needles was comparable to the common use paper made from cellulose and had similarities with art paper.

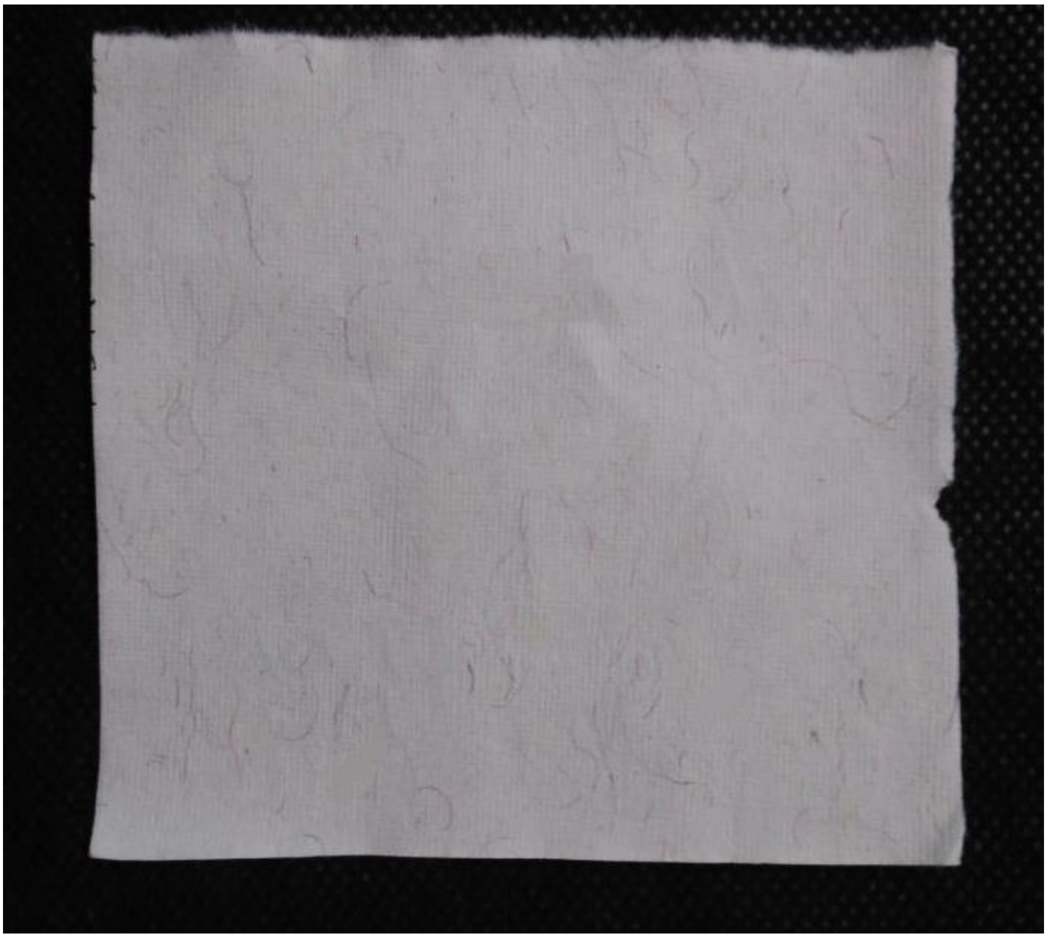


Figure.4.12 – Paper made from Pine needles

CHAPTER V: DISCUSSION & CONCLUSION

Biopackaging deals with the technology related to conception and materialization of biodegradable packaging materials majorly applicable in the food industry. It includes biocomposites, biopolymer based materials, biodegradable plastics, edible films etc. In the past few decades, research has been on a spurt in the field due to its applicability, harmless nature towards environment and food. Cellulose microfibrils are one such type of substrate which have huge applications in the field of biopackaging.[4]

Pine needles are available in the Himalayan and Sub Himalayan regions in large quantities annually. The shed pine needles have more disadvantage than advantages and therefore it is only reasonable to look for ways to utilize the wasted biomass. Since pine needles have considerable amount of cellulose, it is feasible to look for utilization for the same. The establishment of pine needles as MFC substrate will help in efficient utilization of the freely available biomass which is otherwise a source of menace.

From the experiments performed in this project, it was observed that pine needles can be utilized effectively for the isolation of Microfibrillated cellulose. The average yield of MFC was found out to be 41.8% which is comparable to the cellulose content of Pine needles reported earlier. This also implies that the isolation method of Zuluaga et al. which was originally optimized for Banana rachis is equally effective on Pine needles used as substrate. MFC isolated in the present experiment needs further characterization studies like FTIR analysis and SEM imaging etc. Since the major principle involved in separation of cellulose microfibrils, the grinding steps in the pre treatment preparation ensures that.

Although it is notable that chemical isolation procedures are demanding and require large quantities of water after almost every step which makes the process not so environmentally friendly in itself and therefore deviates from the objective of a green source for a green technology. Moreover some chemicals used in the process are also toxic to the environment. Keeping aside these considerations, it is noteworthy that even though the process is not so harmless but the end product is far more useful and less harmful than the substrate left idle itself. The applications of MFC are limitless and an optimum protocol is the first step in the direction.[6] It is observed from the previous research review that pine needles are not explored much in the area and yield study performed in the current project is vastly helpful.

Biocomposites are also a large part of biopackaging studies as they help in overcoming the limitations of pure biopolymers used in packaging. The biocomposites prepared in the present study are comparable to those reported earlier and hence prove that pine needles can have applications in the area as well. The study although requires barrier properties and biodegradability analysis. Further structure analysis of the produced films is also needed. From the currently obtained results it is evident that biocomposite agar film is heavier than that of sodium alginate. Sodium alginate films also have lesser solubility in water and are considered edible. The agar films at the same time are more transparent and can be helpful in packaging foods that require visual monitoring. Biocomposites are significant green packaging candidates and also have a huge application in edible films and packing, since these are generally made up of one or more edible polysaccharides or biopolymers. Edible films help in the significant increase in the shelf life of fresh as well as processed foods and are a safer alternative to wax and lipid coatings.[10]

The experiment performed for binding study did not yield satisfactory results, which is possibly due to lower gum to powder ratio i.e. binding agent to substrate ratio. Another explanation could be the improper distribution of the gum throughout the solution. Even then some level of binding shows that the area can be explored for further optimizations to come up with better results.

The successful use of pine needles in making paper paves way for limitless possibilities in the research area and points towards its applications in the paper and pulp industry as well as packaging industry where it can be used to make green packing material and be further explored for manufacturing of similar products. A Dutch designer Tamara Orjola has successfully utilized pine needles in making furniture and carpets which points towards limitless possibilities in the area. Another arena that opens up is the use of pine needles in the textile industry.

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Publication

- **A healing plate from sacred lotus: Biopackaging application of *Nelumbo nucifera* leaves**

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  Poster presentation: National Conference on *Recent Advances in Ayurvedic Herbal Medicine – From source to manufacturing* organized by
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NATIONAL CONFERENCE ON RECENT ADVANCES IN AYURVEDIC HERBAL MEDICINE – FROM SOURCE TO MANUFACTURING

A HEALING PLATE FROM SACRED LOTUS:
Biopackaging application of *Nelumbo nucifera* leaves

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AIM

To find out the possibility of using lotus leaf in production of environment friendly, biodegradable utensils and containers and observe their effect in enrichment of food material with respect to active compounds possessing healing properties hence establishing the neutraceutical potential of these utensils

INTRODUCTION

Lotus leaves used as an effective drug;[1]
- hematemesis
- epistaxis
- metrorrhagia
- hemoptysis
- hematuria

Extracts of lotus leaves have shown;[1]
- antihelmintic
- antidiabetic
- anti-angiogenic

Lotus leaf possesses high medicinal value which can be utilized in enrichment of the food products and preparation of special biodegradable utensils and containers

Upto 6.3 mg of *2-hydroxy-1-methoxyaporphine*, 1.1 mg of *pronuciferine*, 8.5 mg of *nuciferine*, and 2.7 mg of *roemerine* have been isolated from 100mg lotus leaf extracts in previous studies

The sacred lotus *(Nelumbo nucifera)* has long been used in traditional Ayurveda, Chinese, Japanese and Korean medicine for various purposes

Biopackaging is a new age technology that focuses on development of environment friendly, biodegradable packaging material derived from biological sources

Ideal packaging acts as a barrier between the food material and environment & biological fouling agents such as water vapour, oxygen, solute migration and microorganisms.

Majority of packaging available is non-biodegradable & poses great threat to ecosystem

Packaging materials derived from biodegradable natural sources and edible films are the major parts of biopackaging techniques

Self cleansing nature - aided by *superhydrophobicity* of lotus leaf surface - water droplets take other contaminants along with them roll off the surface rendering it clean

The *"lotus leaf effect"* or the superhydrophobicity of the lotus leaf points towards its potential usage in biopackaging

PROOF OF CONCEPT

Figure 1: Scanning electron micrographs of the adaxial leaf surface of lotus (Nelumbo)[4]

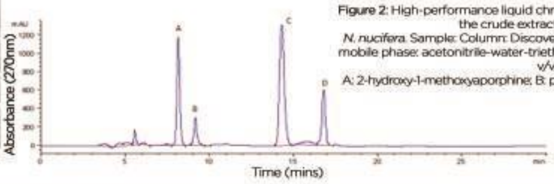

Figure 2: High-performance liquid chromatography (HPLC) chromatogram of the crude extract from the leaves of *N. nucifera*. Sample: Column: Discovery C18 column (25 cm × 4.6 mm, 5 μm); mobile phase: acetonitrile-water-triethylamine-glacial acetic acid (55:44:1:0.15, v/v/v/v); A: 2-hydroxy-1-methoxyaporphine; B: pronuciferine; C: nuciferine; D: roemerine[3]

Lotus leaves for making biodegradable utensils & containers by traditional methods (e.g. leaf plates called as *pattal* in Hindi)

Bioactive / Healing compounds enrichment by containers/utensils such as;

Roemerine, Nuciferine, Rutin, Isoquercitrin, Hyperin, Nornuciferine, Quercetin

Effectiveness for short term storage of food material

Qualitative tests to observe the transfer / leaching out of bioactive and healing compounds.

CONCLUSION

Lotus leaf -*superhydrophobic*; promising candidate for biopackaging application

Biodegradable containers and utensils; Natural & produced at low costs ($)

New employment opportunities (tribal & rural communities)

Manufactured in cottage industries supported by a scientific proof of concept

RESULTS

Biopackaging potential of biodegradable utensils/containers from Lotus leaf will be established

Neutraceutical potential of these utensils by enrichment of food by bioactive compounds like *Hyperin, Roemerine, Nuciferine, Quercetin, Nornuciferine, Isoquercitrin* can be checked

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