Sustainable development of an ultrafiltration membrane from banana pseudo stem for use in water filtration

The thesis is submitted in partial fulfilment of the requirement for the degree.

Master of Science

In

Microbiology

By

Swastik Manibhushan Mondal (217854)

Under the supervision of

Dr. Abhishek Chaudhary

&

Co-supervision of

Dr. Jitendraa Vashistt

to

Department of Biotechnology & Bioinformatics

Jaypee University of Information Technology Waknaghat, Solan-173234, Himachal Pradesh

Certificate

This is to certify that thesis entitled **"Sustainable development of an ultrafiltration membrane from banana pseudo stem for use in water filtration"**, submitted by **Swastik Manibhushan Mondal** in partial fulfilment for the award of degree of **Master of Science** in **Microbiology** to Jaypee University of Information Technology, Waknaghat, Solan has been made under my supervision.

Dr. Abhishek Chaudhary (Guide)

Assistant Professor (SG)

Dr. Jitendraa Vashistt (Co-guide)

Associate Professor

Candidate's Declaration

I hereby declare that the work presented in this thesis, entitled **"Sustainable development of an ultrafiltration membrane from banana pseudo stem for use in water filtration,"** in partial fulfilment of the requirements for the award of the degree of **Master in Science** in **Microbiology** submitted in the Department of Biotechnology & Bioinformatics**,** Jaypee University of Information Technology, Waknaghat is an authentic record of my work carried out over a period from August 2022 to May 2023 under the supervision of **Dr. Abhishek Chaudhary** (Assistant Professor, BT & BI) & Co-supervision of **Dr. Jitendraa Vashistt** (Associate Professor, BT & BI).

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

(Student Signature)

Swastik Manibhushan Mondal, 217854.

This is to certify that the above statement made by the candidate is true to the best of my knowledge.

(Supervisor Signature)

Supervisor Name: Dr. Abhishek Chaudhary

Assistant Professor (Senior Grade)

Biotechnology/Bioinformatics

Dated: 13.05.23

(Co-Supervisor Signature)

Supervisor Name: Dr. Jitendraa Vashistt

Associate Professor

Biotechnology/Bioinformatics

Dated: 13.05.23

ACKNOWLEDGEMENT

First and foremost, I thank "ALMIGHTY GOD" for his innumerable mercies on me.

I would like to convey my heartfelt gratitude to **Dr. Sudhir Kumar**, Professor and Head of the Department of Biotechnology and Bioinformatics, JUIT, Solan, Himachal Pradesh, for his kind assistance in completing the project work successfully.

I would like to thank my research supervisor, **Dr. Abhishek Chaudhary**, Assistant Professor (SG), Department of Biotechnology and Bioinformatics, JUIT, for his guidance, great support from start to finish, and constant ideas during this thesis work. I offer my heartfelt gratitude to him for his gracious assistance in all circumstances.

I would also like to thank my co-supervisor, **Dr. Jitendraa Vashistt**, Associate Professor (BT-BI Department), for his unwavering support and direction, as well as his belief and encouragement in me.

I would like to thank our Ph.D. scholars, **Dr. Monika Chaudhary**, **Ms**. **Sandhya Tegta**, and my dear friend **Purba Kundu,** for their ongoing encouragement, support, and direction during my thesis work.

I am grateful and lucky to have received encouragement, support, and advice from all of the lab technicians of the Department of Biotechnology and Bioinformatics, which assisted me in completing my thesis work.

Last but not least, I would like to express my gratitude to my friends and family for their invaluable company, suggestions, and counsel.

Swastik Manibhushan Mondal

(217854)

CONTENT

LIST OF ABBREVIATION

Abbreviation	Full forms
HMs	Heavy metals
NC	Nanocellulose
MF	Microfiltration
UF	Ultrafiltration
NF	Nanofiltration
RO	Reverse osmosis
CNF	Cellulose nanofibril
FTIR	Fourier transform infrared
PEF	Polyethylene film
CF	Cellulose film
FP	Filter paper
TBPS	Treated banana pseudostem
W.H.O.	World health organisation
LCB	Lignocellulosic biomass
H_2SO_4	Sulphuric acid
GRAS	Generally recommended as safe
NaOH	Sodium hydroxide
V/V	Volume/Volume
W/V	Weight/Volume

Abbreviation	Full forms
EMB	Eosin methylene blue agar
H.A.O.	Hot air oven
Gly.	Glycerol
SSW	Spiked sterile distilled water
UV-Vis	Ultraviolet visible

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Water pollution is a prominent cause of death globally, especially in rural and undeveloped areas where access to resources and municipally cleaned water is limited. Water pollution also contributes to the spread of many chronic, debilitating diseases. 25% of the world's population does not have access to clean drinking water at this time. Since the beginning of humanity's existence, nature has given us a variety of resources for our survival. Among them is cellulose, a biopolymer that is widely available, found in higher plants, and produced by some acetic acid bacteria. It has the potential to be employed as a water filtration membrane. We attempted to make a nanocellulose membrane from a banana pseudo stem by isolating and reducing the cellulose fibrils utilising several physicochemical approaches in this study. A suitable approach was developed, taking both efficacy and cost into mind. Following the curated approach, 50% filtration efficiency was obtained; however, based on the test results, additional optimisation is required to double the filtration efficiency.

Chapter-1

INTRODUCTION

The water crisis is a global concern that needs immediate attention. Water quality deterioration has indeed played an important role in several global challenges, including human access to drinking water and species survival. As of date, approximately 25% of the global population doesn't have access to potable water [1]. Water pollution is a leading cause of death worldwide, and it causes or contributes to the transmission of several chronic enfeebling diseases in people compelled to consume contaminated water. Water contamination has led to several critical implications, especially in rural and underdeveloped regions where the feasibility of resources and municipally treated water is lacking [2]–[4]

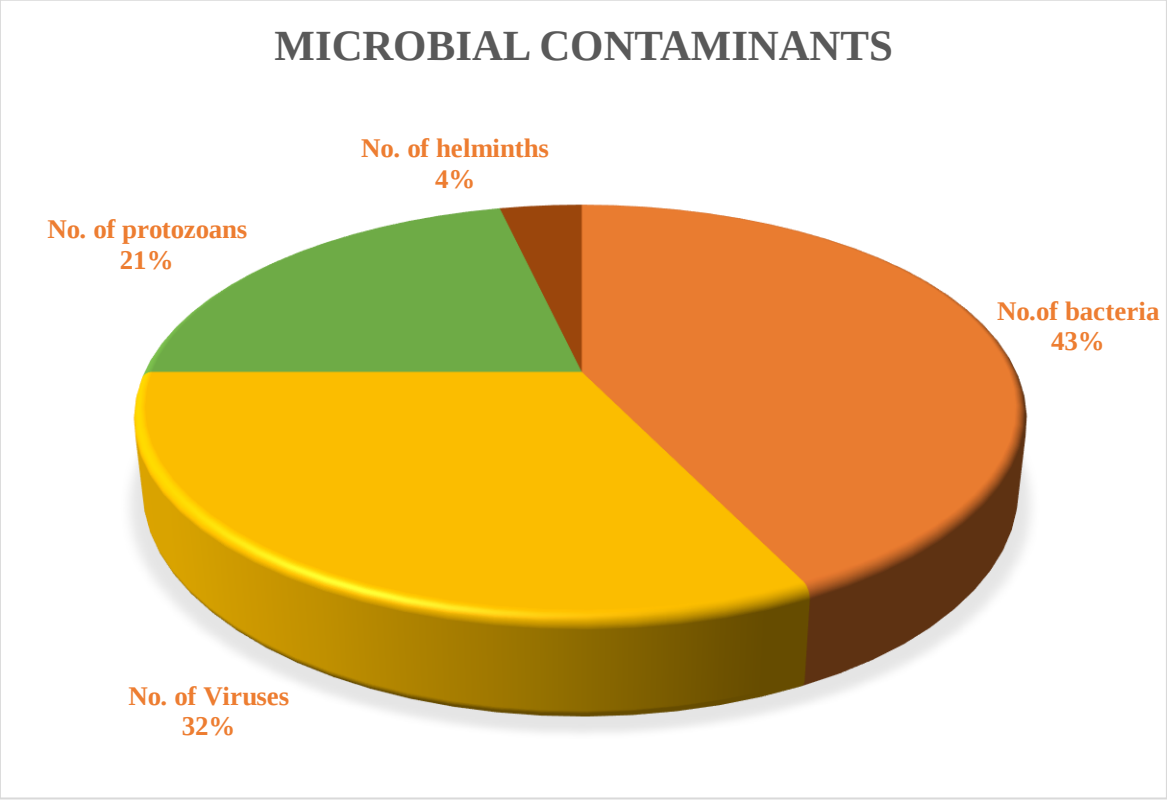


Fig. 1: Representing the prevalence of microbial type in water contamination

Potable water is simply one mode of transmission for diseases transferred via the colonic-oral pathway. Microbial drinking water safety is not solely concerned with contamination from faeces. Some organisms (e.g., Legionella) thrive in piped water distribution networks,

while others (e.g., guinea worms [*Dracunculus medinensis*]) occur in source waters and can cause outbreaks and cases by themselves. Furthermore, microbes, such as toxic cyanobacteria, necessitate specialized management strategies [2]

Diarrhoea, being the second leading cause of death in children of age below 5 years is mainly infested by a host of bacteria, viruses, and some protozoal pathogens and spread through fecally contaminated water [3], [4]. The occurrence of these diseases has been seen as more prevalent in lower-income underdeveloped countries [3]. Globally it accounts for approximately 525,000 deaths of children under the age of 5 years annually and is the main cause of malnutrition in children under the same age group [3], [4].

Along with microbiological contaminants, heavy metal pollution and its entrance into trophic levels of the food chain have exacerbated the situation. Heavy metals are elements of relatively high density ranging from 3.5 to 7 g cm^{-3} and show toxicity against cells at relatively low concentrations [5]. Fig.**2** represents the minimum concentration limit of some profoundly found heavy metal contaminants in water bodies.

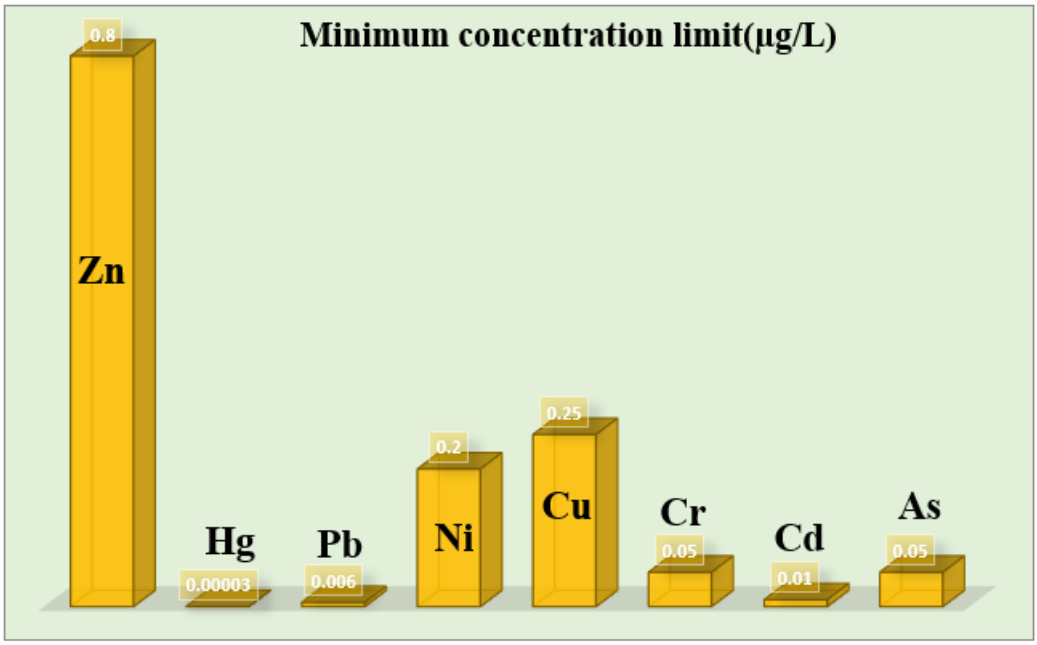


Fig. 2: Heavy metals contaminants & their permissible limit

These HMs contaminants mainly infiltrate the water bodies via. mining activities, mismanagement of industrial effluents, municipal wastes, and residential applications which further get biomagnified by entering into various trophic levels of the food chain [6] The primary reason for their toxicity is attributed to their biomagnification and bioaccumulation

tendencies which lead to interference with the native function of various proteins and enzymes on a molecular level [7]

Various medically critical conditions of acute and chronic status have been represented in the following table.1[5]

Heavy metals	Harmful effects
Zn	Skin irritation, nausea, depression, anaemia, neurological symptoms
Hg	Neurotoxin, Kidney dysfunction, Circulatory & Neurological Disorder
Pb	Central Nervous System Damage, Cerebral Disorders, Kidney, Liver Reproductive System Dysfunction
Ni	Carcinogen, Dermatitis, Gastrointestinal Disorder, Lung, Kidney Damage
Cu	Liver Damage, Convulsions, Insomnia
Cr	Carcinogen, Nausea, Diarrhea
Cd	Carcinogen, Kidney Dysfunction
As	Skin Problems, Visceral Cancer

Table 1. Heavy metal pollutants and their possible medical complications

Besides water treatment, water filtration has also proven an effective strategy to deal with these aforementioned issues. Various strategies have been explored for water filtration, especially by figuring out pore size and its applicability in the removal of various contaminants ranging from biological to inorganic metals.

Membrane filtration is a term most commonly used to refer to a thin single or multilayer material working as a sieve and allowing water along with a few suspended solids to pass through it [8]. The solids are allowed to pass depending on the pore size and the properties of the membrane which can be altered depending on one's objective. These membranes have been grouped based on their pore size as MF (0.5-5µm), UF (5×10^{-3}-5×10^{-1}µm), NF (7×10^{-4}-5×10^{-3}µm), and RO (>0.27nm) [9].

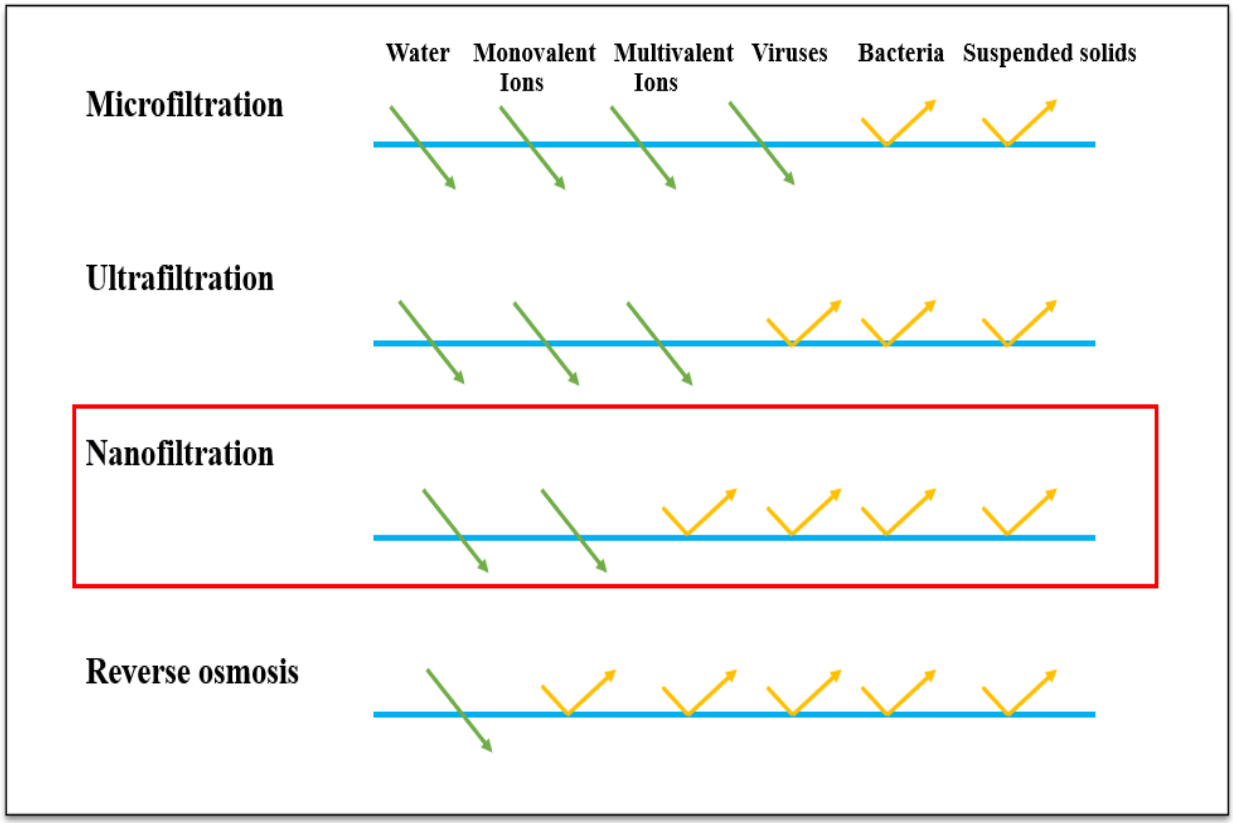


Fig. 3 Shows the range of entities being filtered out at a particular pore range

Chapter-2

2.1 Valorising of biomass “an emergence to save nature”

The idea behind utilizing unused biomass is becoming increasingly relevant in today's world as the cost of waste residue management can be circumvented and the alarming condition of environmental damage can be mitigated [10]. Furthermore, the usage of these residues could not only minimize waste but also have an economic impact by producing value-added products [11].

Cellulose, the most prevalent biopolymer, is a primary component of biomass and is estimated to be produced at a global scale of 75-100 billion tonnes annually [12]

This study aims to explore the nano-dimension range and develop a cellulose-rich membrane for water filtration such that some of the standards of water potability recommended by W.H.O. is attained.

Advancement in human resource development and the ambitious nature of the human species has led to ease of human life. However, it also has led to various challenges in recent and for upcoming generations. One such issue is climate change which endangers sustained global development and is mainly fuelled by the unsustainable use of fossil fuels along with other anthropogenic activities [10]. The quickest way to overcome this is to gradually replace fossil fuel and its derivatives consumption with renewable energy sources [10]. Biomass valorisation has emerged as a potential concept to strategically shift the exploitation of finite fossil fuels consumption and provide some feasible alternatives to its derived products [13].

The overall worldwide biomass supply through agriculture and forestry is projected to be around 11.9 billion tonnes of dry matter per year, with agriculture producing 61% and forestry producing 39% (fig. 4) [14]

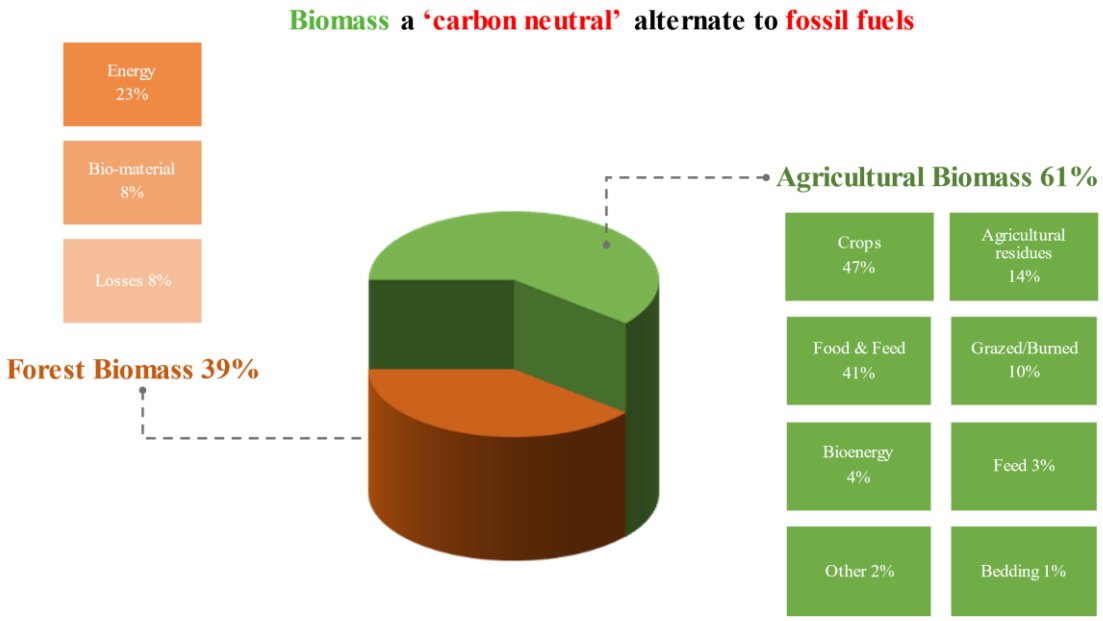


Fig. 4: Worldwide biomass supply through agriculture and forestry

These biomass sources can serve as an impeccable infinite resource for various purposes like fodder, paper, pulp, biofuels, chemicals, and other economically relevant biobased products [13].

One such utilization has been observed in the application of water potability where several scientific literatures has been published for processing cellulose-rich biomass into a functionalized membrane [15]–[17]

Cellulose is an abundant biopolymer having β-D-Glucose as its monomeric constituent linked via. β (1 ⟶ 4) glycosidic linkage with varying degrees of polymerization [18]. It is present in various sources such as hardwood, softwood, agricultural biomass, bacteria, algae, tunicates, etc [19]

Nanocellulose is a derivative of cellulosic biomass having one of its dimensions in the nanoscale i.e. (1-100nm) [19]. Depending on their constituent's morphology and origin nanocellulose can be categorized into three major groups namely cellulose nanocrystals (CNCs), cellulose nanofibrils (CNFs), and bacterial cellulose [20], [21]

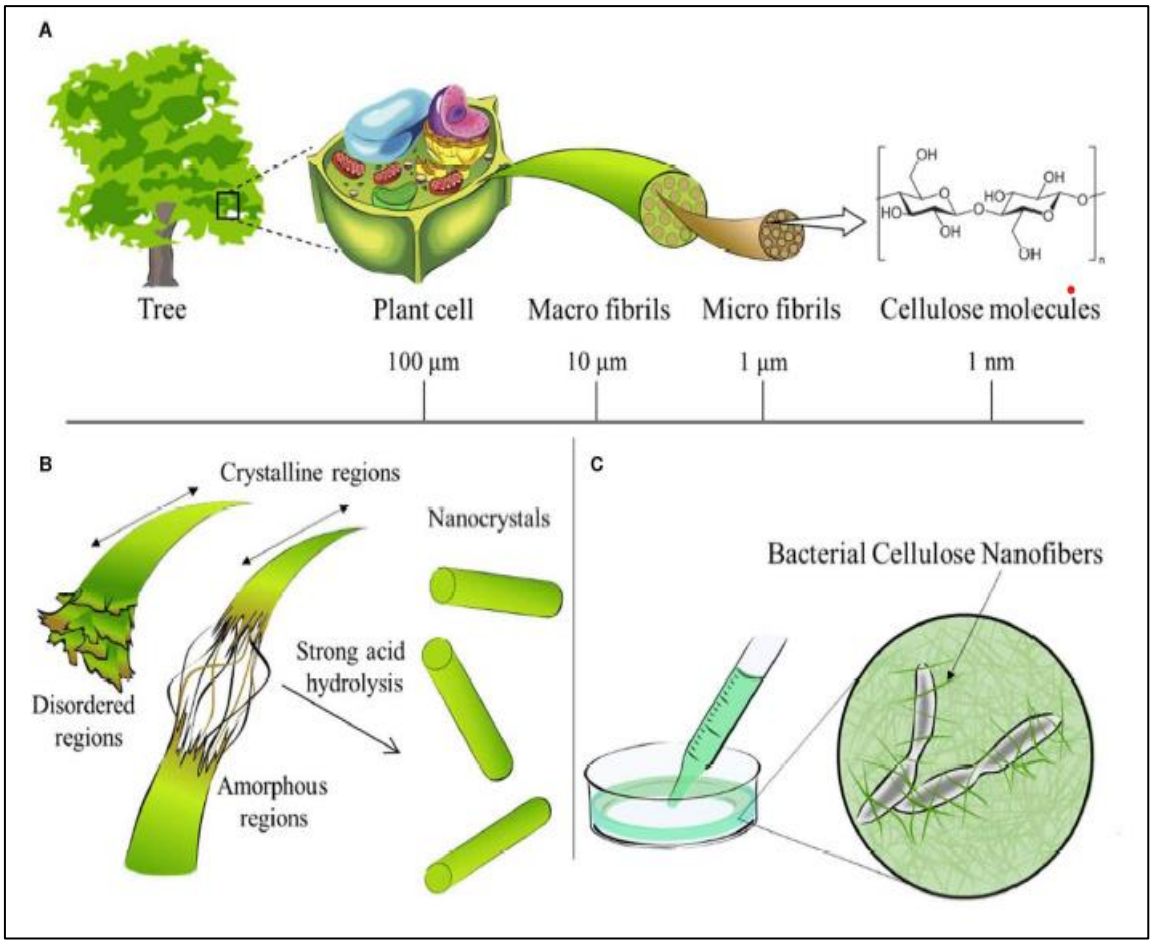


Fig. 5 (a) the hierarchal structure of cellulose, (b) cellulose nanocrystals formed via. acid hydrolysis, (c) synthesis of cellulose nanofibers by bacterial cells [22]

Working on the nanoscale offers various features such as mechanical strength, thermal stability, high surface area, high aspect ratio, ease of surface modifications, and unique solvent flow and membrane deformations which overall improve membrane stability [19], [23], [24]

2.2 Methods being explored to extract nanocellulose from LCB

Various techniques have been mainly focused on improving the crystallinity index along with hydrolysing the other secondary constituents, mainly hemicellulose, lignin, pectin, or other metal contaminants from LCB.

Depending on the kind of cellulosic biomass, these characteristics have been achieved using various combinations of physical, chemical, and enzymatic techniques for processing precursor cellulose into nanocellulose.

However, these methods can be grouped into two major categories either using a top-down mechanical shearing strategy or chemical disruption of the precursor cellulose fibres. Each of these methods has some limitations, thus an effective strategy of combining them can provide a more optimum solution for nanocellulose production with a higher yield and lower cost.

One such example is mechanical defibrillation of cellulose precursors, which involves high shearing forces that defibrillate the cellulose bundles, resulting in micro-fibrillated cellulose production. The method's main shortcoming is the high energy required for appliance operation. Various methods involving high-pressure homogenization, microfluidization, grinding, cryo-crushing, and ball-milling have been used [19].

Some commonly used strategies to defibrillate LCB have been summarised in the table below.

Processes	Mechanism of synthesis
High-pressure homogenization	Passage of precursor from a small orifice under high pressure resulting in exposure of the sample to sudden intense turbulence and hydraulic shear.
Microfluidization	Passage of cellulose precursor under high pressure through a channel result in the disintegration of fibres via. a higher constant shear force. Less prone to clogging due to constant shear rate [19]
Grinding	Mechanical shearing between two rough surfaces leads to the defibrillation of NC. During grinding the heat being generated evaporates the water of the suspension [19]
Cryo-crushing	Water-retained cellulose fibres are exposed to N_2 which solidifies the retained water followed by grinding in a mortar pestle [19]
Ball-milling	The collision between balls inside the container defibrillates the NC [19]

Table 2. Degumming strategies of the LCB

Numerous strategies have been investigated to effectively overcome the constraints faced by mechanical defibrillation of cellulose fibre, such as cutting, acid or enzymatic hydrolysis, TEMPO-mediated oxidation, or the insertion of charged groups through carboxylation. All of these procedures prepare the membrane and lower the amount of energy required. [25].

Depending on the synthesis and optimization method the zeta potential value of the nanocellulose may vary. The negative zeta potential value indicates the negative surface charge of the NC mainly imparted from the hydroxyl groups [26]. The value of zeta potential is detrimental in maintaining the NC in a dispersed form mainly through repulsion between like charges. A value less than 15mV usually causes agglomeration, but a value more than 30 allows NC to remain suspended [19]

NC generated using the TEMPO oxidation approach has the highest zeta potential value of (-69mV), followed by acid hydrolysis at a zeta potential value of (-34mV), but high-intensity ultrasonication has the lowest zeta potential value of (-24mV), resulting in an unstable agglomerated NC form [19]

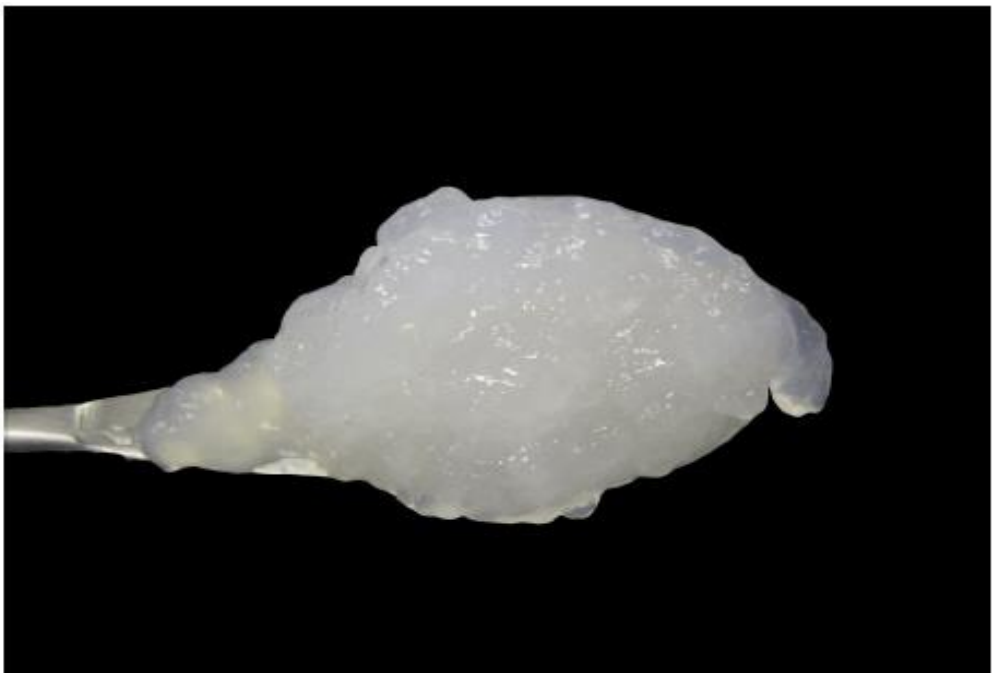


Fig. 6 Picture of a 2 w/v % micro fibrillated cellulose aqueous suspension from eucalyptus, enzymatic pre-treated [25]

2.3 Selection of LCB as an initial substrate

As the synthesis on an industrial scale requires a competitive price point for the product to be easily marketed, the substrate becomes an intrinsic part of determining the overall production cost and its acceptance to the general population.

It has been a challenge to narrow down to a substrate that can be used, giving the optimal yield and requiring minimum processing. The choice of a particular biomass will differ depending on the region and its availability.

Various scientific literature has already been published where a vast array of biomass has been used as a precursor for NC synthesis.

Natural source	Methodology	References
Filter paper and microcrystalline cellulose	Solution plasma-chemical processing as an oxidation-hydrolysis strategy	Surov et al., 2018
Cotton linters	Single step ammonium persulfate-assisted swelling, followed by oxidation	Wang et al., 2019
Eucalyptus hardwood	Irradiation oxidation and organosolv solubilization	Zhang and Liu, 2018
Wood sawdust	Sono-chemical synthesis using acid hydrolysis	Shaheen and Emam, 2018
Blue agave leaves and bagasse fibers	Sonochemical acid hydrolysis enhanced with sonication	Robles et al., 2018
Lignocellulosic biomass	Hydrolysis by Ni(II)-transition metal salt followed by washing with distilled water, centrifugation, sonication and dialysis	Yahya et al., 2018
Oil palm	Sono-assisted TEMPO oxidation	Rohaizu and Wanrosli, 2017

Table 3 Various cellulosic biomass used as a precursor for NC synthesis[22]

Bananas plants are cellulosic-rich biomass especially abundant in tropical and subtropical regions. Approximately 70 million metric tons of bananas are produced every year by the tropical and subtropical regions of the world and India is the leading producer of bananas yielding an annual output of about 14.2 million tonnes [27].

It shows some significant intrinsic properties which can be exploited to get a good yield of NC, especially in countries like India, China, and other tropical and sub-tropical countries where the conditions for banana plant growth are optimum [28]

Fiber	MFA (degrees)	Cellulose crystallinity	Cellulose content (%)	References
Hemp	6	50–90	70–74	Gassan et al. (2001)
Flax	6–10	50–90	64–71	Gassan et al. (2001)
Jute	8	50–80	61–72	Gassan et al. (2001)
Sisal	10–25	50–70	66–78	Gassan et al. (2001)
Banana	11	45–55	44–64	Gassan et al. (2001)
Coir	30–49	27–33	32–43	Gassan et al. (2001)
Sisal	10–25	50–70	66–78	Gassan et al. (2001)
Ramie	8	64	76	Gassan et al. (2001)
Abaca	22.5	52	62	John and Sabu (2012)
Cornstalk	10.9	52–59		John and Sabu (2012)
Soybean straw	12	47	85	John and Sabu (2012)
Oil palm	46	20–30	40–50	Mohanty et al. (2005a,b)
Bagasse	14–15	51.1	52	John and Sabu (2012)
Pineapple leaf fibers	12–14	44–60	70–82	Mohanty and Fatima (2015)
Bamboo	2–10	40–60	26–60	Mohanty and Fatima (2015)
Softwood fiber	3–45	52–62	40–45	John and Sabu (2012)
Eucalyptus	3–44	68	40–50	John and Sabu (2012)
Pine (kraft pulp)	N.D	68	76	John and Sabu (2012)

Table 4 Intrinsic properties of some studied fiber [22]

Banana stem is waste biomass produced in vast quantities after fruit harvesting due to each plant bearing fruit only once. The inedible portions, including pseudo-stems and leaves, account for around 88% of the total weight of the plant and have a high cellulose fiber content [29]. Besides their abundance properties like low microfibrillar angle, cellulose

crystallinity, and cellulose content are also some promising features making it a good precursor material [22]

Other properties like their fibre tensile strength, low density, and high Young's modulus value give them some excellent mechanical properties which can further be optimized using various techniques [22].

Physical property	Hemp	Sisal	Jute	Banana	Kenaf	Ramie
Density (g/cm^3)	1.2	1.3–1.6	1.3–1.5	1–1.5	1.2–1.4	1–1.55
Tensile strength (kN/mm^2)	270–900	540–720	610–780	500	223–930	180–1627
Stiffness (kN/mm)	–	30–40	15–35	–	–	–
Elongation at break (%)	1–3.5	2.2–3.3	1.0–1.9	4.5–6.5	9.1–12.3	1.6–14.5
Maximum elongation (mm)	5–7	5–10	10–14	9	10	15
Tensile modulus (GPa)	23.5–90	10–40	12–60	12	14.5–53	1.44–82.5
Specific modulus (approximate)	40	18	32	9	24	35
Young's modulus (GPa)	4.8	13	15–30	20	20	–
Cellulose (%)	68–74.4	65–75	59–70	63–64	51–52	70–83
Hemicellulose (%)	15–22.4	10–15	15–20	19	20.3–21.5	–
Lignin (%)	3.7–10	7–13	11–15	5	17	5–12.7
Lumen size (mm)	–	11	13	–	–	–
Fiber length (mm)	50–500	10–150	120–900	300–900	150–700	900–1200
Microfibrillar angle (deg)	2–6.2	11–20	8–9	11	–	14
Moisture absorption (%)	10–14	11	12	10–11	12–14	11.8

Table 5 Physical properties of commonly used substrate [22]

The low microfibrillar angle and high cellulose fraction are critical criteria that primarily define fibre strength; other elements, such as the crystallinity index, can be important aspects that primarily influence the membrane's hygroscopic nature. A high crystallinity index is generally suggested to enhance membrane strength and stability.

2.4 Industrial and Environmental concerns related to NC Synthesis

On a small scale, numerous industries throughout the world produce cellulose-based films for a variety of uses. The primary hurdles, however, are in upscaling manufacturing, which is hampered by energy demand, substrate type, and production cost.[19]

One essential element of NC production by opting for a chemical-based strategy, especially H_2SO_4 carried acid hydrolysis necessitates the requirement of acid-stable equipment which increases the cost of production. Along with it the neutralization of acidic pH via. centrifugation or dialysis further increases the time and cost of manufacturing.

To effectively counter the economic and environmental concerns related to nanocellulose production enzymes have been an excellent choice nevertheless their optimization of suitable enzyme concoction and concentration is needed.

Along with the economic element, another difficulty is to limit secondary environmental harm, which is primarily caused by the use of chemicals such as H_2SO_4 during the bleaching or acid hydrolysis step of chemical deconstruction of cellulose precursor.[19]

To add onto this is the struggle to obtain a high yield of NC so that the production could be feasible and the concept of valorising the biomass can be made successful. Continuous efforts are being made towards optimizing the production and scaling it up from laboratory to pilot standard. Industries are constantly looking for an optimized protocol that will reduce production costs to the point where the products will be marketable and profitable.[19]

In this work the effort has been aimed at properly degumming the agro-waste to obtain NC while keeping the cost of preparation in mind.

Multiple approaches can be utilized in conjunction for successful defibrillation and NC synthesis. Beginning with prior mechanical grinding increases the surface area and allows subsequent reagents to access the inside of the fiber more effortlessly. One common approach widely employed for reducing the recalcitrance nature of LCB is mercerization, also known as alkali treatment, which partially eliminates the substrate's pectin, lignin, and hemicelluloses. NaOH is the most commonly employed reagent for this, but other compounds like potassium hydroxide, calcium hydroxide, ammonium hydroxide, and aqueous ammonia have also been studied. Following the mercerization event, successive treatment of the partially delignified LCB can be carried out using an acidified sodium chlorite solution, which solubilizes the lignin fraction by converting it to lignin chloride.

Another chemical-based strategy to downsize the bleached LCB microfibers into nanoscale is through a chemical disruption strategy which entails carefully hydrolysing the amorphous part of cellulose's microfibrillar structure with strong acid. The process referred to as acid

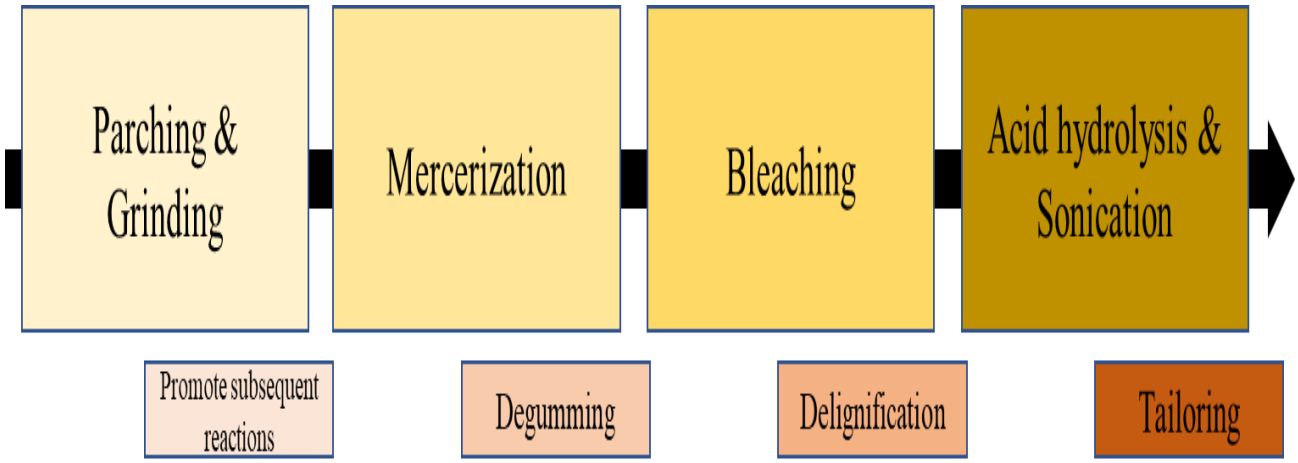


Fig. 7 Overview of the strategy being followed in this work

hydrolysis, involves the absorption of H^+ ions from a strong acid, such as H_2SO_4 or HCl, which hydrolyses the glycosidic bonds before mechanically releasing the individual crystallites. The most commonly employed acid for the following purpose is H_2SO_4 [30]–[33]. However other acids like HCl, phosphoric, hydrobromic, and nitric have also been employed [33]–[35]. However, using H_2SO_4 over other acids provides some additional benefits such as high negative zeta potential by increasing the negative surface charge of NC which mainly helps in maintaining the dispersion state stability [25], [36].

Sulphuric acid-based hydrolysis is effective in preventing agglomeration of the NC being synthesized however, the major limitation remains with the compromised thermal stability due to the presence of sulphate esters on the surface of the NC which can be improved by alkali treatment with sodium hydroxide solution [25]

HCl as an alternative to H_2SO_4 provides better thermal stability however as the CNF does not possess any surface charge from HCl treatment the dispersed state of the suspension is compromised and the NC is more prone to agglomeration [12].

Following the event of acid hydrolysis further, post-treatments such as solvent elimination, neutralization, washing, purification, filtration, centrifugation, sonication, dialysis, fractionation, surface modification, stabilization, and drying (freeze-drying, spray-drying) must be conducted to neutralize the substrate and recover CNC product [22]

In concert with the acid-hydrolysis step, another effective strategy for downsizing the hydrolysed fibers to sub-micron scale is to use high-intensity ultrasonication. The ultrasonic waves that generate cavitation bubbles around the sonication probe gradually reduce the fibre length, which eventually shrinks the individual fibres and modifies their properties based on the time and intensity of treatment.

As aspect ratios increase, tuning of fibres for membrane preparation becomes increasingly challenging. The series of procedures utilised in this work expose the surface hydroxyl groups of the cellulose fibre, which results in the formation of collectively strong hydrogen bonds between the adjacent fibrils. The increase in hydrogen bonding makes it difficult to overcome barriers like compromised membrane flexibility and other issues like membrane fouling and flux rate.

Pore size, however, will also be impacted by insufficient lignin removal from the biomass during degumming. Therefore, usage of plasticizers was chosen to overcome these hurdles.

Glycerol, often known as glycerin, is a GRAS triol molecule that has no smell, no colour, is viscous, and has an effective plasticizing behaviour. Its use enhances the system's flexibility and flux rate, two factors necessary for the membrane's efficiency.

2.5 Biodegradability of cellulose film

The biodegradability rate of the prepared cellulose film has been studied in the referenced work, where they calculated it to be 7%, showing an excellent biodegradability rate when compared to the cellulose filter paper and the polyethylene film. It was found that the degradation of conventionally used PEF was nearly unnoticeable due to its long-sustained life, while the cellulose filter paper showed a comparatively slower rate of degradation. The inference of such a result where the prepared cellulose film showed a high rate of biodegradability can be explained based on its lower crystallinity when compared to cellulose-based filter paper, as the treatment followed in the protocol lowers its crystallinity, making it more susceptible to degradation. The second important reason can be the high surface (-OH) groups present post-processing, which also make them vulnerable to microbial degradation [29].

Aside from the experimental evidence in the cited work, the biodegradability of any prepared cellulose-rich membrane from LCB can be rationally justified by the presence of surface hydroxyl and the susceptibility of glycosidic linkage to various enzymes produced by a variety of naturally occurring soil microbes commonly referred to as cellulolytic bacteria and fungi mainly with the help of cellulase [37] In soil, the half-life of cellulose and hemicellulose is predicted to be between days and weeks [37].

2.6 Characterization

The NC property will vary greatly depending on the technique used for its synthesis. Various aspects related to membrane physical, chemical, and functional aspects are assessed post-synthesis using different experiments and analytical tools. In this section, a quick overview of the key tools and techniques required for the fundamental comprehension of the product feature is highlighted.

Aspects related to membrane characterization like chemical composition, operational stability, hydrophilicity, surface morphological analysis, and filtration efficiency have been conducted to get a clear outlook on the optimisation needed for the protocol being used and the quality of the NC membrane being synthesised.

2.6.1 Optimisation of casting material and temperature

For effective casting of the sonicated TBPS, various materials like glass, polystyrene, and polypropylene were tested to avoid any peeling or damage to the membrane post-drying. Beside selecting the casting material, the temperature required for drying the membrane was also investigated by placing samples at 50 ºC in H.A.O. and at 25 ºC at ambient room temperature.

2.6.2 F.T.I.R analysis

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful analytical technique used to identify and characterize chemical compounds. It utilizes infrared light to detect the vibrational and rotational energy of molecules, allowing for the identification of unknown compounds or changes in known compounds over time. FTIR offers high sensitivity and accuracy with minimal sample preparation requirements making it an ideal tool for many analytical tasks.

The samples at different treatment stages of NC preparation were collected and dried for their investigation using F.T.I.R. (Agilent Cary 630) at Shoolini University.

2.6.3 Flux rate determination

Another critical criterion that controls any prepared membrane's applicability in the real world is its flux rate. A membrane with a more significant flux rate is considered a superior alternative in general; nonetheless, there is a direct relationship between pore size and flux rate; as pore size drops, the flux rate decreases. The treatment approach and time used to degum the LCB are detrimental in influencing the filtration performance and pore size of the membrane.

In the following work, the flux rate is determined under normal gravitational impact using a temporarily created setup and the stated formula.

2.6.4 Water stability & membrane integrity

For evaluating the membrane stability in water by understanding the minimum TBPS volume required for casting without compromising filtration efficiency, the membranes were cut roughly into 2 cm x 2cm squares and submerged in 25 ml of distilled water in petri plates,

where they were further observed. The following test gives us an indication of the casting volume required to utilise the membrane's stability under regular operating conditions. The casting volume of the fibre employed affects the membrane's integrity. Following 16 days of observation, the minimum TBPS volume for casting was determined.

2.6.5 Filtration efficiency/ pathogen's removal

To evaluate the filtration efficacy of the developed membrane, 400 µl of *E. coli* broth was added to 20 ml of sterile distilled water, and its optical density at 600nm wavelength was measured using UV-Vis spectroscopy. The spiked sample was then filtered by passing it through the prepared membrane inserted in a temporary filtering system inside the LAF under gravity force. The difference in turbidity before and after the sample was filtered was used to calculate the rejection percentage of the membrane, which indicates its filtration effectiveness.

$$\text{Rejection \%} = \frac{\text{Control } A_{600} - \text{Sample } A_{600}}{\text{Control } A_{600}} \text{ X } 100\%$$

Further, the result was consolidated by culturing the *E. coli* on an EMB culture medium at 37°C. The coliform bacteria were detected and enumerated.

2.6.6 Water contact angle/ hydrophilicity

Water contact angle, a measurement of a material's surface energy, is based on the angle at which water droplets make contact with the material's surface. It can be used to evaluate a material's prospective applications and wettability. In order to create goods and materials that are resistant to water damage, it can be helpful to understand how liquids interact with various surfaces by measuring the water contact angle.

Chapter-3

3.1 MATERIALS REQUIRED:

Raw material:

- Banana pseudo stem

Laboratory equipment:

- Pressure convection oven
- Blender
- Water bath
- Hot plate with magnetic stirrer
- Digital weighing machine

Laboratory apparatus:

- Aluminium foil
- Muslin cloth
- Glass Petri plates
- Beaker
- Graduated cylinder 250ml, 50ml & 10ml
- Laboratory sieve (180 µm pore size)

Chemical reagents:

- Sodium hydroxide
- Glacial Acetic acid
- Sodium chlorite
- Sulphuric acid
- Glycerol

3.2 METHODOLOGY:

3.2.1 Pre-treatment

3.2.1.1 Parching and Mercerization:

- Partially dried banana pseudo-stems were roughly chopped into manageable pieces and further parched in a pressure convection oven to remove residual water at 60ºC for 24 hours.
- After drying them, the pseudo-stems were transferred to a mixer grinder (230 V~50 HZ) and ground at the maximum setting for 30 seconds with ten-second pauses to avoid excess heat generation.
- The grounded PS was sieved using a laboratory test sieve of 180 µm mesh size.
- 20 gm of the grounded filtrate of banana PS were immersed in 300 ml of 7.5% w/v NaOH solution in a hot water bath at a temperature of 80ºC for 1 h. A technique referred to as the mercerization for partial degradation of lignin.
- The fibres were collected using muslin cloth and thoroughly washed under running hot tap water to lower the pH.
- The residual NaOH from the fibres from the treatment was removed by neutralizing the treated residue with 10% v/v of acetic acid.

Fig. 8 Banana pseudo stem (1); Parched BPS (2); Mercerized BPS (3)

3.2.1.2 Acid hydrolysis

- The bleached fibre was hydrolysed in a dilute sulphuric acid solution, a process referred to as acid hydrolysis, at a 10% v/v concentration at 80ºC for 3hours.
- Following the hydrolysis reaction, the solution was strained through a muslin cloth and washed thoroughly with distilled water and then with running hot tap water.

Fig. 9: Post acid hydrolysis (1), Post mercerization (2)

3.2.1.3 Bleaching

- An acidic solution of pH 5 of 1% (w/v) of sodium chlorite was prepared. The pH of the solution was acidified using acetic acid of 10%v/v.
- The fibres were immersed in the prepared solution of $NaClO_2$ for 2 hours.
- The fibres were then washed with distilled water followed by running hot tap water to neutralize the pH before further treatment.

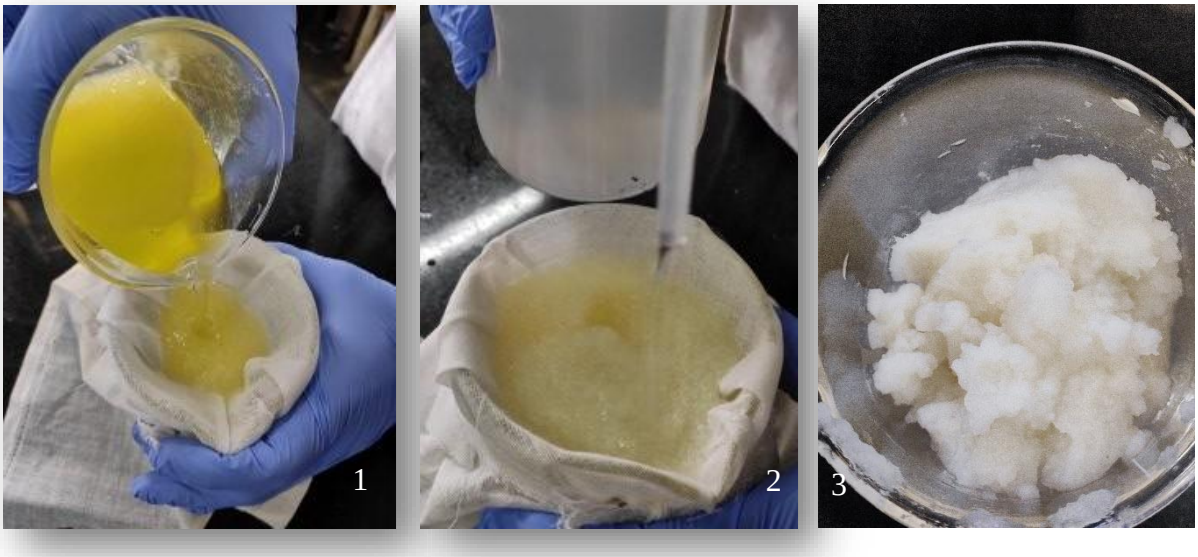


Fig. 10 Post bleaching (1); Neutralization (2); Bleached BPS (3);

3.2.2 Grinding

- The treated sample was grounded to reduce the fiber size and prepare a nearly gel like consistency as shown in the fig. X and were further sonicated.

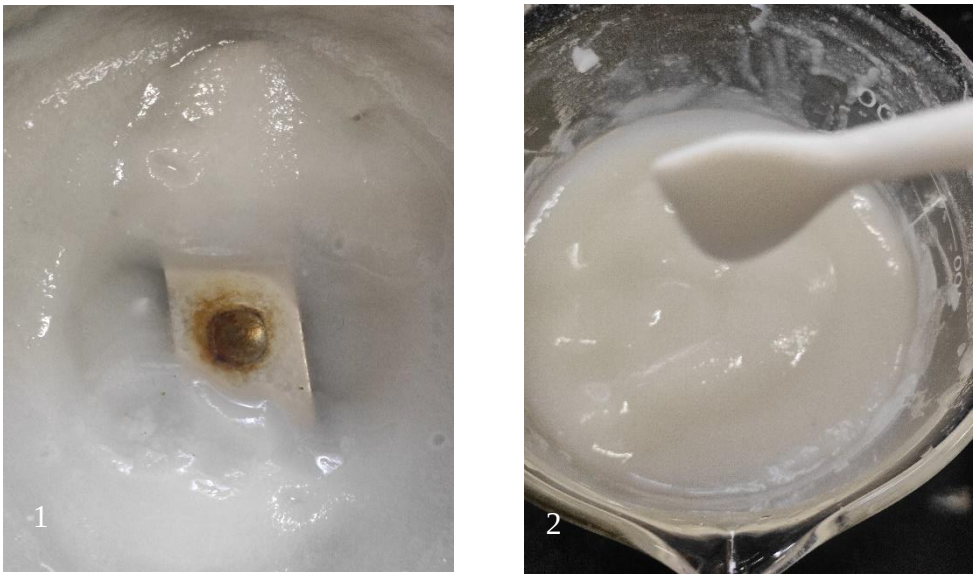


Fig. 11 Grinding (1); Post bleaching (2)

3.2.3 Sonication

- Roughly 40 ml of the grounded suspension was transferred to a 100ml beaker to which 0.4 & 0.8% w/v of glycerol was added and sonicated at 38% amplitude for 15minutes.
- The suspension was mixed thoroughly for 1minute after each 5minutes of sonication cycle for a total of 3 times to maintain homogeneity of the suspended fibres.

3.2.4 Casting

- 10cm X 10cm muslin cloth were cut, to which sonicated suspension of the treated LCB was poured onto a polypropylene based petri plate, and were incubated overnight at 40ºC inside incubator.

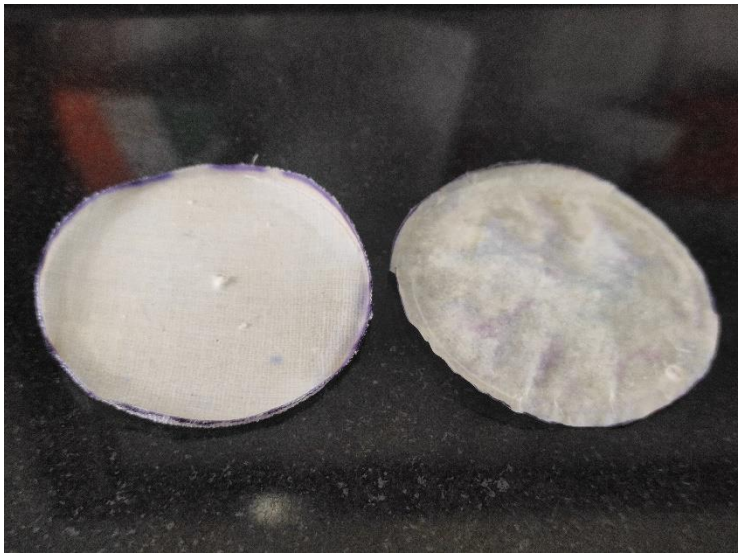
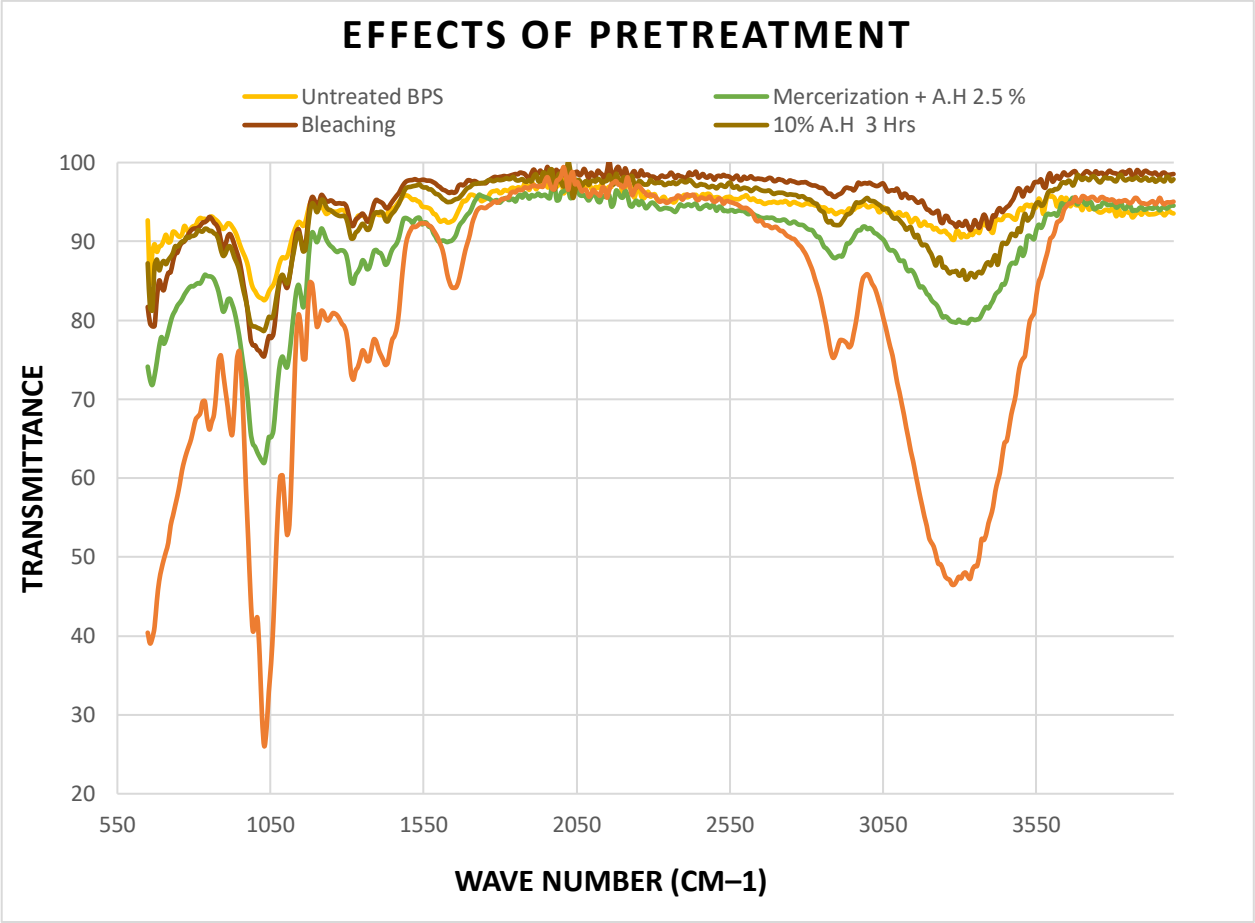


Fig. 12 Prepared membrane filter

Chapter-4

RESULTS & DISCUSSION

4.1 FTIR analysis



The spectral image of the TBPS showed strong absorption bands for intermolecularly bonded hydroxyl groups at around 3200–3300 **cm^{-1}**. Which were observed in every pre-treatment approach being followed. Especially in the sample (10% A.H., 3Hrs) due to the additional sonication and grinding step which minimised the recalcitrance nature of untreated BPS.

Peaks at ~2900 has been assigned to the C-H stretching vibration from the -CH_2 group of celluloses and hemicelluloses it is a characteristic peak observed in natural fibres.

4.2 Optimisation of casting material & temperature

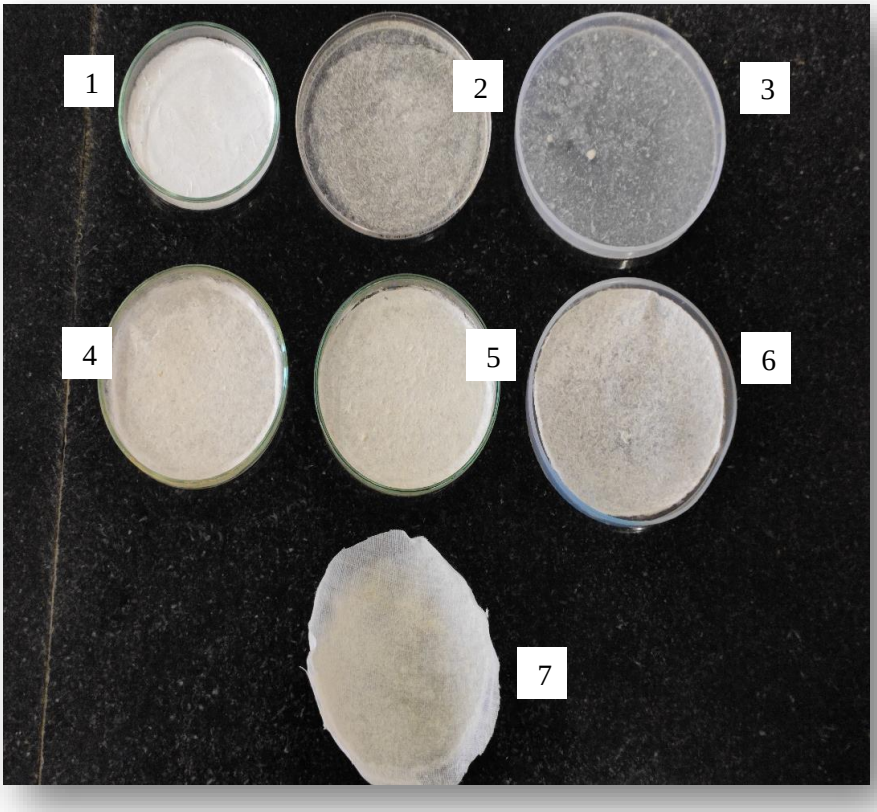


Fig. 14 Optimisation of casting material, temperature and treated BPS loading

Different materials for casting were tested for effective peeling of membranes after drying at different temperatures as given in the table below.

Sample No.	BPS volume	Muslin	Casting material	Drying temp.
1	4% w/v	No	Glass plate	H.A.O.
2	4% w/v	No	Polystyrene plate	H.A.O.

3	4% w/v	No	Polypropylene plate	Ambient temp.
4	6% w/v	No	Glass plate	H.A.O.
5	6% w/v	No	Polystyrene plate	H.A.O.
6	6% w/v	No	Polypropylene plate	Ambient temp.
7	6% w/v	Yes	Polypropylene plate	Ambient temp.

Table 6 Optimisation of casting material, temperature, and treated BPS loading.

The polypropylene based Petri plates were most suitable for casting of the membrane as the membrane being casted were much easier to peel without any damages.

Whereas other material-based Petri plates (glass and polystyrene) easily damaged the membrane due to sticking of the membrane with the surfaces post-drying.

For drying, the membranes were placed in a hot air oven, and a few samples were kept at room temperature. It was found that the effect of temperature was not considerable on the membrane properties. However, it took longer for the membranes kept at room temperature to dry due to the slow evaporation rate.

4.3 Water contact angle examination

Serial No.	Membrane type	Contact angle
1	7% w/v	65.69 º
2	5% w/v	66.06 º
3	5% w/v + 0.8% Gly	63.89 º
4	5 % w/v + 0.4% Gly	57.54 º

Table 7 Contact angle values of prepared membranes

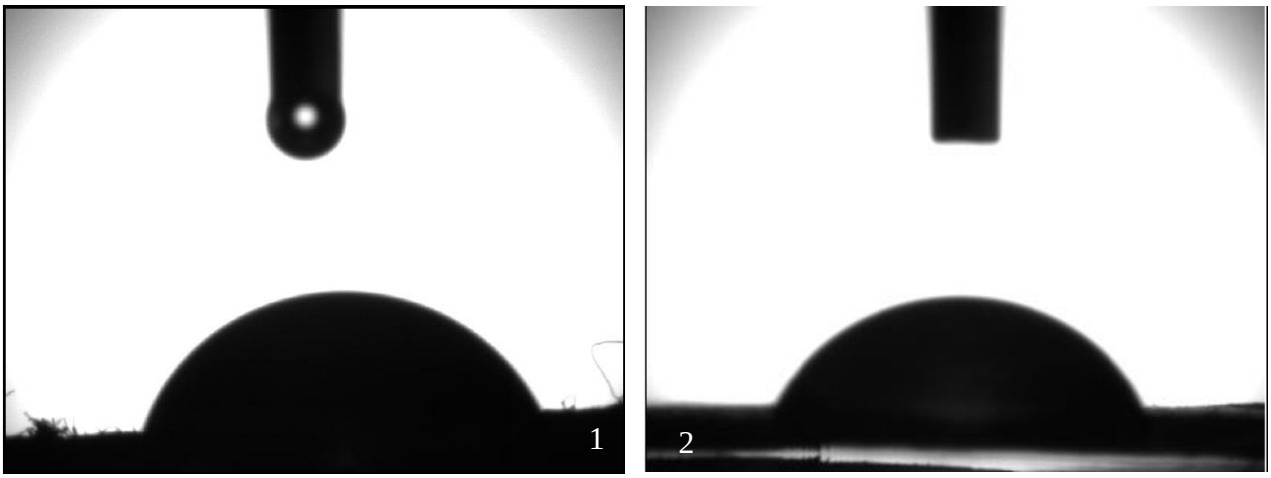


Fig.15 Images of contact angle of prepared membranes 7% w/v (1);

5% w/v (2)

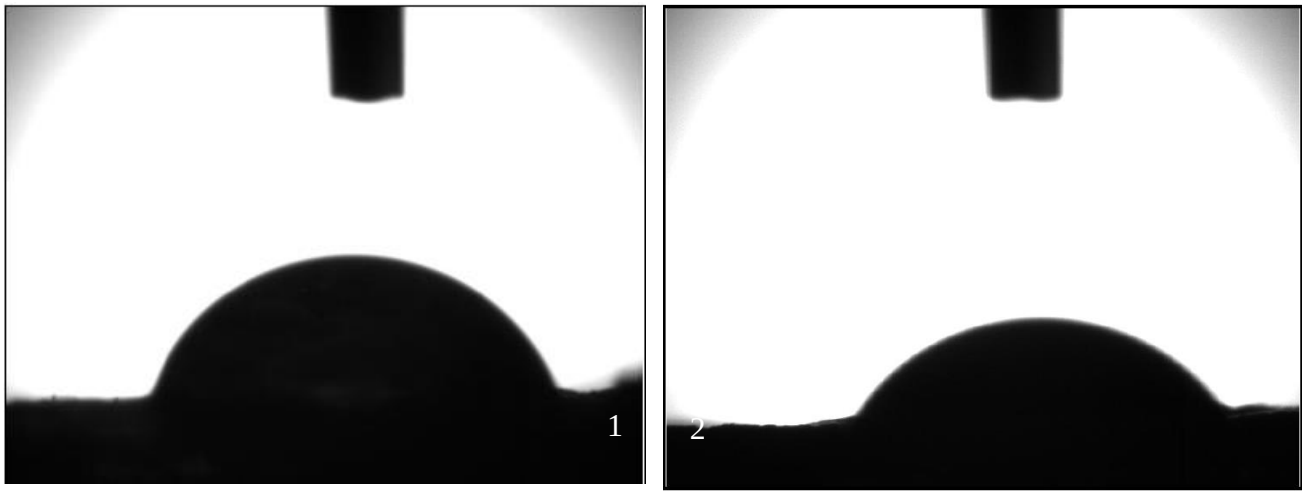


Fig.16 Images of contact angle of prepared membranes 5% w/v + 0.8% (Gly) (1); 5% w/v + 0.4% (Gly) (2)

The degree of hydrophilicity of the prepared membrane was investigated using contact angle measurements. From the given images and results, it can be inferred that with the increase in the concentration of the bleached fibres, the surface roughness was also increased, which ultimately increased the contact angle as well. Whereas in the membrane, where bleached fibres were maintained at a concentration of 5%, the contact angle was reduced.

The membrane to which additionally glycerol was added as a plasticizer, as mentioned in Table 7, tends to be more hygroscopic, which results in a decrease in the contact angle as shown in Fig. 16.

4.4 Membrane stability

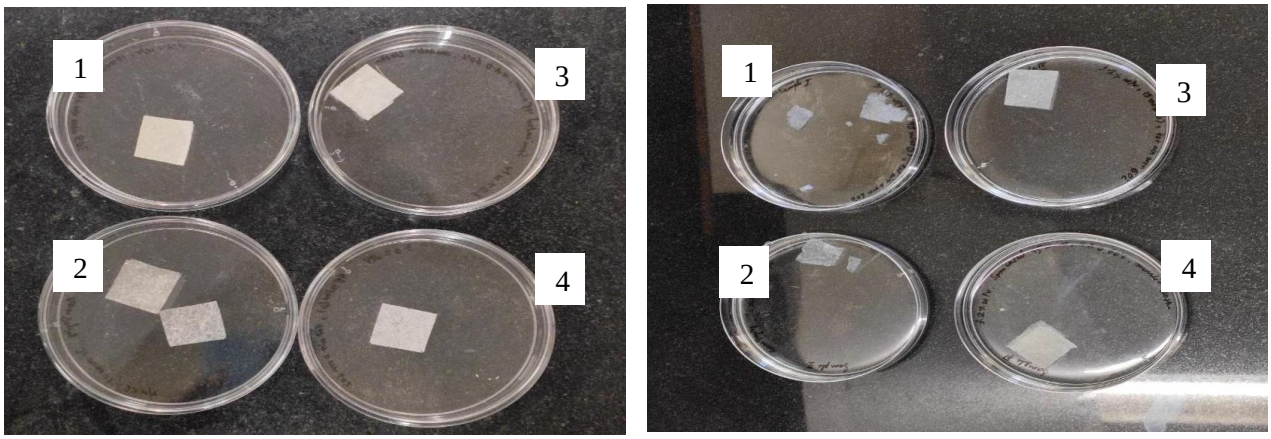


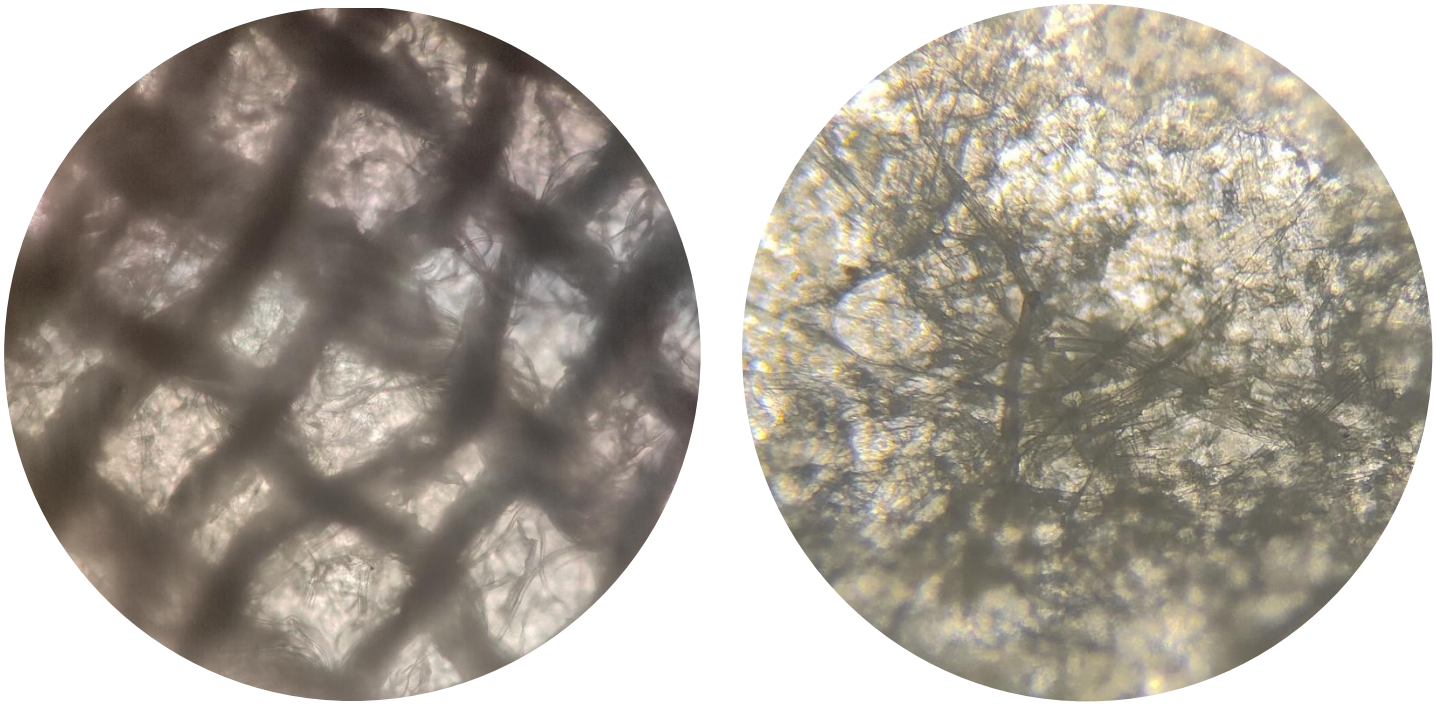
Fig.17 Membrane stability analysis; Day 1 (left); Day 16 (right)

Sample no.	Casting Volume	Stable after 16 days of observation
1.	2% w/v	No
2.	2% w/v	No
3.	4 % w/v	Yes
4.	4% w/v	Yes

Table 8 Membrane stability at different TBPS casting volume

The treated BPS volume for casting the membrane was tested for water stability, and it was found that a solid loading of above 4% w/v was stable for prolonged usage. Whereas concentrations below 4% w/v are easily disintegrated.

4.5 Surface morphology and pore size



(1) **(2)**

Fig. 17 Fibre networking observed under 20 X magnification, of 5 % w/v + 0.4% Gly with muslin (1); Fibre networking observed under 10 X magnification of 5 % w/v without muslin.

The surface morphology and fibre networking of the casted material were examined using a phase contrast microscope at a maximum magnification of 20X because images recorded at higher magnifications were blurred. The images depict the visible networking of interconnected cellulose fibres, which form a mesh-like structure capable of eliminating any particulate pollutants larger than the pores generated by the individual strands.

The results were more consolidated by the later experiments on investigating the filtration efficiency of the membrane explained detailed in subsequent pages.

4.6 Flux rate

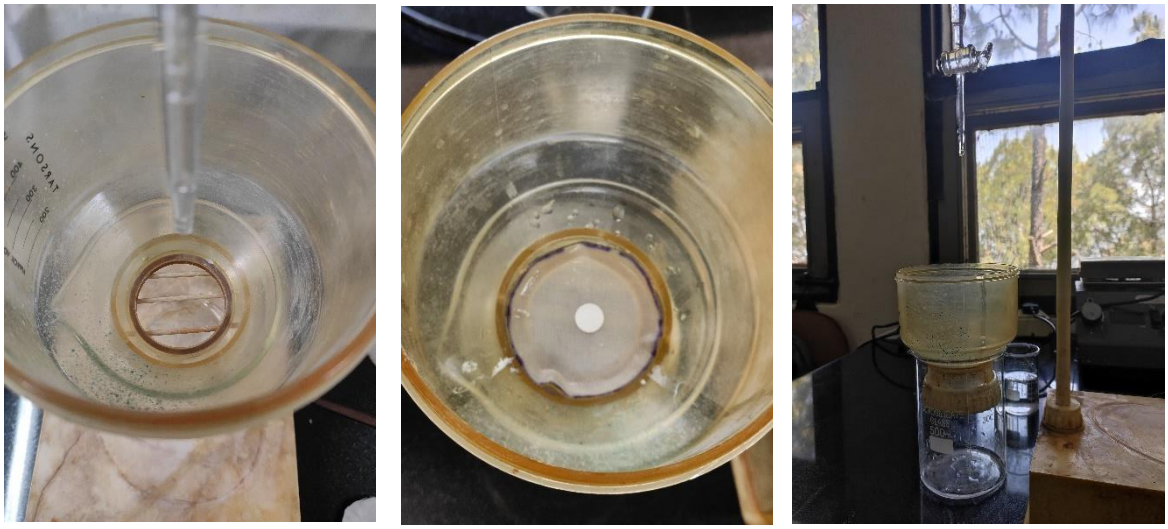


Fig. 18 Temporary setup for flux rate determination

Serial No.	Membrane type	Flux rate
1.	5% w/v + 0.4% (Gly) + muslin	10 ltr/h
2.	5% w/v without muslin	9.74 ltr/h
3.	5% w/v + muslin	5.39 ltr/h

Table 9 depicts the flux rate of different membranes

The addition of glycerol increased the flux rate, which can be rationally justified since the glycerol molecules entered between the adjacent fibres and reduced their hydrogen bonding, resulting in an increase in distance between two adjacent fibres and increased pore size, which ultimately raised the flux rate.

The loading volume of treated fibres and the additional layer of muslin cloth used were the key determining factors for flux rate in the membranes where glycerol was not used as a plasticizer. As shown in the table, in the membrane with 5% w/v of bleached fibre additionally having a muslin support, the flux rate is significantly lower than the membrane prepared without any additional muslin support.

4.7 Filtration efficiency

Fig. 19 Filtration unit

The filtration efficiency was tested by evaluating the rejection percentage of *E. coli* cells from the spiked distilled water using a UV-Vis spectrophotometer.

$$\text{Rejection \%} = \frac{\text{Control } A_{600} - \text{Sample } A_{600}}{\text{Control } A_{600}} \text{ X } 100\%$$

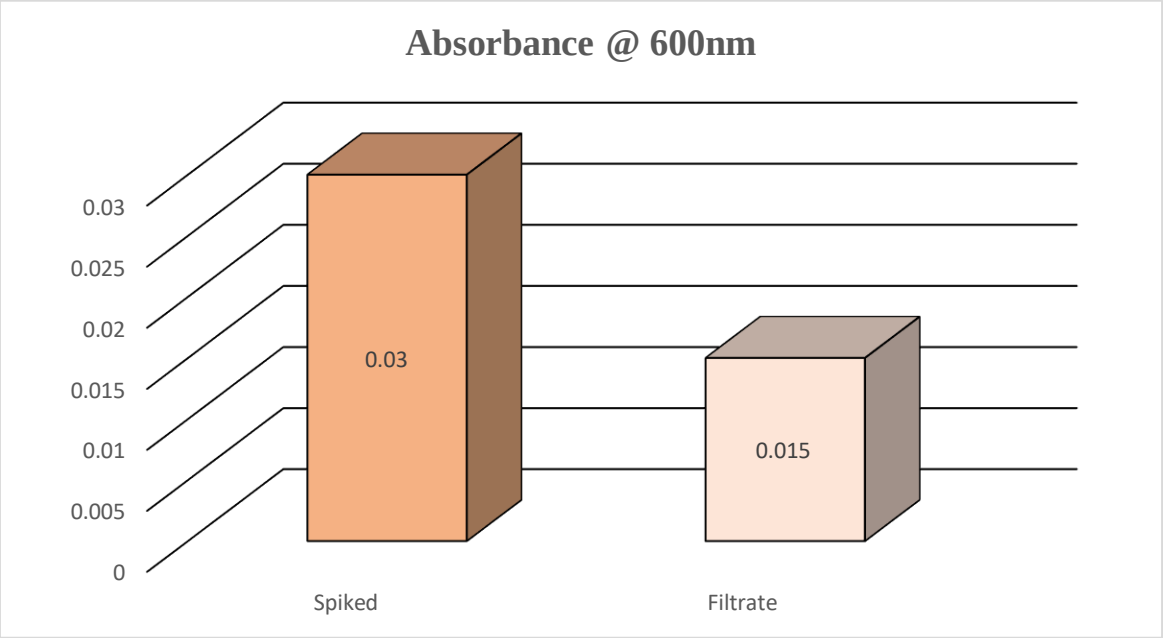


Fig. 20 O.D. value of filtrate before & after filtration

$$\textbf{Rejection\%} = \frac{0.030 - 0.015}{0.030} \text{ X } 100\% = \textbf{50 \%}$$

When the H_2SO_4 concentration was increased during the acid hydrolysis treatment from 2.5% v/v to 10% v/v for 3 hours, there was a considerable reduction in the size of the pores, which was evident from the increase in filtration efficiency. The most substantial effect was likely caused by an additional grinding step performed before sonication. Even though pore size analysis was not possible, the increase in filtration efficiency was indirectly correlated to a reduction in pore size.

4.8 Membrane filtration

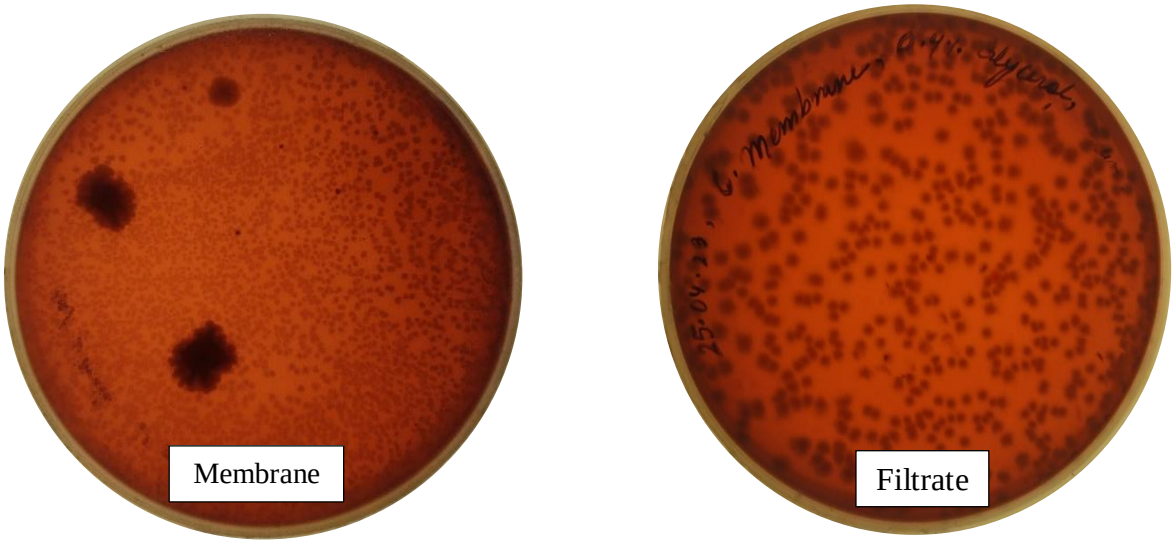


Fig.21 Colony growth observed in EMB plates

The membrane filtration study was carried out to further consolidate the evidence of filtration.

Colonies were observed in both EMB mediums, as shown in figure 21. Colonies growth in the plates on which the prepared membrane was stroked up after filtration ensured that the membrane was able to filter some of the coliform from the spiked sterile distilled water which according to the UV-Vis Spectro analysis came out to be 50% of the total *E. coli* cells in the SSW.

Due to the achievement of 50% filtration efficiency, growth was observed in the plates where filtrate was spread on the surface. **Therefore, further refinement is required to double the membrane's efficiency.**

Chapter-5

CONCLUSION

With an alarming 25% of the global population compelled to consume water contaminated with faeces, particularly in impoverished or developing nations, immediate attention is needed to overcome such a crisis and provide potable water access to people at a reasonable cost.

In this work, the previously stated issue was tackled by exploiting the intrinsic properties of the agro-waste banana pseudo stem, which is being discarded after a fruiting period of 9–12 months.

The prime focus while devising the methodology was to create a membrane with an effective cost without compromising its water purification ability. The methodology includes a combination of both chemical and physical approaches to effectively reduce the recalcitrance nature of the substrate so that the nanocellulose being isolated can be tailored to prepare a membrane with expected features. The objective was satisfied to some degree, as the efficiency of the membrane for water purification came out to be 50% for eliminating particulates up to 2-micron size. The additional introduction of the grinding step in conjunction with increased sulfuric acid concentration and treatment duration prior to sonication has led to major changes in the pore size of the membrane, as it was seen that efficiency increased by 14%. The major challenges while downsizing the cellulose microfibrils were primarily the lower flux output and brittleness of the membrane, possibly due to the elimination of the amorphous region of the cellulose chain, which were overcome to some degree using glycerol as a plasticizer. Further detailed investigation is needed to understand the membrane's changes in properties under different conditions and increase the system's efficiency.

REFERENCES

[1] "Drinking-water." https://www.who.int/news-room/fact-sheets/detail/drinking-water (accessed May 09, 2023).

[2] "Guidelines for Drinking-water Quality FOURTH EDITION INCORPORATING THE FIRST ADDENDUM."

[3] "Diarrhoeal disease." https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease (accessed May 09, 2023).

[4] R. M. Hartman *et al.*, "Risk Factors for Mortality Among Children Younger Than Age 5 Years With Severe Diarrhea in Low- and Middle-income Countries: Findings From the World Health Organization-coordinated Global Rotavirus and Pediatric Diarrhea Surveillance Networks," *Clinical Infectious Diseases*, vol. 76, no. 3, pp. e1047–e1053, Feb. 2023, doi: 10.1093/cid/ciac561.

[5] R. K. Gautam, S. K. Sharma, S. Mahiya, and M. C. Chattopadhyaya, "CHAPTER 1. Contamination of Heavy Metals in Aquatic Media: Transport, Toxicity and Technologies for Remediation," in *Heavy Metals In Water*, Royal Society of Chemistry, 2014, pp. 1–24. doi: 10.1039/9781782620174-00001.

[6] N. Verma, M. Rachamalla, P. S. Kumar, and K. Dua, "Assessment and impact of metal toxicity on wildlife and human health," *Metals in Water*, pp. 93–110, Jan. 2023, doi: 10.1016/B978-0-323-95919-3.00002-1.

[7] M. J. Tamás, S. K. Sharma, S. Ibstedt, T. Jacobson, and P. Christen, "Heavy metals and metalloids as a cause for protein misfolding and aggregation," *Biomolecules*, vol. 4, no. 1. pp. 252–267, 2014. doi: 10.3390/biom4010252.

[8] S. Jeong, G. Naidu, T. Leiknes, and S. Vigneswaran, "4.3 Membrane Biofouling: Biofouling Assessment and Reduction Strategies in Seawater Reverse Osmosis Desalination," *Comprehensive Membrane Science and Engineering: Second Edition*, pp. 48–71, Jan. 2017, doi: 10.1016/B978-0-12-409547-2.12261-9.

[9] J. Hoslett *et al.*, "Surface water filtration using granular media and membranes: A review," *Science of the Total Environment*, vol. 639. Elsevier B.V., pp. 1268–1282, Oct. 15, 2018. doi: 10.1016/j.scitotenv.2018.05.247.

[10] E. N. de Freitas *et al.*, "Challenges of biomass utilization for bioenergy in a climate change scenario," *Biology*, vol. 10, no. 12. MDPI, Dec. 01, 2021. doi: 10.3390/biology10121277.

[11] M. Herrera Rodríguez, "Preparation and Characterization of Nanocellulose Films and Coatings from Industrial Bio-Residues,"

[12] H. Xie, H. Du, X. Yang, and C. Si, "Recent Strategies in Preparation of Cellulose Nanocrystals and Cellulose Nanofibrils Derived from Raw Cellulose Materials," *International Journal of Polymer Science*, vol. 2018. Hindawi Limited, 2018. doi: 10.1155/2018/7923068.

[13] J. A. Okolie *et al.*, "Waste biomass valorization for the production of biofuels and value-added products: A comprehensive review of thermochemical, biological and integrated processes," *Process Safety and Environmental Protection*, vol. 159, pp. 323–344, Mar. 2022, doi: 10.1016/J.PSEP.2021.12.049.

[14] J. Popp, S. Kovács, J. Oláh, Z. Divéki, and E. Balázs, "Bioeconomy: Biomass and biomass-based energy supply and demand," *N Biotechnol*, vol. 60, pp. 76–84, Jan. 2021, doi: 10.1016/j.nbt.2020.10.004.

[15] H. F. Tan, B. S. Ooi, and C. P. Leo, "Future perspectives of nanocellulose-based membrane for water treatment," *Journal of Water Process Engineering*, vol. 37, p. 101502, Oct. 2020, doi: 10.1016/J.JWPE.2020.101502.

[16] A. S. Norfarhana, R. A. Ilyas, and N. Ngadi, "A review of nanocellulose adsorptive membrane as multifunctional wastewater treatment," *Carbohydr Polym*, vol. 291, p. 119563, Sep. 2022, doi: 10.1016/J.CARBPOL.2022.119563.

[17] B. Aoudi, Y. Boluk, and M. Gamal El-Din, "Recent advances and future perspective on nanocellulose-based materials in diverse water treatment applications," *Science of The Total Environment*, vol. 843, p. 156903, Oct. 2022, doi: 10.1016/J.SCITOTENV.2022.156903.

[18] T. Heinze and T. Liebert, "Celluloses and Polyoses/Hemicelluloses," *Polymer Science: a Comprehensive Reference: Volume 1-10*, vol. 1–10, pp. 83–152, Jan. 2012, doi: 10.1016/B978-0-444-53349-4.00255-7.

[19] P. Kaur *et al.*, "Nanocellulose: Resources, Physio-Chemical Properties, Current Uses and Future Applications," *Frontiers in Nanotechnology*, vol. 3. Frontiers Media S.A., Nov. 15, 2021. doi: 10.3389/fnano.2021.747329.

[20] M. Hazwan Hussin, D. Trache, C. T. Hui Chuin, M. R. Nurul Fazita, M. K. Mohamad Haafiz, and M. Sohrab Hossain, "Extraction of cellulose nanofibers and their eco-friendly polymer composites," in *Sustainable Polymer Composites and Nanocomposites*, Springer International Publishing, 2019, pp. 653–691. doi: 10.1007/978-3-030-05399-4_23.

[21] J. Pennells, I. D. Godwin, N. Amiralian, and D. J. Martin, "Trends in the production of cellulose nanofibers from non-wood sources," *Cellulose*, vol. 27, no. 2. Springer, pp. 575–593, Jan. 01, 2020. doi: 10.1007/s10570-019-02828-9.

[22] D. Trache *et al.*, "Nanocellulose: From Fundamentals to Advanced Applications," *Frontiers in Chemistry*, vol. 8. Frontiers Media S.A., May 06, 2020. doi: 10.3389/fchem.2020.00392.

[23] M. Rajinipriya, M. Nagalakshmaiah, M. Robert, and S. Elkoun, "Importance of Agricultural and Industrial Waste in the Field of Nanocellulose and Recent Industrial Developments of Wood Based Nanocellulose: A Review," *ACS Sustainable Chemistry and Engineering*, vol. 6, no. 3. American Chemical Society, pp. 2807–2828, Mar. 05, 2018. doi: 10.1021/acssuschemeng.7b03437.

[24] P. Phanthong, P. Reubroycharoen, X. Hao, G. Xu, A. Abudula, and G. Guan, "Nanocellulose: Extraction and application," *Carbon Resources Conversion*, vol. 1, no. 1. KeAi Publishing Communications Ltd., pp. 32–43, Apr. 01, 2018. doi: 10.1016/j.crcon.2018.05.004.

[25] A. Dufresne, "Nanocellulose: A new ageless bionanomaterial," *Materials Today*, vol. 16, no. 6. Elsevier B.V., pp. 220–227, 2013. doi: 10.1016/j.mattod.2013.06.004.

[26] P. Kaur *et al.*, "Nanocellulose: Resources, Physio-Chemical Properties, Current Uses and Future Applications," *Frontiers in Nanotechnology*, vol. 3. Frontiers Media S.A., Nov. 15, 2021. doi: 10.3389/fnano.2021.747329.

[27] "BANANA." https://nhb.gov.in/report_files/banana/BANANA.htm (accessed May 09, 2023).

[28] D. Reay, "Climate-Smart Food."

[29] B. Ai *et al.*, "Biodegradable Cellulose Film Prepared From Banana Pseudo-Stem Using an Ionic Liquid for Mango Preservation," *Front Plant Sci*, vol. 12, Feb. 2021, doi: 10.3389/fpls.2021.625878.

[30] P. Kumar, D. M. Barrett, M. J. Delwiche, and P. Stroeve, "Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production," *Industrial and Engineering Chemistry Research*, vol. 48, no. 8. pp. 3713–3729, Apr. 15, 2009. doi: 10.1021/ie801542g.

[31] G. Brodeur, E. Yau, K. Badal, J. Collier, K. B. Ramachandran, and S. Ramakrishnan, "Chemical and physicochemical pretreatment of lignocellulosic biomass: A review," *Enzyme Research*, vol. 2011, no. 1. 2011. doi: 10.4061/2011/787532.

[32] V. B. Agbor, N. Cicek, R. Sparling, A. Berlin, and D. B. Levin, "Biomass pretreatment: Fundamentals toward application," *Biotechnology Advances*, vol. 29, no. 6. pp. 675–685, Nov. 2011. doi: 10.1016/j.biotechadv.2011.05.005.

[33] Y. H. Jung and K. H. Kim, "Acidic Pretreatment," *Pretreatment of Biomass: Processes and Technologies*, pp. 27–50, Jan. 2015, doi: 10.1016/B978-0-12-800080-9.00003-7.

[34] T. Zhang, R. Kumar, and C. E. Wyman, "Sugar yields from dilute oxalic acid pretreatment of maple wood compared to those with other dilute acids and hot water," *Carbohydr Polym*, vol. 92, no. 1, pp. 334–344, Jan. 2013, doi: 10.1016/J.CARBPOL.2012.09.070.

[35] X. Zhao, Y. Morikawa, F. Qi, J. Zeng, and D. Liu, "A novel kinetic model for polysaccharide dissolution during atmospheric acetic acid pretreatment of sugarcane bagasse," *Bioresour Technol*, vol. 151, pp. 128–136, Jan. 2014, doi: 10.1016/J.BIORTECH.2013.10.036.

[36] M. Roman and W. T. Winter, "Articles Effect of Sulfate Groups from Sulfuric Acid Hydrolysis on the Thermal Degradation Behavior of Bacterial Cellulose," 2004, doi: 10.1021/bm034519.

[37] K. Killham and J. I. Prosser, "The Bacteria and Archaea," *Soil Microbiology, Ecology and Biochemistry*, pp. 41–76, Jan. 2015, doi: 10.1016/B978-0-12-415955-6.00003-7.