Development of xylem-based water filters

by

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Abstract

Diarrheal diseases caused due to microbial contamination are one of the leading causes of fatalities amongst children. Despite the availability of numerous commercial products for water filtration and treatment, there continues to be a need for technological solutions that can make safe drinking water affordable and accessible. Due to their low cost, high filtration rate per unit weight and the ability to be manufactured locally with little infrastructure, xylem-based filtration devices have potential to address the challenge of microbial contamination of water in resource-limited settings. Previous studies by Boutilier et al. have demonstrated the ability of sapwood xylem from conifers to achieve up to 99.9% rejection of bacteria from water.

However, it has been reported that drying of xylem after extraction leads to a drop in permeability by a factor of over 100. This poses a huge challenge in the context of transportation and storage of these filters. Maintaining the filters in a wet state would require special packaging and also reduce their shelf-life. Further, previous tests with the xylem filters at laboratory scale have involved the use of gas-pressure to drive the flow. In practical applications, the use of pumps would drive up the cost of the device negating the primary advantage of these filters. To keep operational costs as minimal as possible, it is critical to operate xylem filters offline.

This thesis aims to address the challenge of dry storage and offline, gravity-based operation of xylem filters. Moreover, the use of xylem for water filtration has not been explored before and little is known about its performance characteristics. This thesis also seeks to advance the understanding of xylem as a filter material through the study of attributes such as degradation of xylem when soaked in water, filter lifetime, its variation with water quality and variation of flow rate with time. Methods to engineer the xylem filters to improve their rejection capability have also been discussed.

In parallel to technology development, efforts were also made to identify avenues for implementation of these filters in India. The insights gathered from field visits to India and discussions with key stakeholders have also been presented.

Thesis Supervisor: Rohit Karnik
Title: Associate Professor of Mechanical Engineering

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Chapter 1

Introduction

1.1 Motivation

Access to safe drinking water remains a major cause of concern across the globe. According to the guidelines on drinking water quality provided by the World Health Organization [1], water that does not present any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages is considered safe for drinking. The Environment Protection Agency (EPA) classifies the contaminants that make water unsafe for consumption into four main categories, physical (sediments and suspended organic matter), chemical (elements and compounds like salts, arsenic, fluoride), biological (microorganisms like bacteria, virus, parasites and protozoan) and radiological (radioactive elements like cesium, plutonium and uranium) [2]. The length scale of these contaminants has been shown in Figure 1-1.

Of these, microbial contamination of water can lead to diseases like diarrhea, cholera and typhoid and poses the most serious threat to human health [1]. In 2013, 1.7 million cases of diarrhea were recorded globally and it was found to be the second leading cause of fatality amongst children under the age of five with an estimated death toll of 0.7 million [4]. The most common cause of diarrhea is intestinal infection or gastroenteritis. Some of the most common causal organisms of diarrhea have been listed in Table 1.1.

Table 1.1: Common water-borne pathogens leading to diarrhea

Class	**Species**	**Morphology**	**Notes/Comments**
Bacteria	Campylobacter spp.	Spiral, rod or comma-shaped, 0.2–0.8 by 0.5–5 μm [5]	Most frequently isolated species from patients, with acute diarrheal disease [6]
	Escherichia coli	Rod-shaped, 1.1–1.5 by 2–6 μm [7]	Enterohaemorrhagic strains cause acute diarrhea, well-studied and documented
	Francisella tularenis	Rod-shaped or coccoid cells, 0.2–0.7 by 0.2–1.7μm [7]	
	Salmonella spp.	Rod-shaped, 0.7–1.5 by 2.0–5.0 μm [7]	Typhoid has had devastating health implications, can be removed by disinfection
	Shigella spp.	Rod-shaped, 1–3 by 0.7–1.0 μm [7]	Sensitive to disinfection, E.Coli is a reliable indicator
	Vibrio cholerae	Small, straight, slightly curved or comma-shaped rods, 0.5–0.8 by 1.4–2.6 μm [7]	Cholera outbreaks are still common in the developing world, sensitive to disinfection, E.Coli is a reliable indicator
Viruses	Adenoviruses	Spherical, 90–100 nm [8]	Resistant to disinfection
	Astroviruses	Spherical, 28–35 nm [9]	
	Enteroviruses	Spherical, 25–31 nm [10]	
	Hepatitits	Cone-shaped, 40–60 nm at the wide end and 20 nm at the narrow end [11]	
	Noroviruses	Spherical, 23–40 nm [12]	Most common cause of viral diarrhea in adults, resistant to disinfection
	Rotaviruses	Spherical, 60–80 nm [13]	Single most important cause of infant death in the world, responsible for 50–60% of acute gastroenteritis in children, resistant to disinfection [1]
	Sapoviruses	Spherical, 27–40 nm [14]	
Protozoa and helminths	Cryptosporidium hominis/parvum	4-6 μm [15]	Demonstrate exceptional resistance to,disinfectants like chlorine, filtration more effective
	Cyclospora cayetanensis	7.5–10 μm [16]	
	Entamoeba histolytica	Spherical, 10–20 μm [17]	Resistant to disinfectants like chlorine
	Giardia intestinalis	Oval shaped thin-walled cysts, 10–20 by 7–10 μm, 0.3–0.5 μm thickness [18]	Most common cause of diarrheal outbreaks in the US, resistant to disinfectants
	Toxoplasma gondii	4–8 by 2–3 μm (Tachyzoites), 5–50 μm [19]	Very little known about the response of T. gondii to water treatment

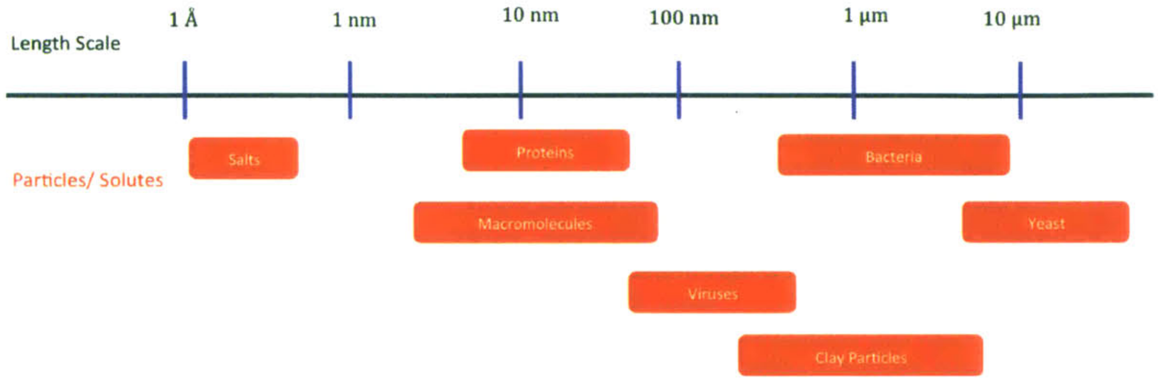


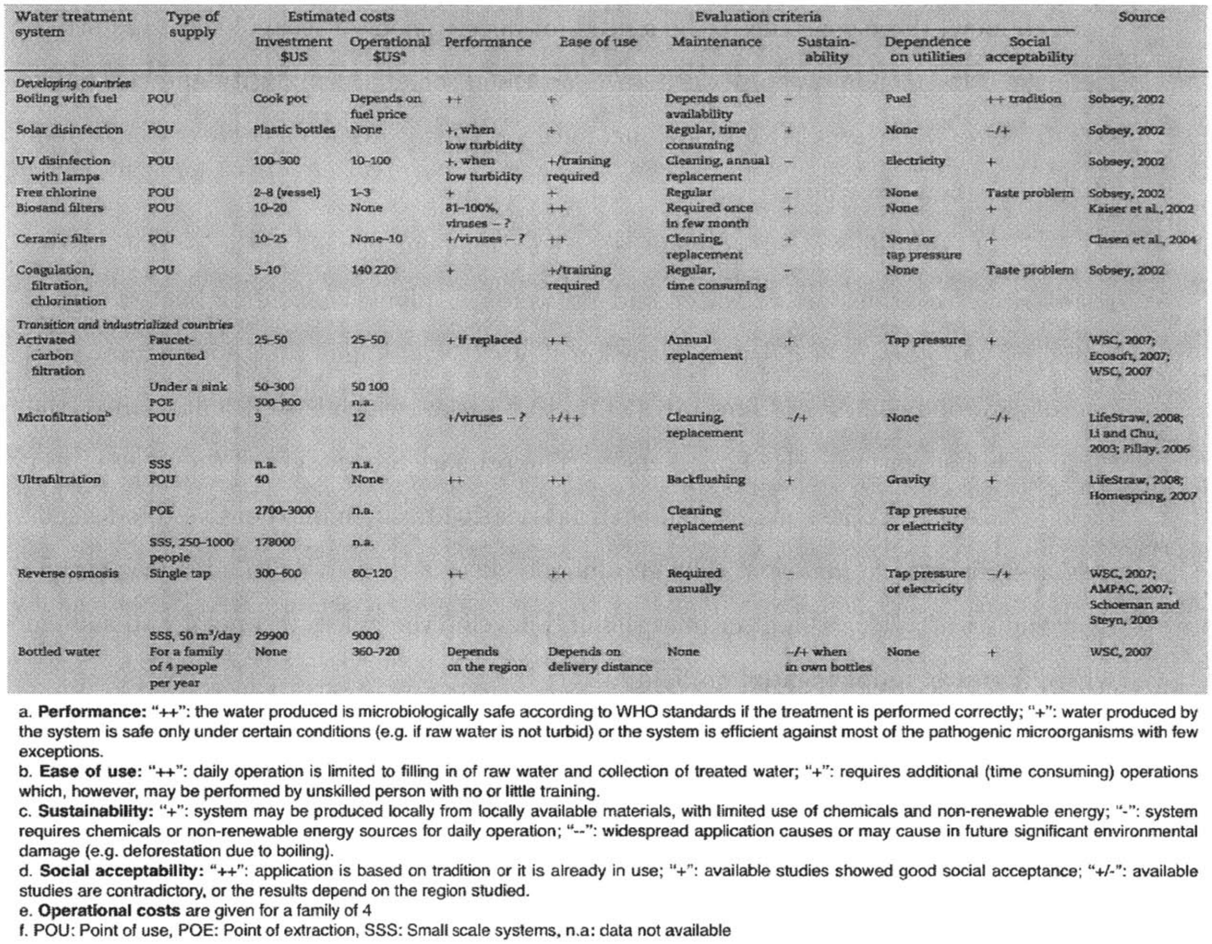
Figure 1-1: Comparison of length scale of different contaminants [3]

It has been shown that household water treatment and safe storage have great potential in mitigation of risks associated with waterborne diseases [20]. The importance of these approaches is even more significant in regions where there is no access to piped water supply systems and dependency on source water is high. Numerous advancements have been in the development of technological solutions for household water filtration. Peter-Verbanets et al. reviewed the commercial technologies available for point-of use water treatment and categorized them as follows [3]:

- Heat and UV-based
    - Boiling with fuel
    - Solar radiation
    - SODIS (combined action of heat and UV)
    - UV disinfection with lamps
- Chemical-based
    - Chlorine tablets
    - Coagulation, flocculation and precipitation
    - Adsorption
    - Ion exchange
- Physical-based
    - Sedimentation or settling
    - Filtration
        - Membranes (Micro, Ultra and Nano)

- * Ceramic and granular media filters including sand filters
- * Aeration

A comparison between these technologies has been shown below in Figure 1-2 [3].



Water treatment system	Type of supply	Estimated costs		Evaluation criteria						Source
		Investment $US	Operational $US[a]	Performance	Ease of use	Maintenance	Sustainability	Dependence on utilities	Social acceptability	
Developing countries										
Boiling with fuel	POU	Cook pot	Depends on fuel price	++	+	Depends on fuel availability	–	Fuel	++ tradition	Sobsey, 2002
Solar disinfection	POU	Plastic bottles	None	+, when low turbidity	+	Regular, time consuming	+	None	–/+	Sobsey, 2002
UV disinfection with lamps	POU	100–300	10–100	+, when low turbidity	+/training required	Cleaning, annual replacement	–	Electricity	+	Sobsey, 2002
Free chlorine	POU	2–8 (vessel)	1–3	+	+	Regular	–	None	Taste problem	Sobsey, 2002
Biosand filters	POU	10–20	None	81–100%, viruses – ?	++	Required once in few month	+	None	+	Kaiser et al., 2002
Ceramic filters	POU	10–25	None–10	+/viruses – ?	++	Cleaning, replacement	+	None or tap pressure	+	Clasen et al., 2004
Coagulation, filtration, chlorination	POU	5–10	140 220	+	+/training required	Regular, time consuming	–	None	Taste problem	Sobsey, 2002
Transition and industrialized countries										
Activated carbon filtration	Faucet-mounted	25–50	25–50	+ if replaced	++	Annual replacement	+	Tap pressure	+	WSC, 2007; Ecosoft, 2007; WSC, 2007
	Under a sink	50–300	50 100							
	POE	500–800	n.a.							
Microfiltration[b]	POU	3	12	+/viruses – ?	+/++	Cleaning, replacement	–/+	None	–/+	LifeStraw, 2008; Li and Chu, 2003; Pillay, 2006
	SSS	n.a.	n.a.							
Ultrafiltration	POU	40	None	++	++	Backflushing	+	Gravity	+	LifeStraw, 2008; Homespring, 2007
	POE	2700–3000	n.a.			Cleaning replacement		Tap pressure or electricity		
	SSS, 250–1000 people	178000	n.a.							
Reverse osmosis	Single tap	300–600	80–120	++	++	Required annually	–	Tap pressure or electricity	–/+	WSC, 2007; AMPAC, 2007; Schoeman and Steyn, 2003
	SSS, 50 m³/day	29900	9000							
Bottled water	For a family of 4 people per year	None	360–720	Depends on the region	Depends on delivery distance	None	–/+ when in own bottles	None	+	WSC, 2007

a. **Performance:** "++": the water produced is microbiologically safe according to WHO standards if the treatment is performed correctly; "+": water produced by the system is safe only under certain conditions (e.g. if raw water is not turbid) or the system is efficient against most of the pathogenic microorganisms with few exceptions.
b. **Ease of use:** "++": daily operation is limited to filling in of raw water and collection of treated water; "+": requires additional (time consuming) operations which, however, may be performed by unskilled person with no or little training.
c. **Sustainability:** "+": system may be produced locally from locally available materials, with limited use of chemicals and non-renewable energy; "-": system requires chemicals or non-renewable energy sources for daily operation; "--": widespread application causes or may cause in future significant environmental damage (e.g. deforestation due to boiling).
d. **Social acceptability:** "++": application is based on tradition or it is already in use; "+": available studies showed good social acceptance; "+/-": available studies are contradictory, or the results depend on the region studied.
e. **Operational costs** are given for a family of 4
f. POU: Point of use, POE: Point of extraction, SSS: Small scale systems, n.a: data not available

Figure 1-2: Overview of available Point-of-Use and Point-of-Extraction water treatment technologies [3]

According to the UNDP standards, the cost of water should not exceed 3% of the household income [21]. As per the data released by the World Bank in 2011, 40% of the world's population lives at less than \$2/day, which translates to a monthly income of \$60 [22]. Technologies for water treatment, if they are to be made accessible to such people, should therefore cost less than \$2 a month. Of all the technologies listed above, only chlorine tablet disinfection, solar disinfection and biosand filters meet the low cost-requirements. While the use of chlorine is highly limited by the unpleasant taste and odor, the efficacy of solar disinfection deteriorates with increase in turbidity and is largely determined by the availability of sunlight. Biosand filters require high maintenance and skill for operation and score low on ease of usage.

As of now, there continues to be a need for development of technologies that strike an optimal balance between performance, cost and social acceptability and can make safe drinking water accessible to low-income groups. It is in such a context that xylem filters find their relevance.

In 2014, Boutilier et al. demonstrated that xylem, which is the porous tissue responsible for transport of water and nutrients in plants, can filter bacteria from water by simple pressure-driven filtration [23]. It was found that approximately 3 cm^3 of sapwood could yield flow rates of up to 4 L/day, which is sufficient to meet the clean drinking water needs of one person. The primary advantages of the xylem filter include the ease of construction and their fabrication from an inexpensive, biodegradable and disposable material. The goal of the current thesis was to advance the understanding of xylem as a filter material and develop the xylem technology to a stage where it can be implemented on field.

In the following sections, a detailed description of the xylem structure, its variation amongst different species and the prior work on xylem-based filtration has been provided.

1.2 Structure of plant xylem

The following description of the plant xylem structure has been reproduced directly from 'Water Filtration Using Plant Xylem' by Jongho Lee and Michael Boutilier [23]:

"The flow of sap in plants is driven primarily by transpiration from the leaves to the atmosphere, which creates negative pressure in the xylem. Therefore, xylem evolution has occurred under competing pressures of providing minimal resistance to the flow of sap, while protecting against cavitation (i.e. nucleation) and growth of bubbles that could stop the flow of sap and kill the plant, and to do this while maintaining mechanical strength . The xylem structure comprises many small conduits that work in parallel and operate in a manner that is robust to cavitation [24, 25] (Figure 1-3). In woody plants, the xylem tissue is called the sapwood, which often surrounds the heartwood (i.e. inactive, non-conducting lignified tissue found in older branches and trunks) and is in turn surrounded by the bark (Figure 1-3b,c). The xylem conduits in gymnosperms (conifers) are formed from single dead cells and are called tracheids (Figure 1-3c), with the largest tracheids reaching diameters up to 80 mm and lengths up to 10 mm [24]. Angiosperms (flowering plants) have xylem conduits called vessels that are derived from several cells arranged in a single file, having diameters up to 0.5 mm and lengths ranging from a few millimeters to several meters [24]. These parallel conduits have closed ends and are connected to adjacent conduits via "pits" [25] (Figure 1-3d,e). The pits have membranes with nanoscale pores that perform the critical function of preventing bubbles from crossing over from one conduit to another. Pits occur in a variety of configurations; Figure 1-3d,e shows torus-margo pit membranes that consist of a highly porous part shaped like a donut (margo) and an impermeable part in the center called torus, occurring in conifers [25]. The porosity of the pit membranes ranges in size from a few nanometers to a few hundred nanometers, with pore sizes in the case of angiosperms tending to be smaller than those in gymnosperms [25, 26]. Pit membrane pore sizes have been estimated by examining whether gold colloids or particles of different sizes can flow through [27, 25]. Remarkably, it was observed that 20 nm gold colloids could not pass through inter-vessel pit membranes of some deciduous tree species [27], indicating an adequate size rejection to remove viruses from water. Furthermore, inter- tracheid pit membranes were found to exclude particles in the 200 nm range [25], as required for removal of bacteria and protozoa.

Since angiosperms (flowering plants, including hardwood trees) have larger xylem vessels that are more effective at conducting sap, xylem tissue constitutes a smaller fraction of the cross-section area of their trunks or branches, which is not ideal in the context of filtration. The long length of their xylem vessels also implies that a large thickness (centimeters to meters) of xylem tissue will be required to achieve any filtration effect at all –filters that are thinner than the average vessel length will just allow water to flow through the vessels without filtering it through pit membranes (Figure 1-3a). In contrast, gymnosperms (conifers, including softwood trees) have short tracheids that would force water to flow through pit membranes even for small thicknesses (< 1 cm) of xylem tissue (Figure 1-3a). Since tracheids have smaller diameters and are shorter, they offer higher resistance to flow, but typically a greater fraction of the stem cross-section area is devoted to conducting xylem tissue. For example, in the pine branch shown in Figure 1-3b used in this study, fluid-conducting xylem constitutes the majority of the cross–section. This reasoning leads us to the conclusion that in general the xylem tissue of coniferous tress –i.e. the sapwood is likely to be the most suitable xylem tissue for construction of a water filtration device, at least for filtration of bacteria, protozoa, and other pathogens on the micron or larger scale.

The resistance to fluid flow is an important consideration for filtration. Pits can contribute a significant fraction (as much as 30-80 %) [24, 25] of the resistance to sap flow, but this is remarkably small considering that pit membrane pore sizes are several orders of magnitude smaller than the tracheid or vessel diameter. The pits and pit membranes form a hierarchical structure where the thin, highly -permeable pit membranes are supported across the microscale pits that are arranged around the circumference of the tracheids (Figure 1-3a). This arrangement permits the pit membranes to be thin, offering low resistance to fluid flow. Furthermore, the parallel arrangement of tracheids with pits around their circumference provides a high packing density for the pit membranes. For a given tracheid with diameter D and length L, where pit membranes occupy a fraction α of the tracheid wall area, each tracheid effectively contributes a pit membrane area of $\pi \mathrm{DL}\alpha/2$, where the factor of 2 arises as each membrane is shared by two tracheids. However, the nominal area of the tracheid is only $\pi \mathrm{D}^2/4$, and therefore, the structure effectively amplifies the nominal filter area by a factor of $2\alpha(\mathrm{L}/\mathrm{D})$ (Figure 1-3f). The images in Figure 1-3c indicate $\mathrm{D} \sim 10$–$15\ \mu$m, $\alpha \sim 0.2$, yielding an effective area amplification of ~20 for tracheid lengths of 1–2 mm. Therefore, for a filter made by cutting a slice of thickness ~L of the xylem, the actual membrane area is greater by a large factor due to vertical packing of the pit membranes. Larger filter thicknesses further increase the total membrane area, but the additional area of the membrane is positioned in series rather than in parallel and therefore decreases the flow rate, but potentially improves the rejection performance of the filter."

1.3 Filtration using plant xylem

Preliminary filtration tests were performed by Boutilier et al using branches having a diameter of ~0.5 inches extracted from Eastern White Pine (*Pinus Srobus*). A pigment dye solution comprising of particles in the size range of 70-500 nm was passed through a 1-inch long xylem filter. The filtrate particle size distribution was found to peak at 80 nm (Figure 1-4) demonstrating the capability of xylem to remove larger aggregates. Further, filtration was found to occur in the top 2-3 millimeters of the filter. This observation hinted towards the possibility of making these filters more compact by reducing filter length [23][8]. The ability of xylem to remove bacteria was tested using rod-shaped, fluorescently labeled, 1 μm Escherichia Coli bacteria and a rejection of atleast 99.99% was observed. Figure 1-5e shows SEM images of bacteria trapped in the the pit membranes [23].

While freshly cut xylem was capable of yielding reasonable flow rates and achieving high rejection, the permeability of the dried samples was found to drop by a factor of 100. Rewetting these samples did not help recover flow rates. This was identified as one of the major challenges associated with the use of xylem filters. Building onto this work, Benjamin Potash, an alumnus from the Department of Mechanical Engineering at MIT, tried to characterize the performance of the xylem filters made from Eastern White Pine. The key conclusions of the study have been listed below:

- Filters having a length of 0.25 inches were determined to be sufficient for complete rejection of bacteria and other particulates in the 1 μm range. For particles below the 100 nm range, rejection increased with increase in length. The optimal length of the filter was found to be dependent on the quality of feed solution.

- Experiments conducted to check for the dependence of flow rates on the direction in which the xylem was mounted (that is, whether the direction of flow of water through the xylem is the same as that under natural conditions in a tree or reverse) showed slight variations.
- In order to address the challenge of loss of permeability due to drying, xylem filters were coated with Polyvinylpyrrolidone (PVP) having different molecular weights (10K, 40K and 360K). The recovery rates of the 10K and 40K PVP samples rewetted the xylem to 12.1% and 14.7% of the initial flow rate respectively and the rewetting in the former case was found to be faster. After re-wetting, both coatings showed rejection rates comparable with fresh cut samples, suggesting they are still able to effectively filter bacteria from the feed solution.

Though coating the xylem samples with PVP 10K and 40K showed promising results, the flow rates were found to be 3×10^{-12} m^2 Pa^{-1} s^{-1} which is quite low when compared to 5-6 $\times$ 10^{-10} m^2 Pa^{-1} s^{-1} obtained by Boutilier et al [23]. Further research into exploring suitable methods for preserving xylem permeability was therefore required.

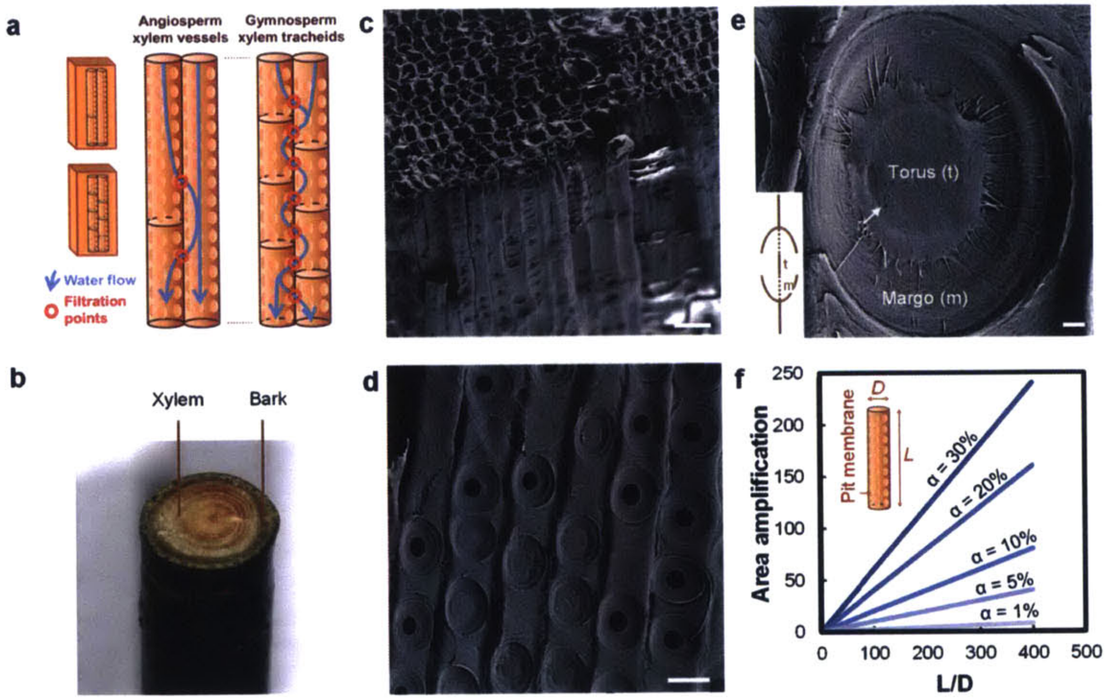


Figure 1-3: Xylem structure. a) Structure of xylem vessels in flowering plants and tracheids in conifers. Longer length of the vessels can provide pathways that can bypass filtration through pit membranes that decorate their circumference. b) Photograph of ~ 1 cm diameter pine (Pinus Strobus) branch used in the present study. c) Scanning electron microscope (SEM) image of cut section showing tracheid cross section and lengthwise profile. Scale bar is 40 μm. d) SEM image showing pits and pit membranes. Scale bar is 20 μm. e) Pit membrane with inset showing a cartoon of the pit cross–section. The pit cover has been sliced away to reveal the permeable margo surrounding the impermeable torus. Arrow indicates observed hole–like structures that may be defects. The margo comprises radial spoke-like structures that suspend the torus that are only barely visible overlaying the cell wall in the background. Scale bar 1 μm. f) Dependence of the area amplification, defined as the pit membrane area divided by the nominal filter area, on the tracheid aspect ratio L/D and fractional area α occupied by pit membranes.

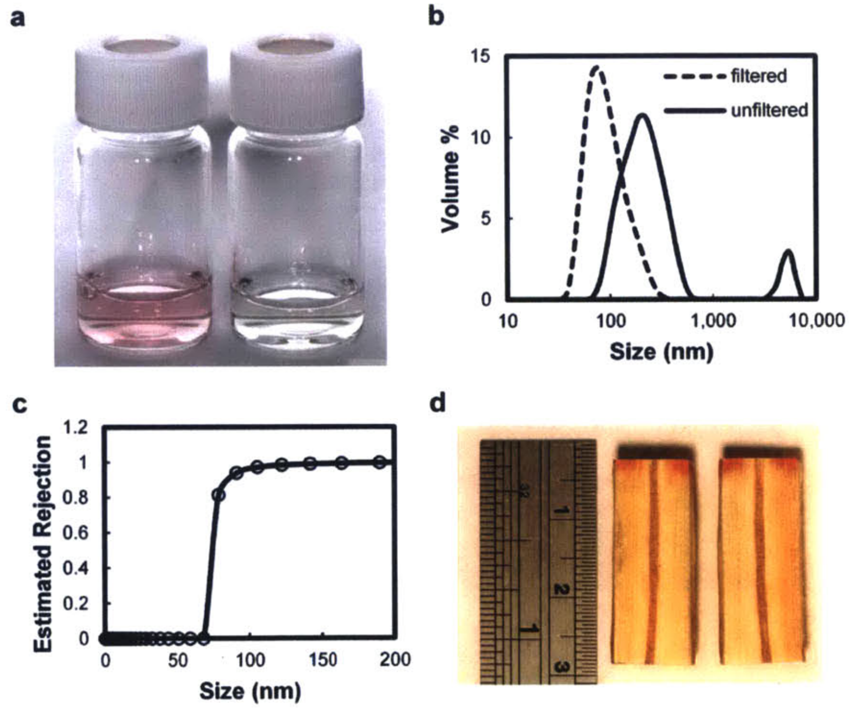


Figure 1-4: Filtration of pigment dye using xylem filter. a) Feed solution of a pigment dye before filtration (left), compared to the filtrate (right). b) Size distribution of the pigment particles in the feed and filtrate solutions measured by Dynamic Light Scattering. c) Dependence of the rejection on the particle size estimated from the data in (b). d) Cross-section of the xylem filter after filtration. Scale is in centimeters and inches [23].

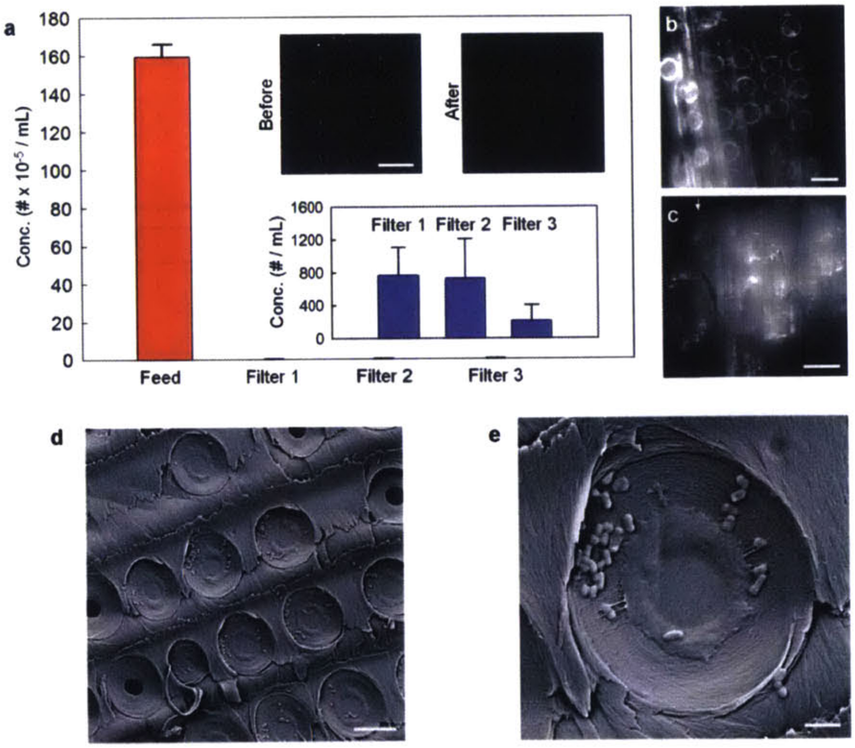


Figure 1-5: Filtration of model bacteria by the xylem filter. a) Concentrations of bacteria in the feed and filtrate solutions. Inset shows fluorescence images of the two solutions. Scale bar is 200 μm. Error bars indicate ± for experiments performed on three different xylem filters. b) Fluorescence image of xylem filter cross-section showing accumulation of bacteria over the margo pit membranes. Scale bar is 20 μm. c) Low-magnification fluorescence image shows that bacteria are trapped at the bottoms of tracheids within the first few millimeters of the top surface. Scale bar is 400 μm. Arrow indicates top surface of the xylem filter and also the direction of flow during filtration. Autofluorescence of the xylem tissue also contributes to the fluorescence signal in (b) and (c). d), e) SEM images showing bacteria accumulated on the margo pit membranes after filtration. Scale bars are 10 μm and 2 μm, respectively [23]

1.4 Objective of Thesis

The main objectives of the thesis are as follows:

1. Develop methods for dry storage of xylem filters - The drop in permeability of xylem upon drying is a major impediment in the context of transportation and storage of these filters. Although it is relatively straightforward to design devices that keep the filter element wet, distribution of filters in the wet state would require special packaging and reduce the shelf-life. Development of methods that enable dry storage of xylem filters and preservation of filter characteristics is critical for the deployment of this technology to the field.
2. Achieve gravity driven filtration through xylem - Although flow rate tests through xylem at the laboratory scale have been conducted using gas-pressure, operating these filters offline without using electricity or pumps is necessary to keep the operational cost of the filter as minimal as possible.
3. Understand operational attributes of xylem as a filter material - The use of xylem for water filtration has not been explored previously. The study of performance attributes as filter lifetime, its dependence on water quality and variation of flow rate with time is an indispensable component of product design and development.
4. Explore ways to improve performance characteristics and enhance the rejection ability of xylem filters
5. Work towards the transition of technology from lab to field - The project is funded by the Tata Center for Technology and Design at MIT which seeks to address the challenges of resource-constrained communities with an initial focus on India. Apart from technology development, efforts to identify pathways for implementation of xylem filters in India were pursued in parallel.

1.5 Outline of Thesis

The outline of the thesis is as follows:

- Chapter 2 describes methods that enable dry storage of xylem filters
- Chapter 3 focuses on gravity-driven filtration through xylem
- Chapter 4 deals with the study of operational attributes and performance characteristics of xylem filters
- Methods to improve the rejection ability and performance characteristics of xylem filters have been discussed in Chapter 5.
- Insights gathered through efforts aimed at implementation of xylem filters in India have been presented in Chapter 6.
- Chapter 7 provides a summary of the thesis and recommendations for future work.

Chapters 2-4 have been presented in manuscript form and can be read independently. Chapter 5 provides an overview of the different methods in which the performance attributes of xylem filters can be improved and can be read independently. Chapter 6 provides an overview of the insights gathered from field visits to India and uses some results from previous chapters.

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Chapter 2

Dry storage of xylem filters

2.1 Introduction

The use of sapwood xylem as a membrane for water filtration has been explored previously. While the xylem filters possess immense potential to make safe drinking water affordable in resource-limited settings, the loss of permeability upon drying is one of the major challenges associated with the use of these filters [1]. The necessity to maintain the filters in wet state to preserve flow rates would require special packaging and impose severe restrictions on the shelf-life. Further research and investigation is therefore required into the development of methods that enable preservation of xylem filter characteristics in the dry state. In order to do so, it is important to understand the mechanisms responsible for the drop in permeability.

The primary function of the xylem is to transport water and nutrients from the root to the leaves.This transport mechanism is widely explained using the Cohesion-Tension theory where transpiration, or evaporation of water from the leaves is expected to be the main driver for water movement [2]. The surface tension force arising from the formation of an air-water interface leads to a negative pressure (or tensile force) in the water below the meniscus. This tension gets transmitted through a continuous water column to the roots causing the water to rise up. The continuity of this water column, which is maintained by the cohesive forces between the water molecules and the adhesive forces between the walls of the xylem conduits and water, is critical for water transport (Figure 2-1) ([2, 3]).

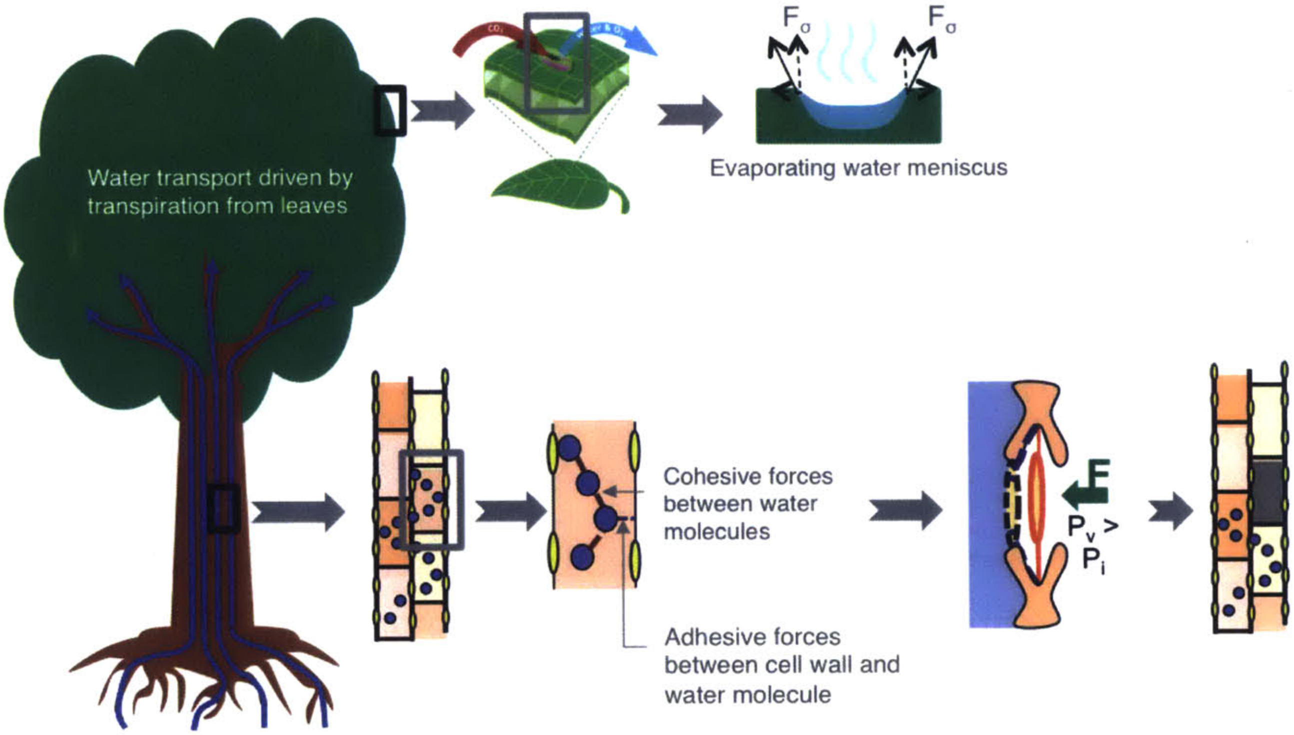


Figure 2-1: Mechanism of water transport in plants

However, as the xylem operates under negative pressures, it is prone to the risk of cavitation or formation of vapor bubbles that can disrupt the interactions responsible for keeping the water molecules together. The structure of xylem has therefore evolved under competing pressures of providing minimal resistance to water transport, while maximizing protection against cavitation that poses a serious threat to plant life. The xylem consists of multiple parallel conduits with closed ends (referred to as tracheids) that are inter-connected through perforations, called 'pits', present along the walls. In conifers, the pits consist of an impermeable central disc called 'torus' surrounded by a highly permeable membrane, 'margo'. The pores in the margo range from a few nanometers in angiosperms to a few hundred nanometers in gymnosperms. (Figure 2-2)

The main function of the pit membrane is to prevent propagation of vapor bubbles from one tracheid to the other. In a nucleation event, if the bubble formed has a radius greater that the critical radius, it continues to grow and fill the lumen of the tracheid.

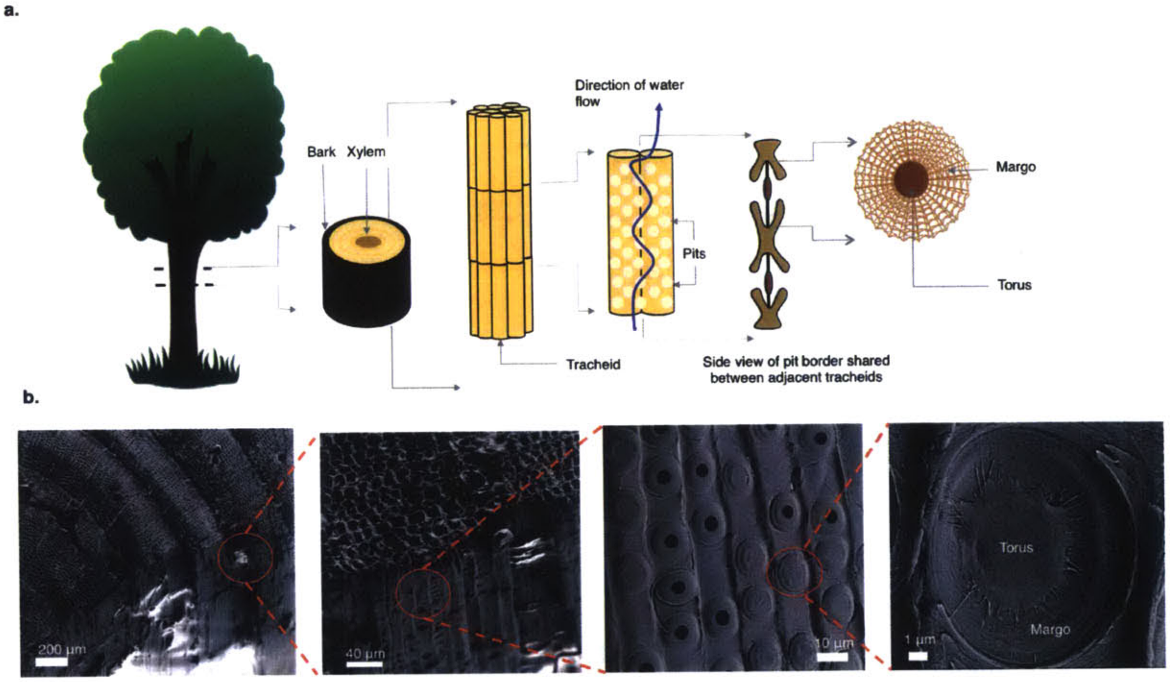


Figure 2-2: a) Schematic depicting the structure of xylem in plants b) SEM images of xylem at different magnifications obtained by *Boutilier et al.*, 2014 [1]

The formation of an evaporating meniscus on the margo leads to a pressure difference across the pit membrane. The force exerted due to the differential pressure pushes the pit membrane against the secondary cell wall. Due to the closure of the pit membrane, the vapor-filled tracheid is isolated from the rest of the transport network and is rendered dysfunctional for water transport. ([4]). This phenomenon, referred to as pit aspiration or embolism, has been widely studied in the past ([4, 5, 6, 7]).

In order to retain xylem permeability after drying, the sealing of the pit membrane against the cell wall should be prevented and the membrane must be retained in the suspended state. Different approaches and techniques to accomplish this task were explored.

1. **Introducing molecules/polymer chains between the pit membrane and secondary cell wall** : The presence of obstacles that act as an intermediate layer between the pit membrane and the secondary cell wall can prevent pit aspiration during drying. These obstacles, which could be molecules of compounds or polymers, can act as a resting support for the pit membrane until

the xylem is re-wetted again. Water would be able to flow through the gaps between the molecule chains, dissolve the compound/polymer and restore the pit membrane back to the suspended position eventually. The key factors to be considered in the process include the following:

(a) Choice of compound/polymer to be introduced which is governed by the parameters listed below:
    i. Size: If the molecule is too large, it will lead to a drop in permeability upon drying due to increased flow resistance. If the size is too small, the molecules will have a weak adsorption and might not have any effect on the drying process.
    ii. Cost: As one of the primary advantages of the xylem filter is the low cost, the polymer/compound chosen should not raise the cost of treatment significantly.
    iii. Suitability for consumption: The compound/polymer should be included in the list of chemicals or additives approved by the Food and Drug Administration
    iv. Stability at room temperature

    Based on the above considerations, sucrose and Polvinylpyrrolidone (PVP) seemed to be promising candidates.

(b) Method of treatment of xylem filters:
    i. By immersing the xylem filter in a solution of polymer/compound in water and subsequent drying to evaporate water
    ii. By immersing the xylem filter in a solution of polymer/compound in water followed by immersion in another solvent that induces precipitation of solute and drying
    iii. Flushing the solution using gas-pressure through the xylem followed by drying

Previously, Benjamin Potash, an undergraduate in the Mechanical Engineering Department at MIT, had studied the effect of introducing PVP molecules of varying molecular weight (10K, 40K and 360K) on the permeability after drying.

Experiments were conducted by immersing the xylem samples in solutions of PVP in water at three different concentrations (5%, 10% and 20%). The key conclusions of the study were [8]:

- The permeability of sample coated with PVP 360K was less than that of the control sample (which was immersed in water and dried)
- Dried samples coated with PVP 10K at all concentrations (5%, 10% and 20%) and PVP 40K (5%) initially had the same permeability as the control but showed increase in permeability as more water was flown through.
- The best results were seen in samples coated with 5% PVP having molecular weights 10K and 40K which were able to regain 12.1% and 14.7% of their initial flow rate after drying respectively. Qualitative rejection tests conducted using red ink showed that the rejection ability of the xylem was preserved.

With this background as the starting point, experiments with PVP having molecular weight 10K and 40K were repeated. The effect of introducing sucrose at different concentrations was also tested. However, sucrose has a strong tendency to undergo crystallization which can lead to the formation of large crystals,rather than uniform coatings that can damage the xylem. In the sugar industry, lime juice is commonly used for suppressing crystallization during production of sugar syrups or sugarcane juice through a process called clarification [9]. To prevent any structural damage to the xylem due to the growth of crystals, the effect of treating xylem samples with sucrose solution mixed with lime and other non-crystallizing sugars, such as corn syrup and maltodextrin was studied. Corn syrup is made from maize starch and has various amounts of maltose and higher oligosaccharides. It is often used as a substitute for sucrose in bakery products due its non-crystallizing nature. Apart from simple immersion and drying, attempts were also made to introduce sucrose into xylem samples by precipitation using ethanol [10].

2. **Replacing water in the xylem with a low surface tension liquid:** Permeability of wood has been previously studied in the context of drying and chemical treatment procedures for manufacturing of wood-based products. Processes such as impregnation of wood with chemical stabilizers and pulping agents require high permeability to facilitate treatment [11]. This has motivated investigation of methods for preventing pit aspiration in order to maintain permeability.

   It has been shown that treatment of xylem with ethanol and acetone, which have a surface tension of 22.39 mN/m and 25.2 mN/m respectively in comparison to 72.9 mN/m for water at 20°C, helps maintain the pit membrane in an unaspirated state [5]. This has been attributed to the reduction of surface tension forces that cause sealing to a level where they can be overcome by the forces arising due to the mechanical rigidity of the membrane [12, 13]. However, Petty et al [6] performed force calculations and demonstrated that the mechanical forces that resist the movement of the membrane are so weak that they cannot counter the surface tension forces even when the evaporating fluid is ethanol or acetone. Further, it was demonstrated that reducing the surface tension of water by adding surfactants still leads to pit aspiration after drying [7]. Consequently, it was proposed that the process of pit aspiration is not dictated by mechanical forces alone but also involves water-mediated hydrogen bonding between the pit membrane and cell wall.

   Though there continues to be a lack of understanding on the exact mechanism responsible for pit aspiration, there is enough evidence to believe that treating xylem with ethanol can prevent the sealing of pit membranes. However, the effect of ethanol on the flow rate and filtration characteristics of the xylem has never been explored before and a thorough, quantitative assessment of xylem permeability post ethanol treatment is lacking.

   To evaluate the efficacy of ethanol treatment in the context of using xylem as a filtration device, the permeability and rejection ability of ethanol-treated xylem samples was studied and the mechanisms underlying the observations were investigated.

3. **Varying filter geometry:** Apart from chemical treatment methods, the variation of permeability with the geometric configuration of the xylem filter was studied. Length is a critical parameter affecting the performance of xylem filters. Samples that are too short might consist of tracheids with truncated ends that would provide a resistance-free path for the water to flow through resulting in no filtration. However, if the sample is too long, it would increase the resistance to flow resulting in lesser flow rates. It is extremely important to strike an optimal balance and arrive at a length that maximizes rejection and permeability, both of which are key performance attributes of the filter. In this regard, experiments were conducted to study and understand the dependence of rejection and permeability on length.

2.2 Materials and Methods

2.2.1 Sample Preparation

Wood for all experiments was extracted from *Pinus Strobus* or Eastern White Pine that is commonly available on the campus of Massachusetts Institute of Technology, Massachusetts, USA. Samples were harvested from trees outside McCormick dorm at MIT and from trees that were specifically bought for research. Straight branches, with a diameter of roughly 0.5 inches were chosen and excised from trees using a tree pruner (Figure ??a). Additionally, the following points were kept in mind while choosing a branch:

1. Branches that do not lead to green parts of the plant undergo inactivation of xylem through a process called compartmentalization [14]. Filters made out of such branches would have low permeability (as shown in Figure 2-3). Branches with leaves would have an active transport system in place and would therefore be more suitable for filtration.

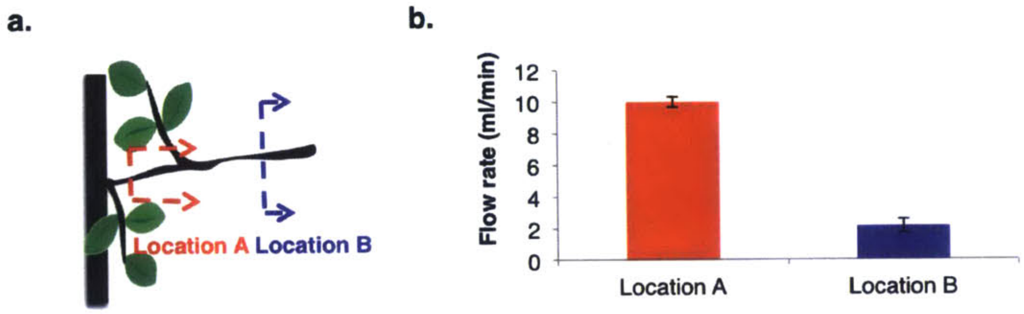


Figure 2-3: Effect of sampling location. Samples with a diameter of 0.5 inches and length of 0.5 inches were tested at 10 psi.

2. Locations near junctions where multiple branches connect to one another or to the trunk of the tree were avoided. Branching leads to bending of inner vasculature causing the xylem to be compressed at the lower sections of the branch and elongated at the top. Junctions have been studied extensively as flow constrictions and are known to have lower hydraulic conductivity [15]. Extraction of wood from junctions for making xylem filters should therefore, be avoided.

To prevent the wood from drying upon excision, the cute end of the branch with leaves intact was immediately placed in a bucket filled with water. It was observed that during winters, storing the branches indoors at 20-22°C for 2-3 days helped in restoring activity and thereby, in achieving improved flow rates. During the experiments, the direction of flow of water through the xylem was kept similar to that under natural conditions. In order to orient the sample correctly during testing, the side of the branch exposed to air was marked by placing a dot with a sharpie at the center of the filter. The center, which is the pith or heartwood, is impermeable and does not play a role in filtration.

The branches were then cut down into smaller sections having the desired thickness using an automated vertical cutting saw while keeping track of the side exposed to air. The lower end of the branch that was dipped in water was discarded, as the effect of water storage on wood degradation and xylem structure is not well understood at the moment. The bark was peeled away by hand and the wood was immediately dipped in a 20 ml vial filled with deionized water until experimentation.

Figure 2-4: a) Corona pruner used for harvesting branches b) Steps involved in constructing a xylem filter [1]

2.2.2 Preparation of polymer-based test solutions

The procurement details of the various chemicals used during these experiments have been summarized in Table 2.1.

Table 2.1: Summary of different polymer lengths used for experiments

Chemical	**Supplier**	**Molecular weight**	**Number of monomers**	**Size/Length of polymer (nm)**	**Cost (USD/kg)**
Sucrose	G-Biosciences	342.3	-	~1 nm	58.60 (very high purity) ~2(common household sucrose)
Polyvinyl-pyrrolidone (PVP)	Sigma Aldrich	10,000	90	12.8	83.17
		40,000	360	51.2	170.4
		360,000	3.243	460.5	125
Maltodextrin	Spectrum Chemicals				152
Corn Syrup	ACH Food Companies				5-6

Sucrose, PVP and maltodextrin solutions having different concentrations were prepared in batches of 50 ml by weighing the required quantity of the powder using a Mettler Toldo AL104 scale and dissolving it in deionized water. The solution was then stirred vigorously, sonicated using a VWR Symphony Ultrasonic Cleaner for 20 minutes to break up large lumps (especially for PVP 40K) and then left on a VWR Analog Vortex Mixer until the solute was completely dissolved. Solutions of sucrose and lime juice were made by first preparing a 20 ml aqueous solution of sucrose of the desired concentration and then adding 5 ml lime juice obtained from fresh lime. Corn syrup solutions in water were prepared in batches of 50 ml at different concentrations on a volume by volume basis and mixed thoroughly using a VWR Analaog Vortex Meter.

2.2.3 Flow rate measurements through xylem samples

The samples were mounted in a rubber tubing with an inner diameter of approximately half an inch. To maintain the natural direction of flow, the un-dotted end of the sample was loaded into the tube. The samples were secured in place using a hose clamp to prevent any leakage. The rubber tube was then filled with deionized water. Nitrogen gas was used to pressurize the water through the xylem sample at 10 psi unless specified otherwise (Figure 2-4). The time required for 5 ml of water to flow through the sample was recorded in all tests except for the samples treated with polymer-based solutions where 2 ml of water was flown through.This was primarily because the permeability of samples was extremely low in the latter case.

2.2.4 Treatment of xylem with polymer-based solutions

All samples were first flushed with 5 ml of water to remove sap.

For the experiments involving immersion, xylem samples were dipped in 20 ml vials containing the test solutions. The immersion time was kept constant at 9 hours across all experiments.

For experiments involving precipitation, samples were first immersed in the test solution followed by soaking in 200-proof ethanol procured from VWR International for 3 hours.

For experiments involving flushing as the method of treatment instead of immersion, the sample was flushed with 5 ml of test solution followed by 3 ml of ethanol (if necessary) at 10 psi.

All samples were subsequently dried.

2.2.5 Drying of xylem samples

All samples were dried in VWR vacuum oven at atmospheric pressure and 45°C unless stated otherwise. The sample weights were continuously monitored with a Mettler Toldo AL104 scale until no further reduction was observed to ensure complete drying.

2.2.6 Treatment of xylem samples with ethanol

Xylem samples were flushed with 5 ml of water to remove sap followed by flushing with 5 ml of 200-proof ethanol procured from VWR at a pressure of 10 psi. All samples preserved with ethanol were tested within 2 weeks of treatment.

2.2.7 Air flow rate measurements

Air flow rates were conducted using the setup illustrated in Figure 2-5. The xylem sample was mounted in a rubber tube that was connected to a nitrogen gas cylinder at one end. The sample was tightly secured using a hose clamp to prevent leakage. Air was flown through the sample at a pressure of 1 psi. A lower pressure was chosen in order to avoid compressibility effects. The air coming out of the sample was routed through a tube into a beaker that was kept inverted in a bucket filled with water. The beaker was placed into its position while ensuring that it was completely filled with water without any trapped air. The flow of air into the beaker caused the formation of air bubbles that got collected at the upper end thus causing the water level in the beaker to fall. The time required for air to displace 250 cm^3 of water was recorded and the air flow rate was calculated.

2.2.8 Rejection tests using fluorescent microspheres

Rejection tests were conducted using 1 μm fluospheres. Fluorescently labeled yellow-green, carboxylate modified, latex microspheres having a peak emission wavelength

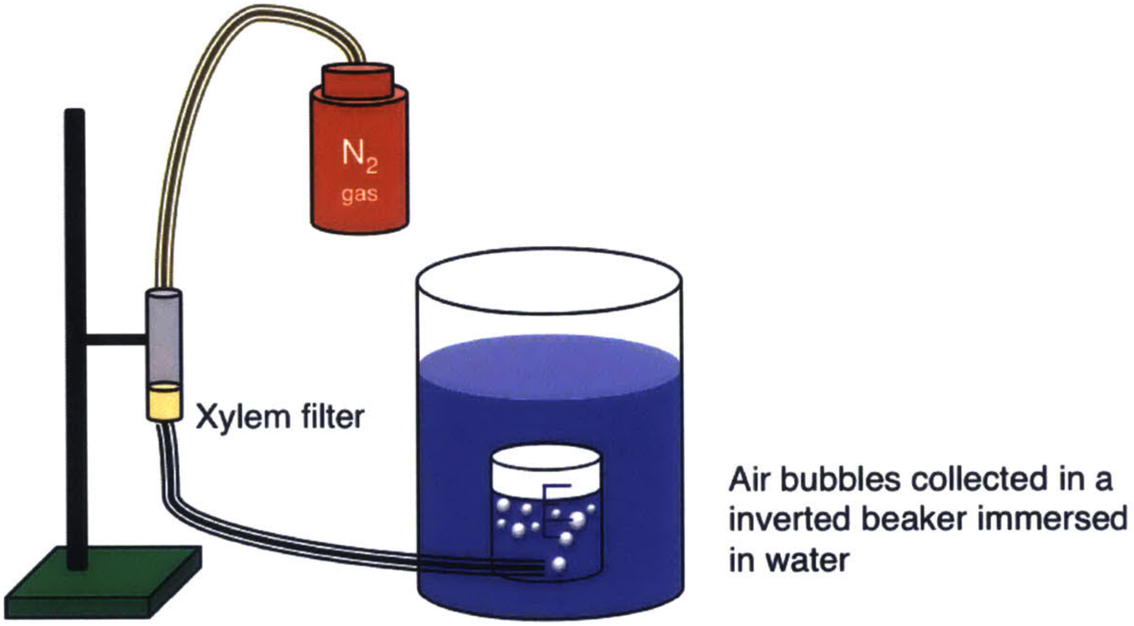


Figure 2-5: Schematic of setup used for airflow rate experiments

of 488 nm were purchased from LifeSciences. The fluosphere solution was sonicated for 1 minute using a VWR Ultrasonic Mixer to break down particle aggregates. The fluospheres solutions were prepared in batches of 50 ml. The required quantity of fluospheres was pipetted out and added to deionized water to achieve concentrations in the range of 10^6 particles/ml. While the goal was to test for the maximum rejection capacity of the xylem, extremely high concentrations would have made counting difficult and time-consuming; increased exposure time could cause bleaching of fluorescent particles leading to inaccuracy in counting as well.

The mixture was then left on a VWR Ananlog Vortex Mixer for 2-3 minutes to ensure homogenous mixing. The input count was evaluated using a hemacytometer (a disposable Neubaeur-modified C-chip procured from InCyto). 10 μL of the fluospheres solution was inserted into the C-chip which was mounted under a Nikon TE2000U inverted epifluorescence microscope and was illuminated with FITC light. FITC has a wavelength in the range of 495-519 nm which is close to peak emission wavelength of the yellow green particles and causes these particles to fluoresce brightly. The particles were counted under a magnification of 30x. The number of particles in each of the four 1 mm^2 corner arrays was enumerated and the final concentration was obtained by averaging over these numbers and multiplying by a factor of 10^4 (Figure 2-6). The same protocol was followed to estimate the concentration of fluospheres in the filtrate as well.

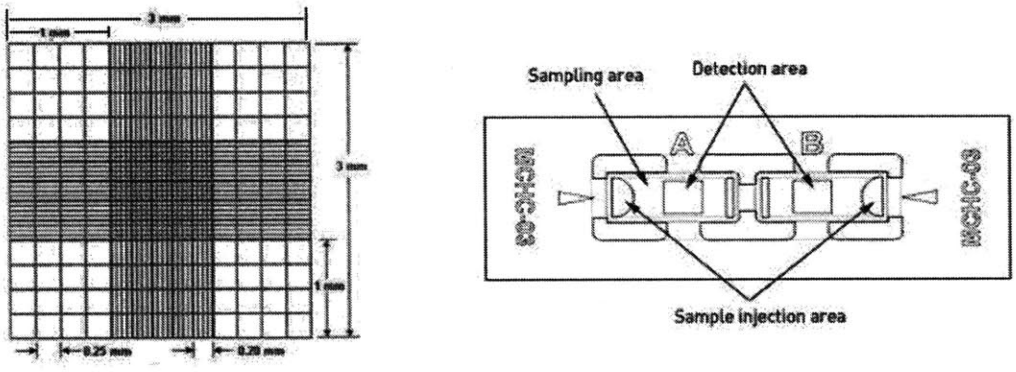


Figure 2-6: Schematic of disposable hemacytometers used for counting fluospheres

2.2.9 SEM imaging

A Zeiss Merlin High Resolution Scanning Electron Microscope was used to acquire images. Xylem samples were sliced into thin vertical sections and imaged under an EHT voltage of 0.7-1.2 kV and a probe current of 75-90 pA. No coating was applied to the samples. The depth-of-resolution mode was used for acquiring images.

2.2.10 Imaging conductive pathways using fluorescent dyes

Xylem cross sections were visualized after staining them with Fluorescent Brightner 28 (FB28) procured from Sigma Aldrich. Dyes such as safranin, fuchsine and toluidine blue are commonly used in visualization of conductive pathways in xylem but have a tendency to diffuse through cells resulting in staining of non-functional vessels. It has been reported that FB28 is a better alternative for such studies as apart from having a lower diffusion rate through the xylem tissue, it binds selectively to cellulose in the secondary cell walls and has strong fluorescence which allows identification of individual functional tracheids with greater accuracy [16].

FB28 was first sonicated using a VWR Symphony Ultrasonic Cleaner for 10 minutes to break up particle aggregates. A 50 ml 0.3% volume-by-volume solution of FB28 was prepared in deionized water and left on a VWR Analog Vorticity Meter for 5 minutes to ensure thorough mixing. Samples with a diameter of 0.5 inches were flushed with 5 ml of this solution followed by 2 ml of DI water to remove the excess dye particles under a gas pressure of 10 psi. The samples was immediately sectioned into thin horizontal slices using a razor blade and were mounted under a Nikon TE2000-U inverted epifluorescence microscope. The entire cross section was divided into 36-40 sub-domains which were imaged under a magnification of 60x and later stitched together to generate the complete image.

2.3 Results

The results for each of three methods described earlier have been presented separately. All results have been averaged over three measurements and the error bars in the graphs denote standard deviation.

2.3.1 Treatment of xylem samples with polymers

All the xylem samples used for experiments in Section 2.3.1 had a diameter and length of 0.5 inches each. The reported flow rates were measured at an applied pressure of 10 psi.

Treatment of xylem samples with PVP

Experiments with PVP having molecular weights 10K (at concentrations of 5%, 10%, 20% and 30%) and 40K (at concentrations of 5%, 10%) were repeated. Increasing the molecular weight (or length of chains) and the concentration of PVP led to a decrease in flow rate (Figure 2-7). It is possible that the longer polymer chains create greater hindrance to the flow of water resulting in reduced permeability. The dried samples coated with PVP showed a 60-400x decrease in permeability with flow rates in the range of 0.1-0.5 ml/min in comparison to 30-40 ml/min for the fresh samples.

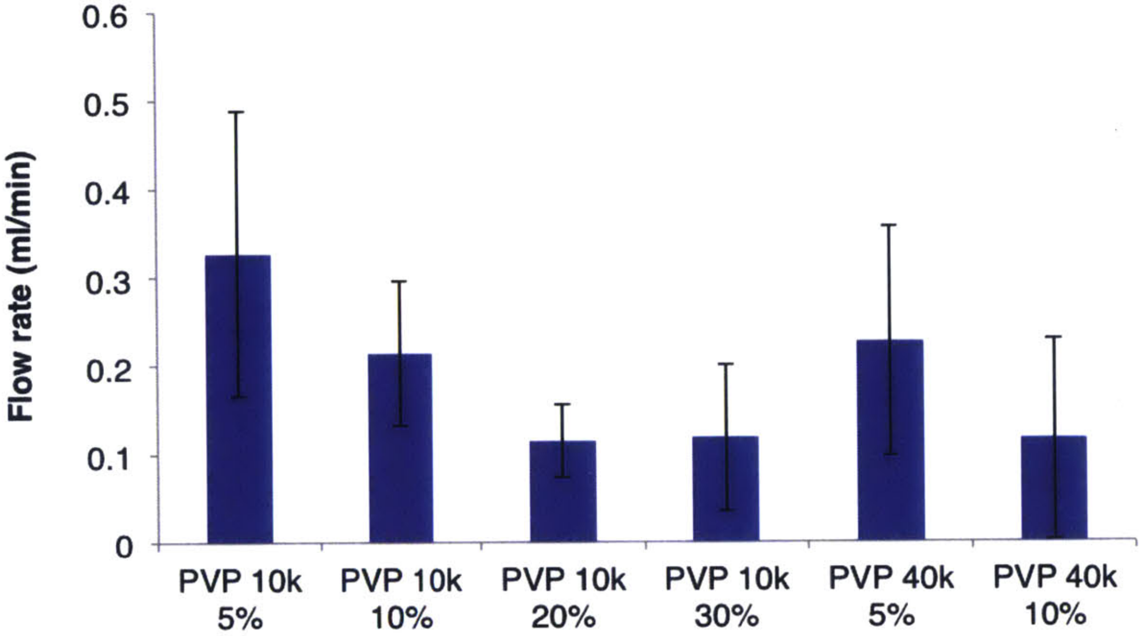


Figure 2-7: Effect of immersing xylem in PVP on flow recovery

Treatment of xylem samples with sucrose

As the flow rates obtained after treatment with PVP were still very low, the effect of introducing sucrose at concentrations of 5%, 10%, 20%, 30% and 40% was studied. The average flow rates were found to be slightly better and were in the range of 0.17-1 ml/min. Increasing concentrations up to 20% led to a reduction in flow rate but at higher concentrations, the flow rates were observed to be higher (Figure 2-8). Introducing sucrose at higher concentrations can have two effects, increased resistance to flow of water as explained previously or formation of larger crystals which could damage the membranes in the xylem creating leakage pathways for water to flow through. It is possible that while the former mechanism is more dominant in concentrations up to 20%, the latter takes over at higher concentrations.

As preserving the structure of xylem is critical for filtration, sucrose solutions having concentrations of 10%, 20%, 30% and 40% were mixed with lime juice, which is know into prevent crystallization of sugars. For reference, control samples which were just dipped in deionized water and dried were also prepared. Xylem samples

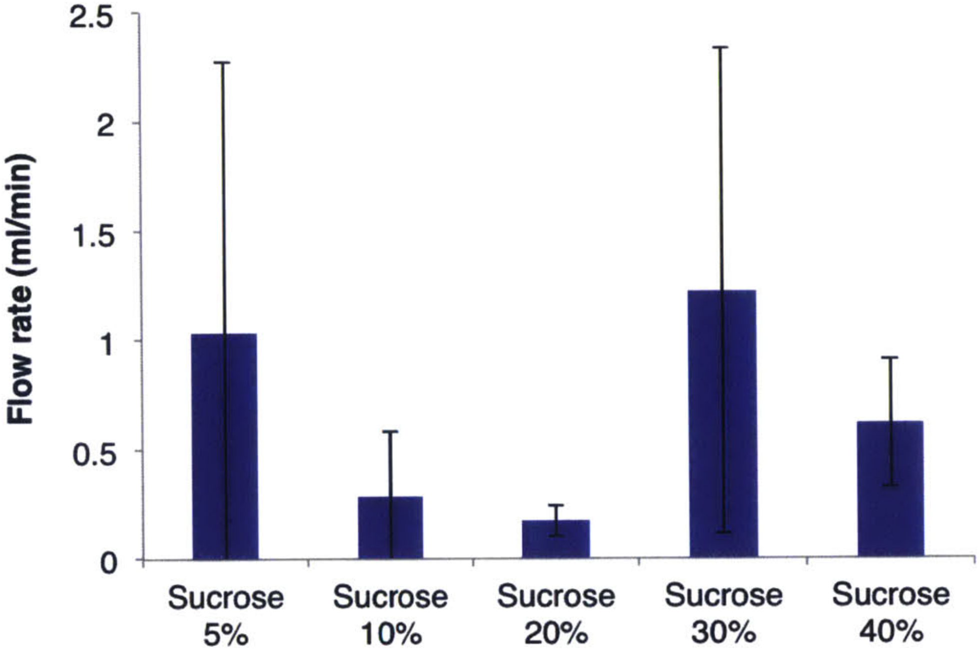


Figure 2-8: Effect of immersing xylem in sucrose on flow recovery

immersed in these solutions had flow rates between 0.051-0.096 ml/min upon drying, which was much lower than that of plain sucrose (Figure 2-9). However, the flow rates of the dried samples did not show an increasing trend with concentration as was observed previously. Nevertheless, no significant improvement in permeability was achieved.

The effect of treating the xylem with non-crystallizing sugars such as light and dark corn syrup was also explored. While light corn syrup refers to corn syrup seasoned with vanilla and salt, dark corn syrup consists of corn syrup, molasses, caramel, salt and sodium benzoate [17]. The results were not found to be particularly encouraging as flow rates after drying were still very low. Samples treated with light corn syrup showed higher permeability and increasing the concentration resulted in a slight drop in flow rate (Figure 2-10).

In comparison to simple immersion of samples in the test solution and drying, attempts were also made to check if antisolvent precipitation allows a better control over the size distribution of the precipitate resulting in improved permeability after drying. Samples immersed in sucrose solutions of concentrations 5%, 10% and 20%

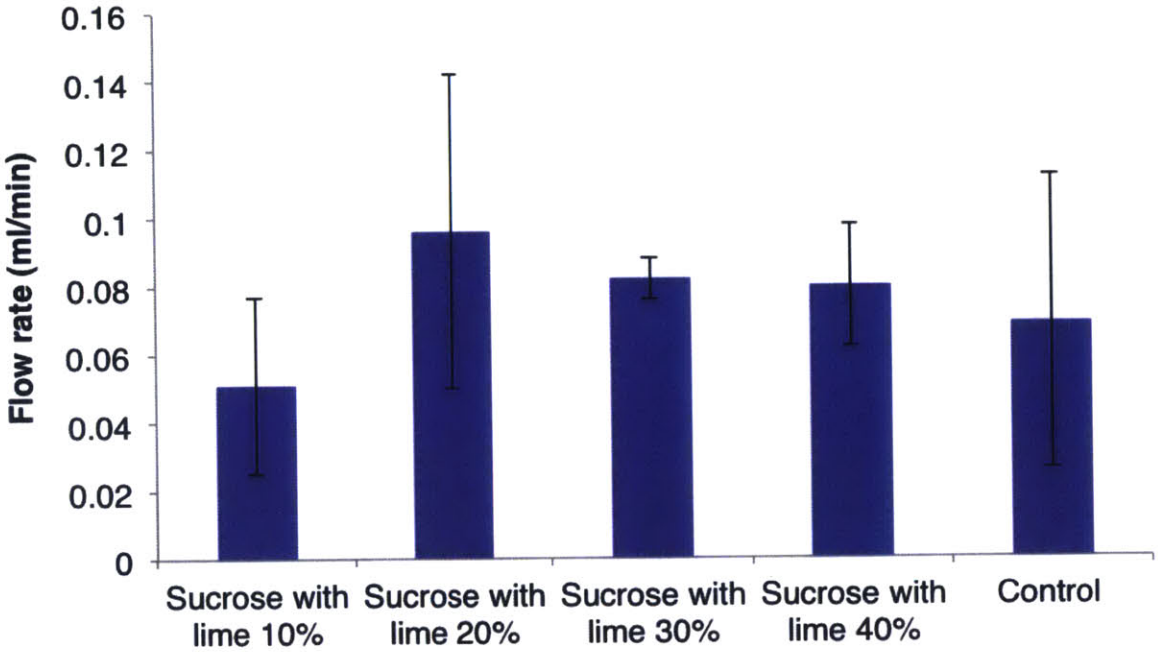


Figure 2-9: Effect of immersing xylem in sucrose-lime juice solution on flow recovery

were soaked in ethanol and dried. As depicted in Figure 2-11, the flow rates were once again an order of magnitude lower than those before drying.

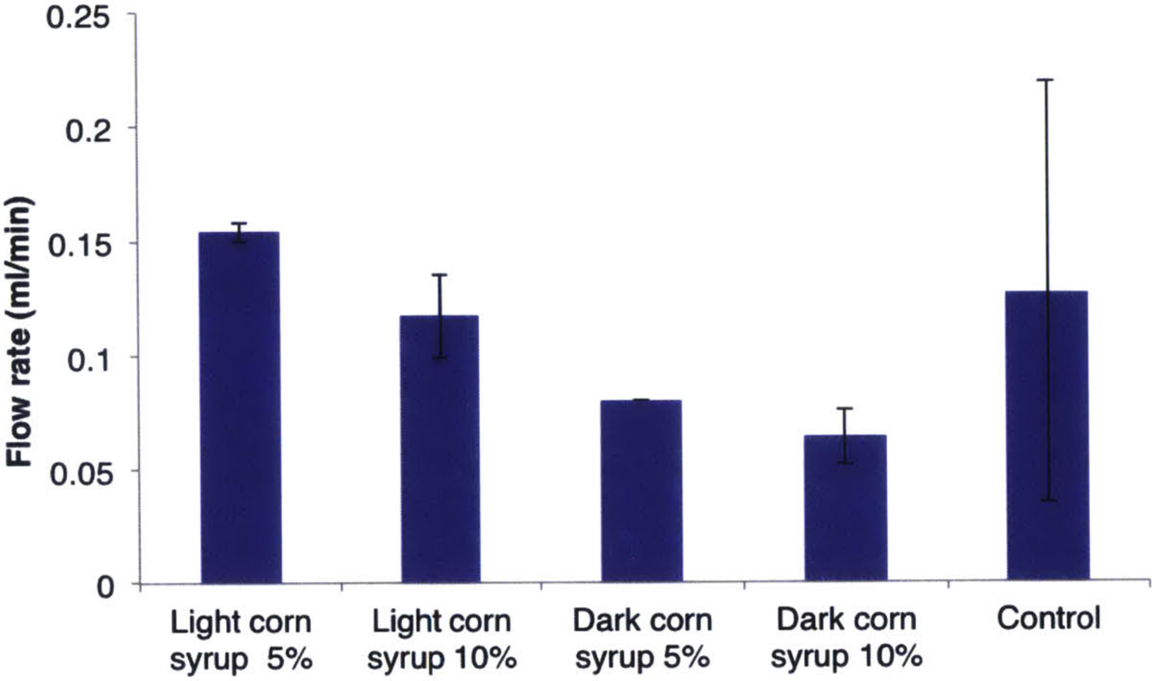


Figure 2-10: Effect of immersing xylem in sucrose-lime juice solution on flow recovery

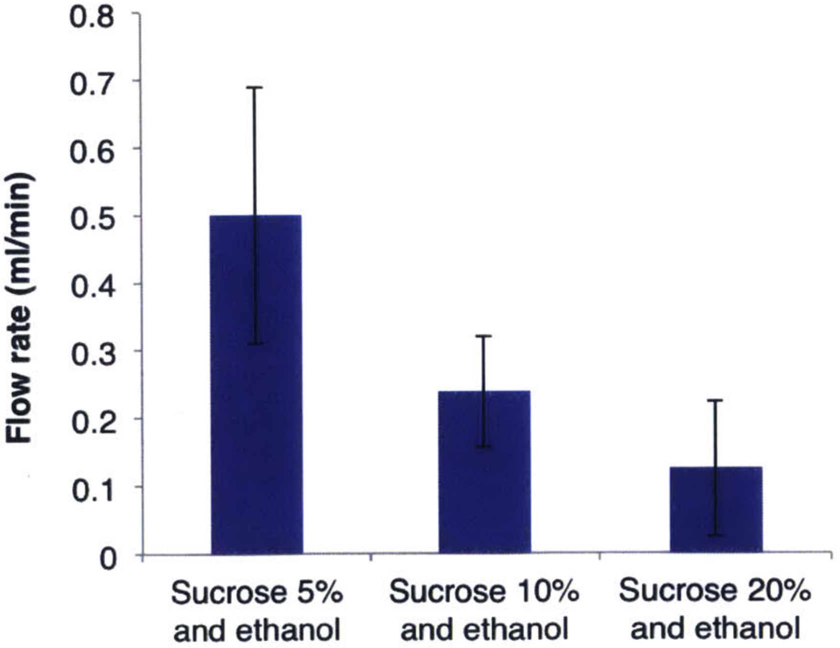


Figure 2-11: Effect of immersing xylem in sucrose-lime juice solution on flow recovery

Conclusion

Treating xylem samples with sucrose, PVP and corn syrup did not yield very encouraging results. The permeability of the dried samples was ~100 times lower than that of the fresh sample even in the best case. This could be due to a variety of factors such as the time available for dissolution of the solute particles during tests being very low. This provided a strong motivation to explore other approaches that might be more effective in preserving xylem permeability after drying.

2.3.2 Treatment of xylem with ethanol

Xylem samples, having a length and diameter of 0.5 inches, were treated with ethanol, both through immersion and flushing (Figure 2-12). These samples showed flow rates that were an order of magnitude higher than the control sample which was soaked/flushed with deionized water. It was observed that the permeability of samples immersed in ethanol was higher that that which was described in Section 2.3.1 for antisolvent precipitation. This could be because of the increase in immersion time from 3 to 9 hours that allowed more time for ethanol to diffuse through the sample and thus, have a stronger effect.

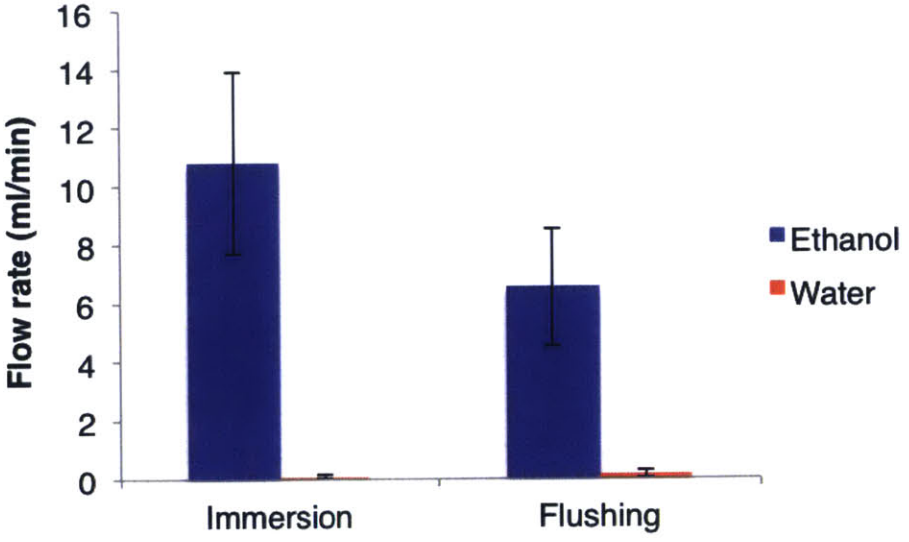


Figure 2-12: Effect of ethanol treatment on flow recovery

The permeability of dried samples after treatment with ethanol was of the same order as that of fresh samples. This approach towards xylem preservation seemed to be very promising and was investigated in greater detail.

It was previously found that 0.25 inch long xylem samples had the same filtration performance as those having a length of 0.50 inches or more [8]. As increase in length adds resistance to flow, subsequent experiments were conducted with xylem samples having a length of 0.25 inches while the diameter was kept constant at 0.5 inches as before. Further, 0.25 inch samples were found to have a much higher recovery in comparison to the ones that were 0.50 inches long. This observation has been discussed in greater detail in Section 2.3.3.

Further, even within the same tree, the permeability of xylem can vary from branch to branch and in fact, may also change within the same branch. This could be due to bending of branches or compartmentalization that leads to structural changes in the xylem. In order to account for the variations in fresh sample flow rates, percentage of recovery as defined by Equation 2.1 was introduced as a parameter for comparing the efficacy of treatment instead of the flow rate post-drying.

$$Recovery = \frac{Flow\ rate\ after\ drying}{Flow\ rate\ before\ drying} \tag{2.1}$$

Further, to ensure that ethanol treatment works for samples from different trees, samples were collected from various branches as well as from trees in different locations. As can be observed from Figure 2-13, ethanol-flushed samples consistently gave higher recoveries in comparison to the ones that were just flushed with water and dried.

After establishing the consistency and repeatability of results obtained post ethanol treatment, experiments were conducted to understand the underlying mechanisms responsible for the improved permeability observed. Though the low surface tension of ethanol and changes in adhesion might play a critical role in preventing pit aspiration, ethanol can have other effects on the physical, chemical and mechanical properties of the xylem. A few experiments that were designed to evaluate the dependence of

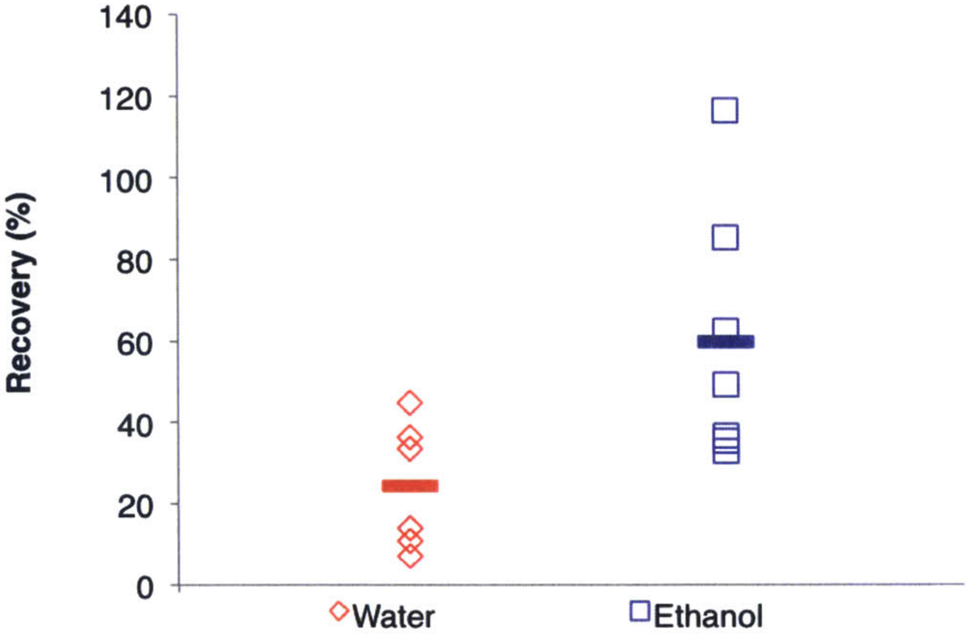


Figure 2-13: Repeatability and consistency of ethanol treatment

permeability on some of these effects have been described below.

Necessity of presence of ethanol during drying

Treatment of xylem samples with ethanol involves two steps : flushing and drying. Ethanol replaces water in biological molecules and is commonly used as a preservative agent. It is known to denature proteins leading to deactivation of live processes [18]. If live processes have any role to play in preserving the permeability of xylem, then flushing with ethanol alone should result in a change in recovery.

To determine the relative importance of exposure of xylem to ethanol versus the presence of ethanol during drying, two sets of experiments were conducted.

1. To verify if flushing with ethanol affects permeability, samples were flushed with ethanol and water and tested for flow rates of water immediately without drying. The flow rates in both cases were similar (Figure 2-14). This showed that flushing with ethanol does not induce changes in permeability of fresh samples.

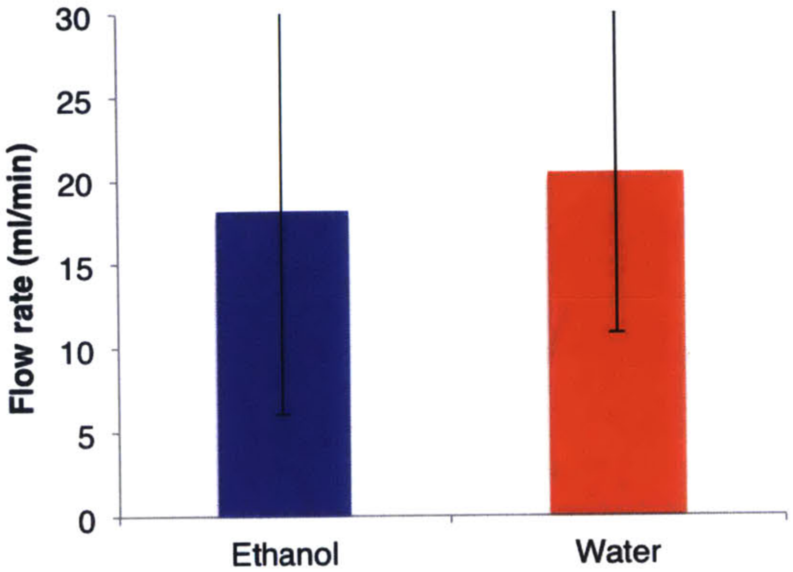


Figure 2-14: Effect of ethanol treatment on permeability of fresh samples

2. To verify if the presence of ethanol is critical during drying, four sets of samples were prepared:
   (a) Flushed with water and dried
   (b) Flushed with ethanol and dried

(c) Flushed with water followed by ethanol and dried

(d) Flushed with ethanol followed by water and dried

Figure 2-15 shows the variation in recovery after drying for different flushing sequences. Samples where water was present during drying had lower recovery than the ones where ethanol was present.

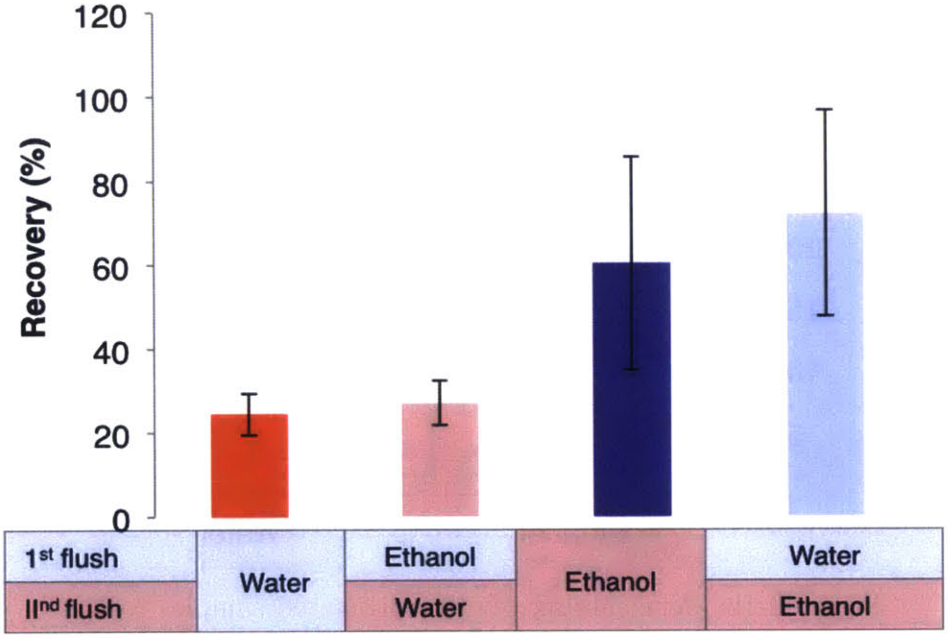


Figure 2-15: Effect of flushing sequence on permeability

These experiments conclusively demonstrated that flushing with ethanol alone does not contribute towards preventing pit aspiration. The presence of ethanol during drying is critical to preserve permeability.

State of pit membranes in ethanol-preserved samples

The increased permeability of samples preserved using ethanol could come from two factors:

- The pores could be left open
- Treatment with ethanol could impede ability of the pit membrane and the cell wall to form strong adhesive bonds with one another. Under such circumstances, the flow of water under external pressure could cause the membranes to detach

and return to the suspended state thereby restoring permeability.

To test which of the above mechanisms dominate, air flow rates at a low pressure of 1 psi through ethanol-treated and water-treated samples were compared. Having a much lower density and surface tension that water, it would be difficult for air to exert pressure and undo any such mechanical changes induced during drying.

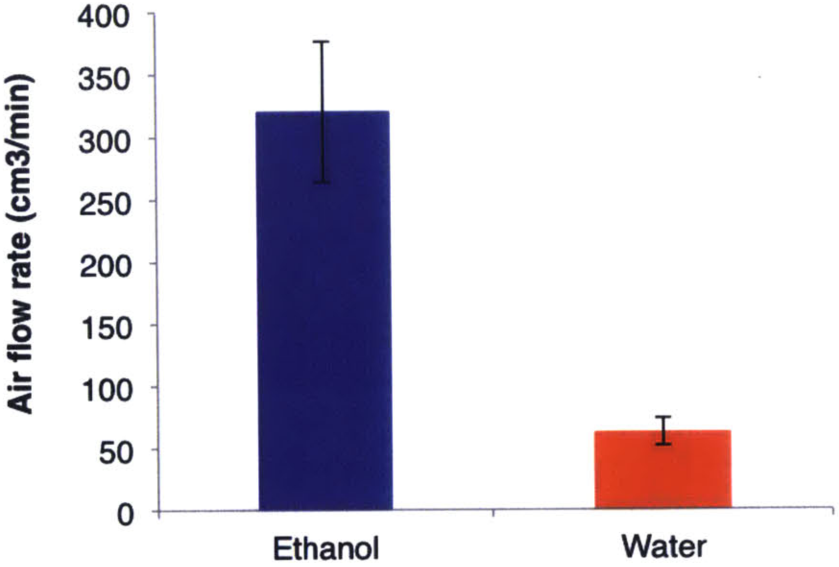


Figure 2-16: Permeability of ethanol-treated samples to air

Ethanol-treated samples demonstrated high permeability to air indicating that the pits stay open post-drying (Figure 2-16).

Interaction of ethanol with sap

Xylem sap consists of water, nutrients, hormones and organic substances. The use of ethanol for extraction of organics from wood is quite common [19]. Interaction of ethanol with the solutes in sap could also affect permeability. To test the validity of this hypothesis, sap was flushed out of the xylem by passing 20 ml of deionized water in both, the standard as well as reverse orientation. As the volume of water passed was approximately 20 times that of the filter, it can be said with a fair amount of certainty that this procedure would have removed sap completely. The samples were then flushed with water and ethanol and dried. The flow rates after drying was compared to samples that were not flushed with DI water initially. The experiment was repeated by flushing the sap with 5 ml of water instead of 20 ml.

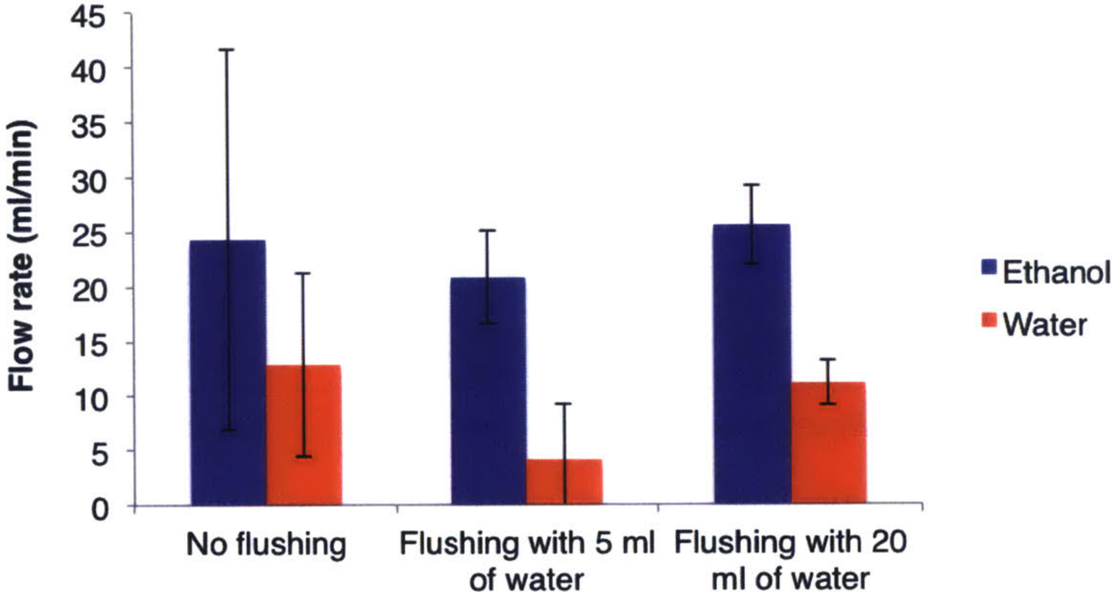


Figure 2-17: Effect of flushing sap on permeability of dried filters

Flushing of sap did not contribute to any significant difference in permeability for both, water-flushed and ethanol-flushed samples upon drying. Increasing the volume of water used from 5 ml to 20 ml did not produce any difference either.

Use of other solvents and ethanol-water mixtures

The possibility of preserving permeability by using other low surface tension solvents was explored by treating xylem samples with heptane. Heptane is an inorganic solvent and has a surface tension of 20 mN/m which is comparable to that of ethanol (22 mN/m).

The recovery rates of heptane-treated samples were similar to that of ethanol (Figure 2-18). This showed that it could be possible to preserve the permeability of xylem through treatment with non-polar low surface tension solvents.

Further, samples were flushed with a 10% volume-by-volume mixture of ethanol and water which would have a surface tension marginally lower than water but sig-

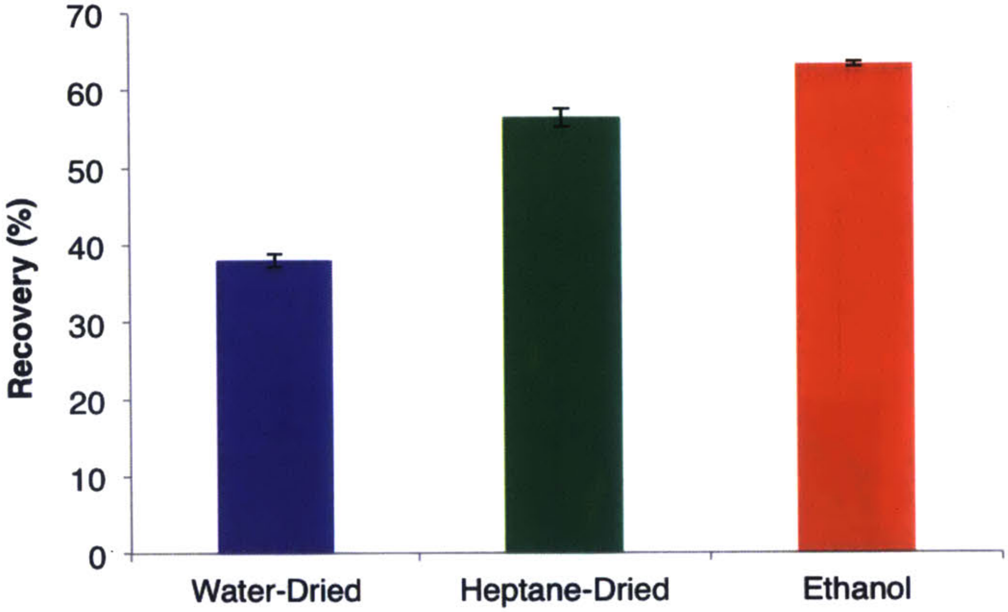


Figure 2-18: Effect of flushing heptane on xylem permeability

nificantly higher than ethanol [5, 12].

The permeability of these samples was found to be more or less similar to that of the water-flushed samples (Figure 2-19). This observation was in line with previous findings where ethanol-water mixtures with concentrations below 70% were found to be ineffective in preventing pit aspiration.

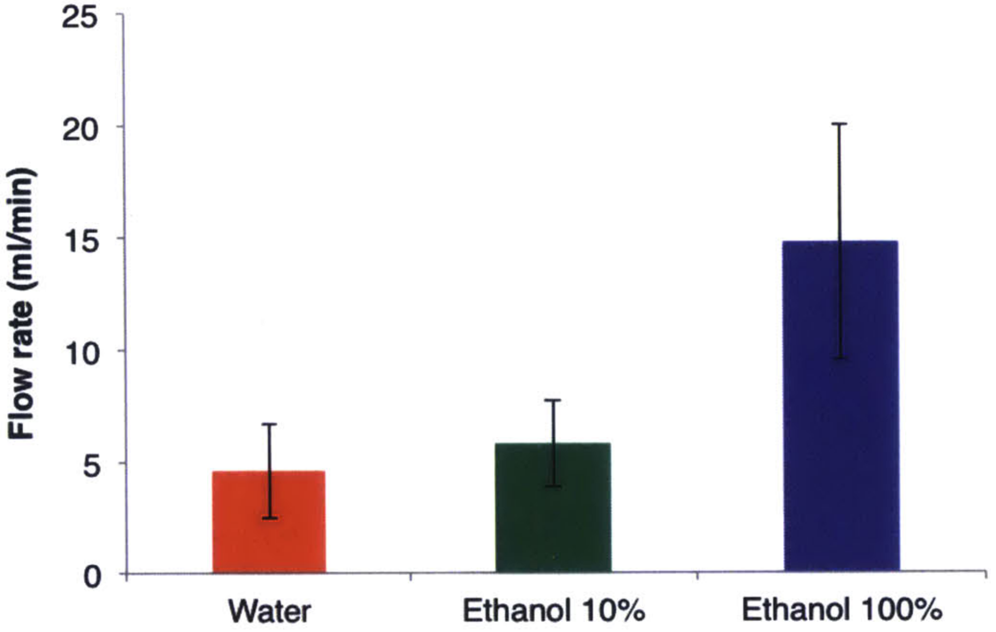


Figure 2-19: Effect of concentration of ethanol-water mixture on xylem permeability

Imaging

Xylem samples treated with ethanol were imaged using a Scanning Electron Microscope (SEM) at the Center for Material Science and Engineering Facility. The images showed that ethanol does indeed help maintain the membranes in their suspended state after drying (Figure 2-20). While majority of the membranes in ethanol-dried samples were found to be in an unaspirated state, as shown in Figure 2-20c, some of them were found to be partially aspirated (Figure 2-20d).

Further, imaging with Fluorescent Brightner 28 (FB28) was done to visualize the conductive pathways in fresh, ethanol-dried and water-dried samples. As illustrated in Figure 2-21, the number of open pathways in ethanol dried samples was more than the water-flushed ones but lesser than the fresh samples.

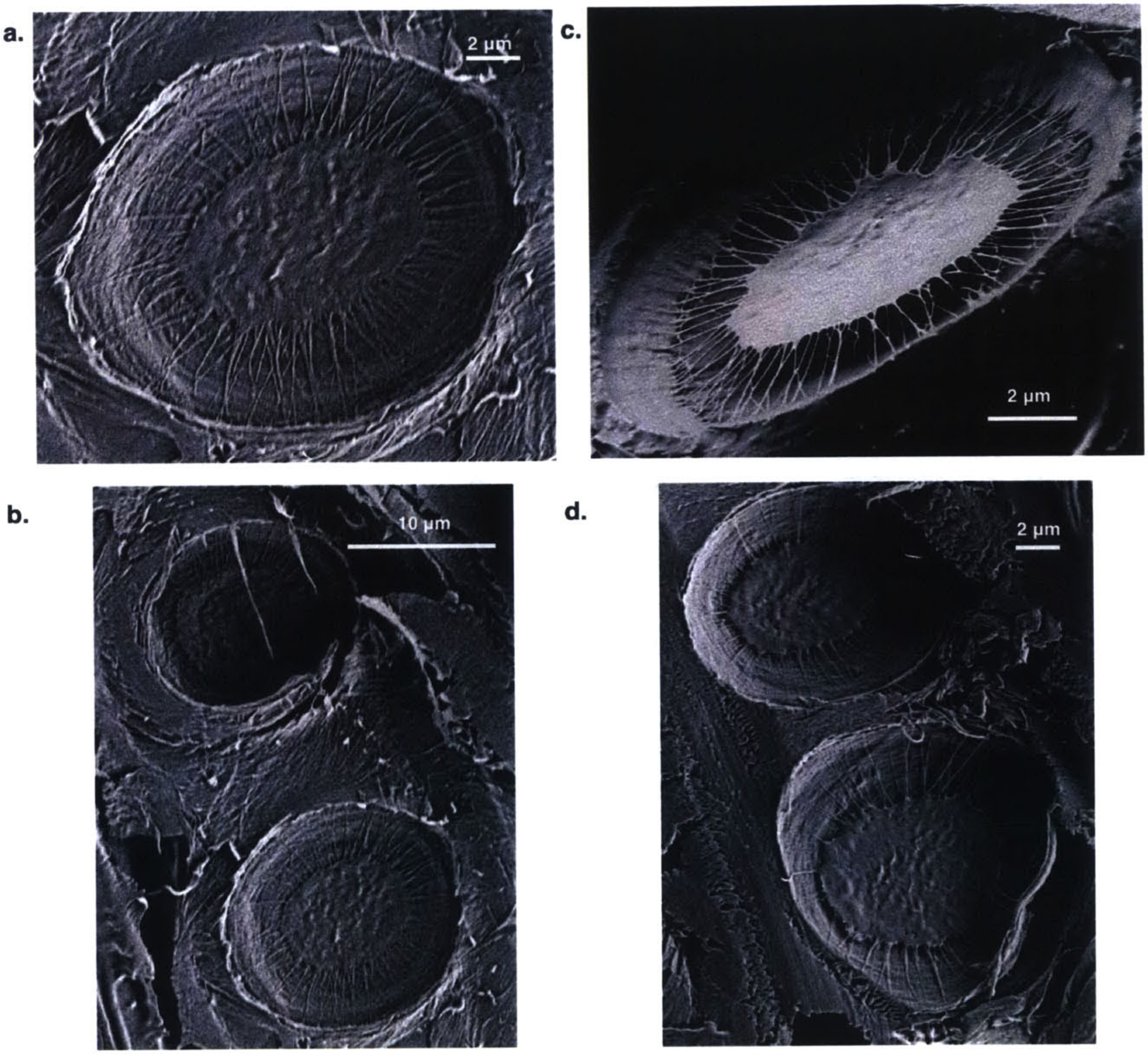


Figure 2-20: a), b) Pit membranes of dried xylem. The images show fibers completely collapsed against the cell wall c), d) Pit membranes of xylem samples treated with ethanol. The membrane is retained in a suspended state and the gaps between fibers and cell wall can still be seen

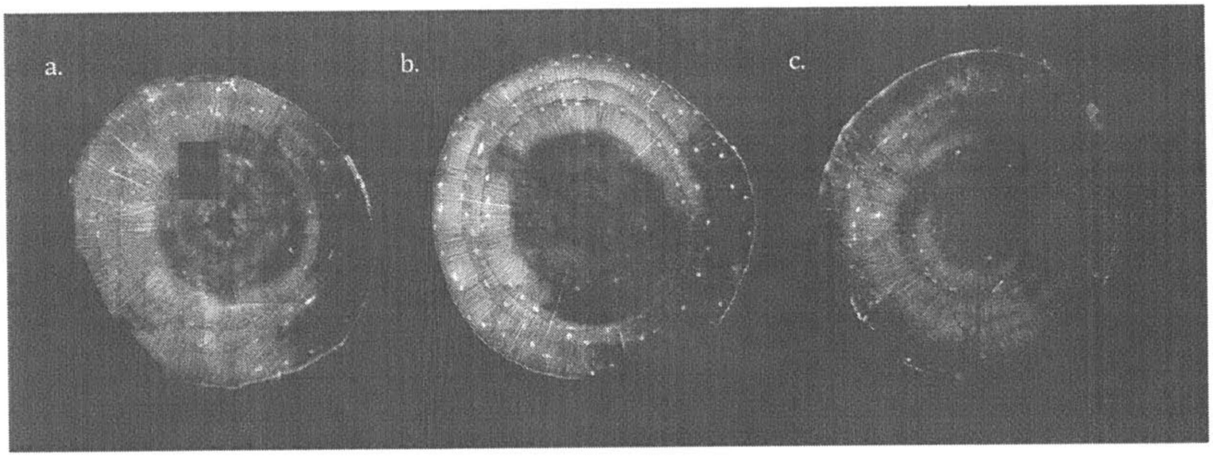


Figure 2-21: a), b) and c) show cross-sections of fresh, ethanol-dried and water-dried samples respectively. The samples had a diameter of 0.5 inches. The illuminated parts of the images show regions through which dye penetration occurred and depict pathways available for flow. The fresh samples show maximum permeability followed by ethanol-dried and finally water-dried samples.

Rejection of xylem filters after ethanol treatment

After achieving improved flow recovery after drying using ethanol, the next step was to check if ethanol also helps preserve the rejection ability of xylem. Experiments done with 1 μm fluospheres showed that the rejection ability of the xylem is not compromised during ethanol treatment.

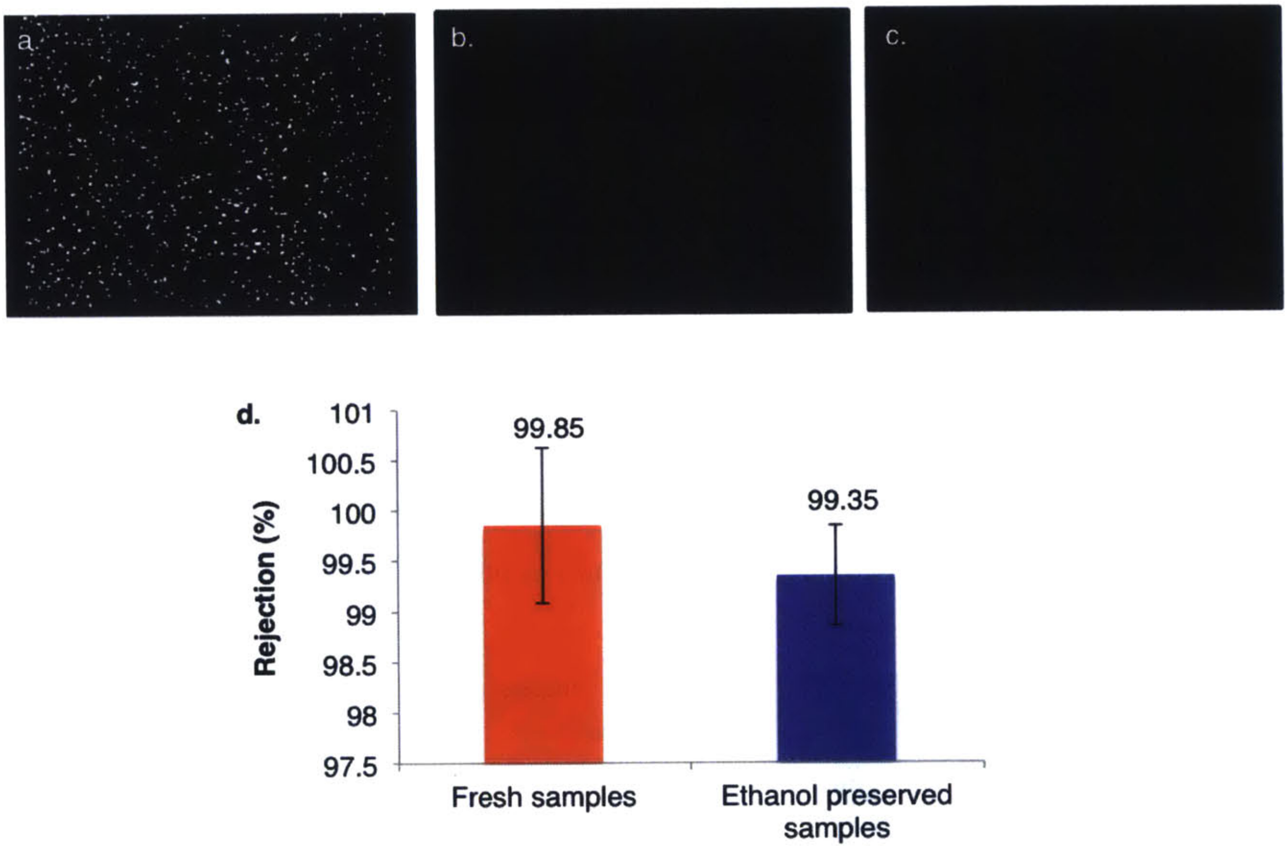


Figure 2-22: a) Feed solution of 1 μm fluorescent microspheres with a concentration of 1.3×10^6 particles/ml b) Filtrate obtained from fresh samples c) Filtrate obtained from ethanol-dried samples d) Comparison of rejection of fresh and ethanol-preserved samples

Conclusion

It was found that treatment of xylem samples with ethanol helps preserve permeability and rejection ability of the filters after drying. Experiments conducted to understand the mechanism of preservation showed that treatment with ethanol prevents the pit membrane from closing during drying resulting in high permeability of xylem samples. Flushing xylem samples with ethanol is not enough to induce changes in permeability and the presence of ethanol during drying is critical to prevent pit aspiration.

2.3.3 Effect of sample length on permeability

All results have been averaged over three measurements.

Variation in recovery and rejection ability with length

The rejection of 1 μm fluospheres and permeability of dried 0.25 inch, 0.50 inch and 1.00 inch long samples were tested. Although the rejection performance remained more or less the same with length, a dramatic decrease in recovery was observed when the sample length was increased from 0.25 inches to 0.50 inches (Figure 2-23). Longer samples showed recovery that were an order of magnitude lower than the shorter ones. Filter length was found to have a profound influence on performance.

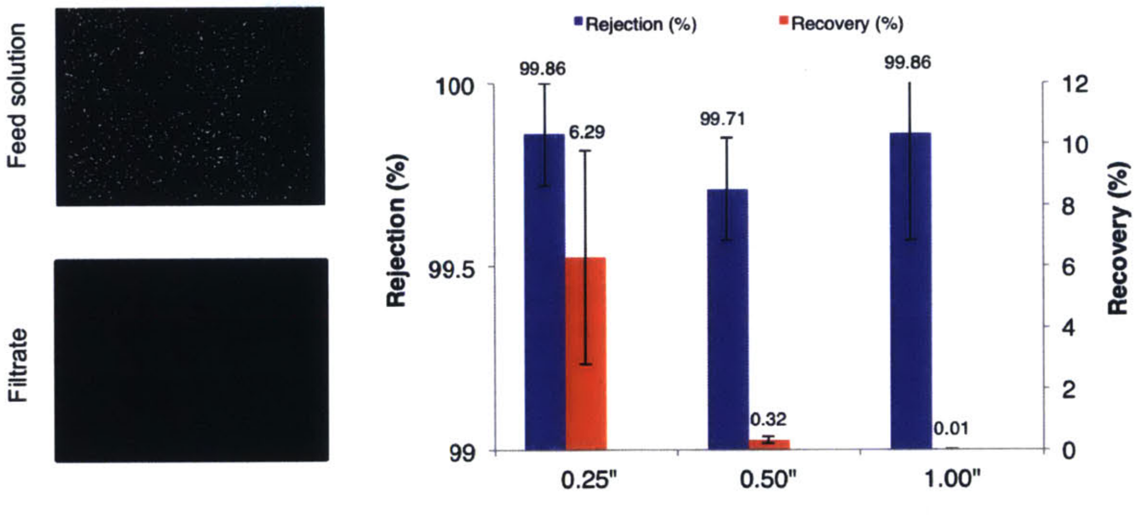


Figure 2-23: Variation in rejection and recovery with filter length. Feed solution was prepared using 1 μm fluorescent microspheres at a concentration of 1.2×10^6 particles/ml.

Factors responsible for strong dependence of length on permeability

The abrupt drop in permeability with increase in length required further investigation. In order to explain this dependence, the following hypotheses were proposed and tested:

1. **Evaporation rate :** During drying, a gradient in the rate of evaporation will be established across the sample length. While the end surfaces, which are completely exposed to air, will dry out faster, the sections in the middle will tend to dry more gradually. This gradient will become steeper with increasing sample length. The rate of evaporation could influence the tendency of pits to aspirate.

   As a first step, experiments were conducted to verify if there exists a gradient in the evaporation rate across the sample length. Samples having a length of 1.5 inches were dried in the oven at 45°C until a 30% reduction in weight was observed. Each sample was then cut into 6 sections measuring 0.25 inches each using an automated vertical cutting saw. These section were then dried completely and their weights monitored as a function of time. In all four samples, the ends showed least reduction in weight post-sectioning indicating that they were comparatively drier than the middle sections to begin with (Figure 2-24). After having established the presence of a gradient in the evaporation rate across the sample length, the next step was to study its effect on permeability. To do so, different methods were adopted to control the rate of evaporation (illustrated in Figure 2-25a-c).

   The sample weights were monitored at regular intervals to estimate the extent to which the rate of evaporation was controlled in each of the arrangements. The drying curves for the samples dried using these three different arrangements (??a,b and c)ave been plotted in Figure 2-26. The third setup where the samples were placed in a closed vial with a hole in the cap was the most effective in slowing down the rate of evaporation. However, no variations could be observed in the recovery rates of the samples dried in the open and closed conditions.

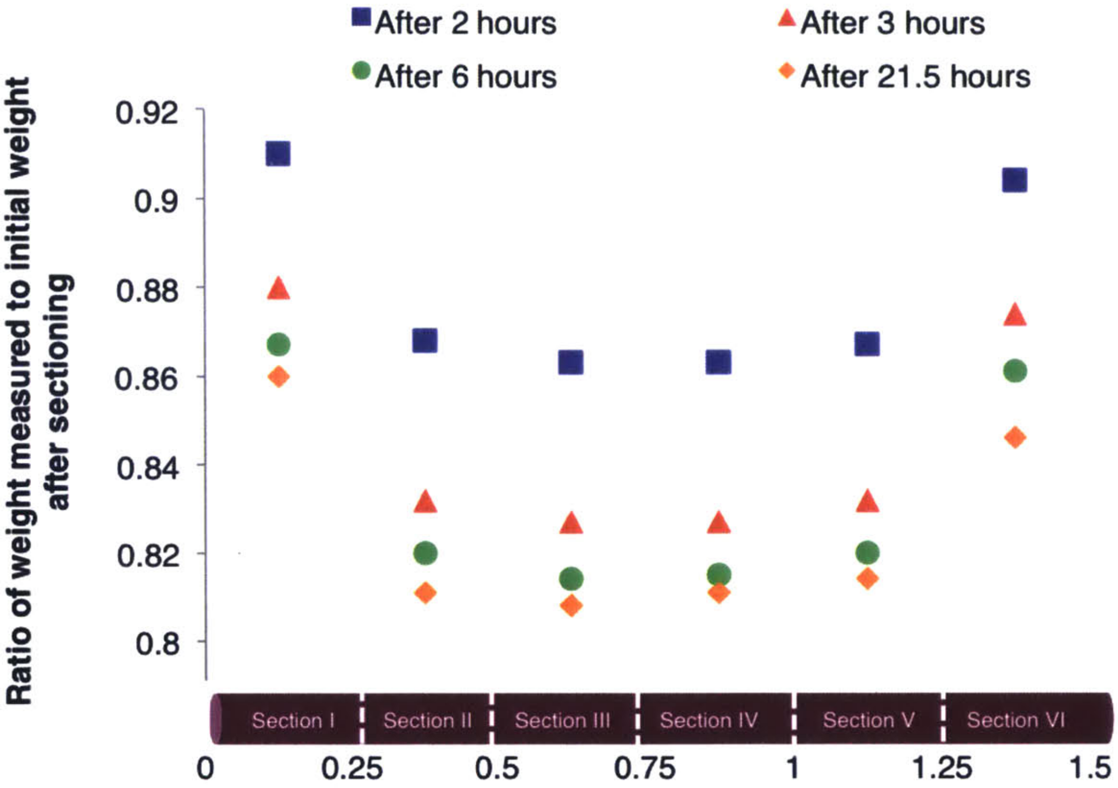


Figure 2-24: Reduction in weight of 0.25 inch pieces cut from partially dried 1.5 inch long samples post-sectioning. The reduction has been plotted against the location from which the smaller sections were extracted from the long sample for four cases

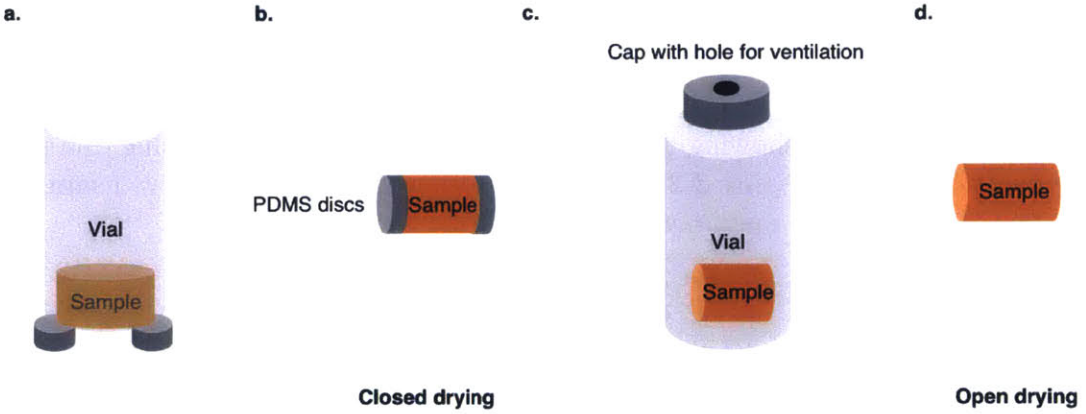


Figure 2-25: Schematic of arrangements used for controlling evaporation rate a) Sample placed on two side supports and covered with a vial on top to restrict exposure to air b) Sample placed between two PDMS discs to control the rate of evaporation from the sides c) Sample placed inside a closed vial with a hole in the cap d) Samples dried in the open

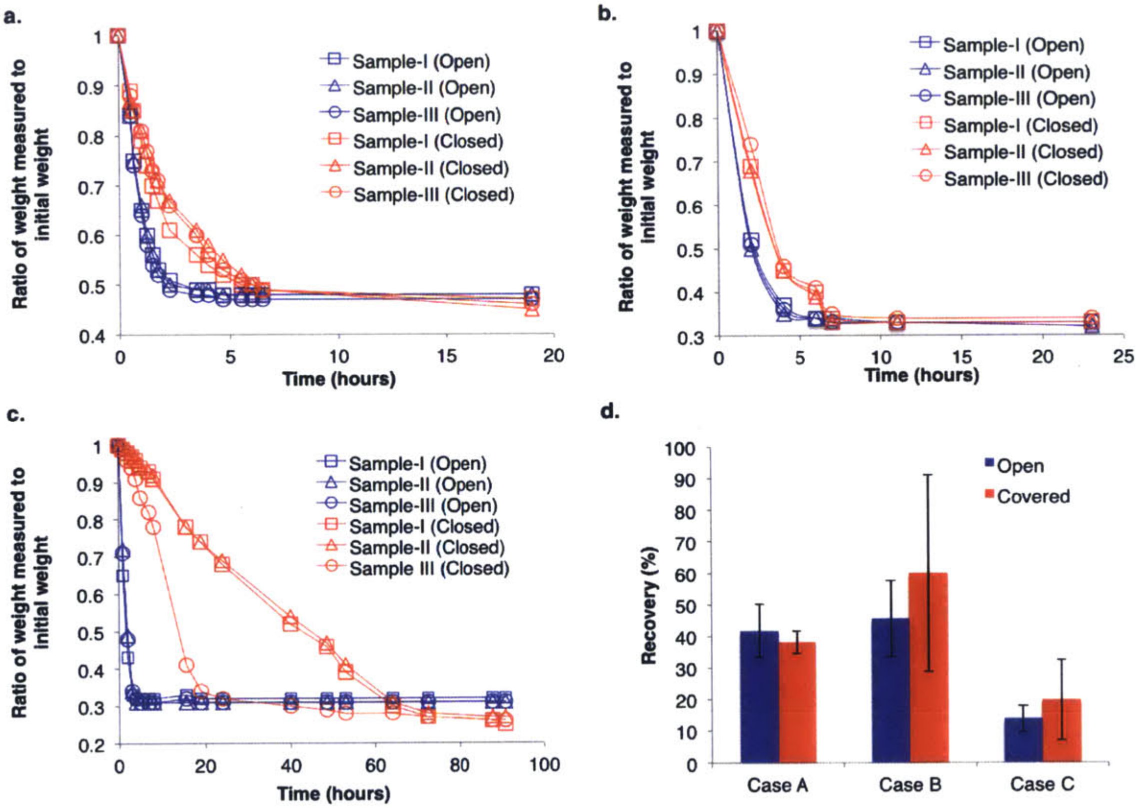
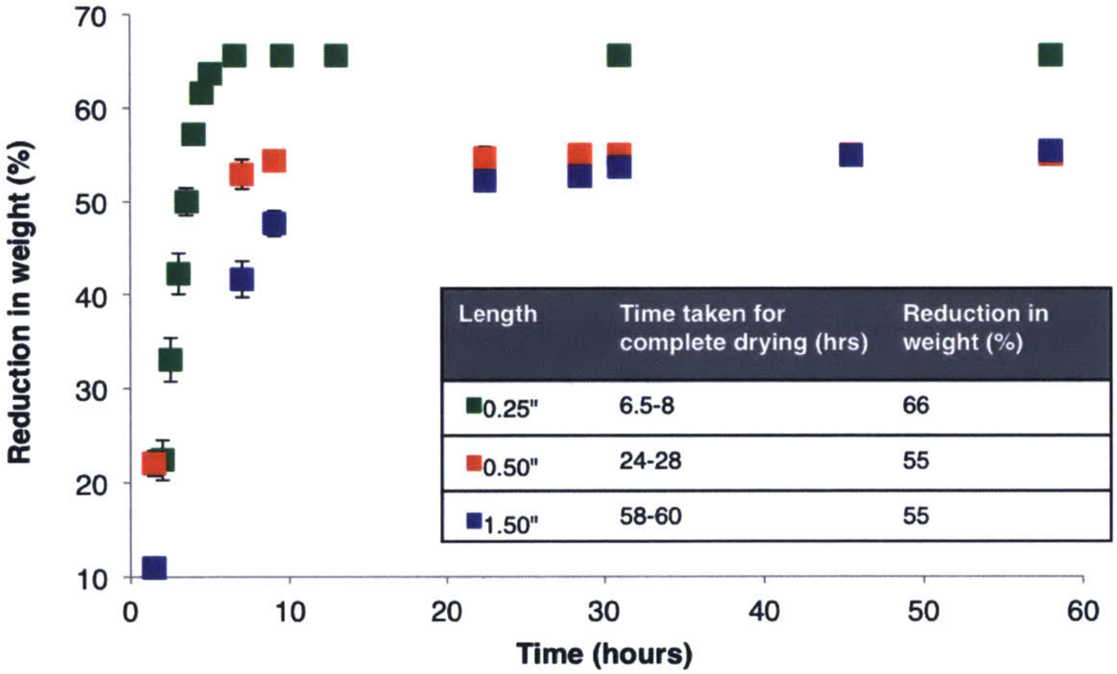


Figure 2-26: a), b) and c) showing the drying curves for samples dried using different arrangements depicted in Figure 2-25 a), b) and c) respectively d) Recovery of samples dried under controlled and open conditions for all

These experiments demonstrated that the rate of evaporation does not play a crucial role in pit aspiration.

2. **Drying period :**The longer samples took much more time to dry out completely than the shorter ones and were therefore kept in the oven for a longer duration at 45°C. The properties of the xylem could be affected by time of exposure to higher temperatures. To check for this effect, 0.25 inch, 0.5 inch and 1.5 inch long samples were flushed with water and dried in the oven at 45°C. The sample weights were continuously monitored to compare the time taken for complete drying. The results have been shown in Figure 2-27. The longer samples took around 10 times as much time as the shorter ones to dry out completely.



Length	Time taken for complete drying (hrs)	Reduction in weight (%)
🟩0.25"	6.5-8	66
🟥0.50"	24-28	55
🟦1.50"	58-60	55

Figure 2-27: Variation in drying period for 0.25, 0.5 and 1 inch long samples

To verify if the time of exposure to higher temperatures affects permeability, samples having a length of 0.25 inches were flushed with water and stored in the oven at 45°C for 19 hours and 100 hours before testing.

The results did show any correlation between the two variables tested for (Figure 2-28).

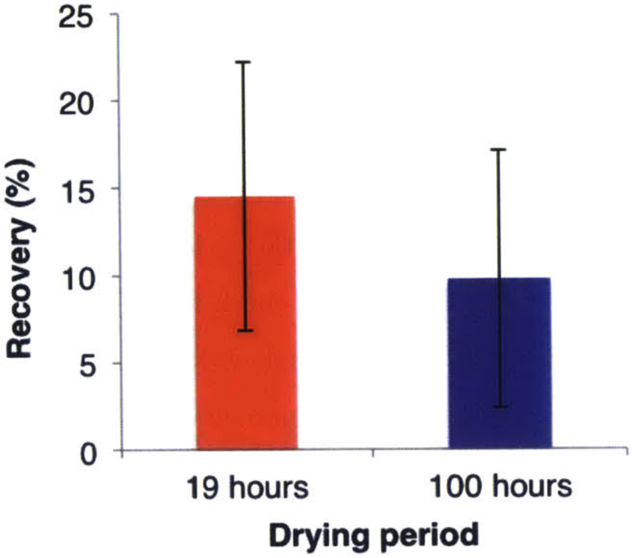


Figure 2-28: Effect of drying period on permeability

3. **Solute concentration :** Wood is mainly composed of cellulose, hemicellulose, lignin and minor amounts of inorganics and extractives. The extractives, which could be monomers, dimers and polymers are a group of cell wall chemicals mainly consisting of fats, fatty acids, fatty alcohols, phenols, terpenes, steroids, resin acids, rosin, waxes, and many other minor organic compounds. These extractives are soluble in a variety of solvents such as water, toluene, ethanol and ethers [19]. The drying process would drive the water-soluble solutes out of their solution form. As the water starts evaporating from the ends, it would cause these solutes to move inwards ultimately resulting in their concentration in the center of the sample. Depending upon the size of the solute molecules, they could add resistance to the flow resulting in a drop in permeability. Previous experiments where xylem samples were treated with Polyvinypyrrolidone (PVP) and sucrose showed that the presence of such molecules in the xylem can result in a significant drop in permeability. As the volume of the solutes would scale with that of the filter, one can expect the effect of solute concentration to be a cause of concern as sample length increases while the cross-sectional area remains constant. The use of ethanol for removing extractives from wood for the purpose

of chemical characterization is a fairly common practice [19, 7]. In order to test if solute concentration plays a critical role in determining filter permeability after drying, 1.5 inch long samples were completely dried. The samples were then cut into 6 smaller sections of 0.25 inches. These sections were weighed individually and flushed with 10 ml of ethanol to remove the ethanol-soluble solutes. They were then dried and weighed again upon completion of drying. If solute concentration took place, the sections cut from the middle portion of the 1.5 inch long samples should have shown the greatest reduction in weight. However, as shown in Figure 2-29, all sections showed an equal reduction in weight and there was no clear evidence to indicate solute concentration in the center.

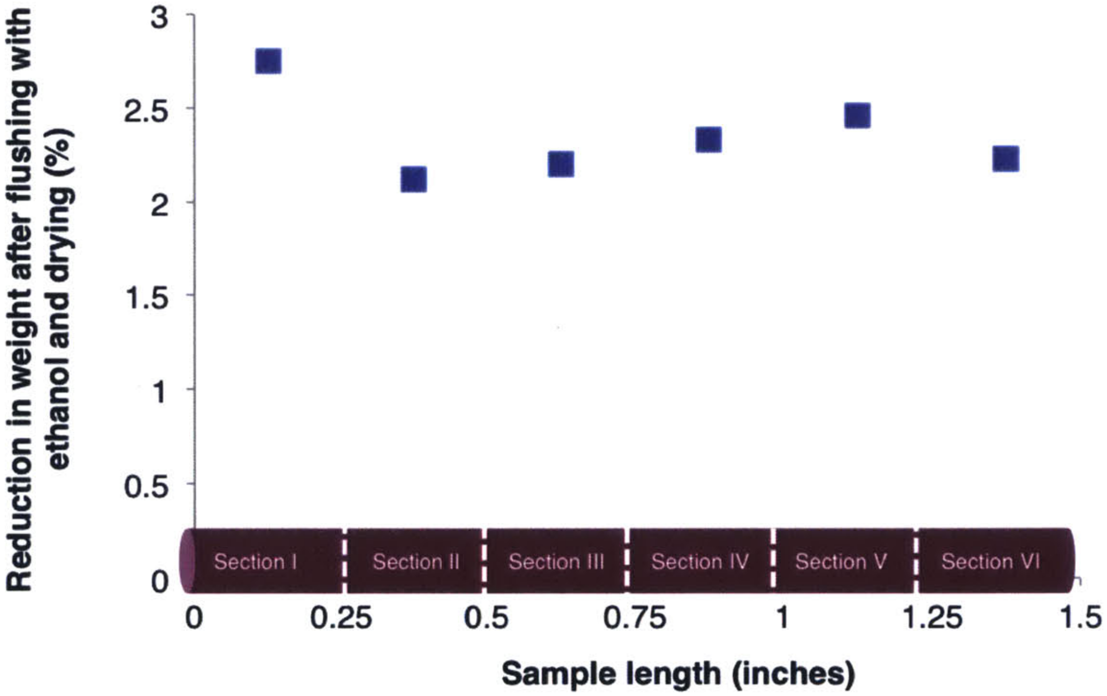


Figure 2-29: Tests for solute concentration in sample center during drying. The reduction in weight of each 0.25 inch section has been plotted against its location along the length of the original sample that was 1.5 inches long

4. **Connectivity between tracheids :** The drying process would cause some of the pores to seal while others would remain open. The permeability of a

dried sample is, therefore, a function of how the open pores link up to generate pathways for the flow of water. This problem bears a strong analogy to the study of percolation in porous media which is a fairly well studied topic [?, ?, ?]. In these studies, the medium is modeled as a collection of nodes arranged in the form of a lattice. A cluster is defined as a group of nodes that are inter-connected. For example, a simple cubic lattice with 16 sites or nodes is depicted in Figure 2-30. The cluster size of the orange, green and purple balls is 3, 6 and 2 respectively. In order to observe percolation through the medium, it is necessary to have at least one cluster extending across the two ends of the medium. The maximum size and distribution of clusters is a function of the probability p with which the nodes are inter-connected. If p is too low, then the chances of finding a cluster that is large enough to match the length scale of the medium is extremely low and no percolation will be observed. However, beyond a critical probability p_c, the probability of observing percolation (denoted by $\theta(p)$) shoots up suddenly (Figure 2-31). The percolation theory can easily be applied to xylem filters if the blocking of a tracheid is not influenced by the blocking of others. The xylem essentially consists of tubular structures called tracheids stacked together (Figure 2-32). The tracheids have sealed ends but are interconnected to each other through pits that are present on their walls. The permeability of the filter to fluid flow depends upon interconnectivity between

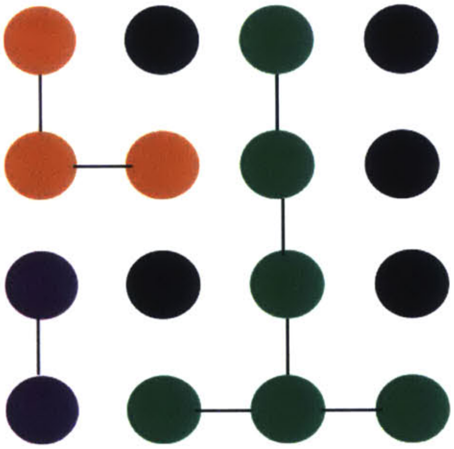


Figure 2-30: Percolation model to study flow through porous media

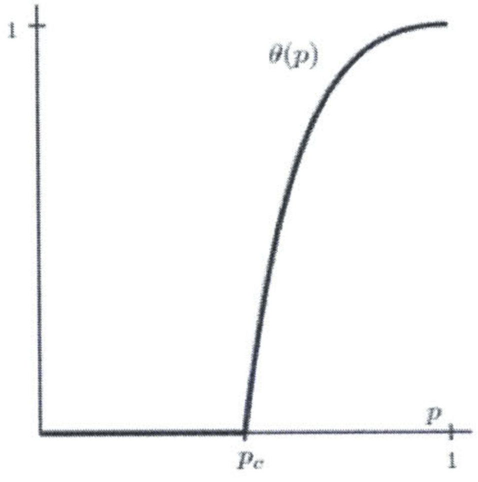


Figure 2-31: Probability of percolation as a function of probability of connectivity between nodes

the tracheids, which in turn, is dictated by the number of open pits. In this scenario, it is possible to compare the tracheids to nodes and the pits to edges. The probability p of an edge existing between nodes will be the probability of a pit remaining open towards the end of the drying process. Assuming p to be constant for all the pits along the sample length, there will be a maximum cluster size S associated with it. If the sample length L exceeds S, then no percolation will be observed. However, if L<S, then water will be able to flow through. The previous experiments demonstrated that while 0.25 inch samples show some flow recovery upon drying, the permeability drops abruptly for samples that are 1.5 inches long resulting in almost no flow. This observation could simply be a result of a purely stochastic process where the delicate balance between sample length and cluster size of the interconnected tracheids dictates permeability.

To verify this hypothesis, samples having a length of 1.5 and 0.25 inches were completely dried. Airflow rates were measured through these samples to check for permeability. The 1.5 inch samples were then cut into smaller sections of 0.25 inches and the airflow rate tests were repeated. It was found that while the 1.5 inch samples showed almost no permeability to air, their smaller sections did allow air to pass through. However, it was found that the permeability

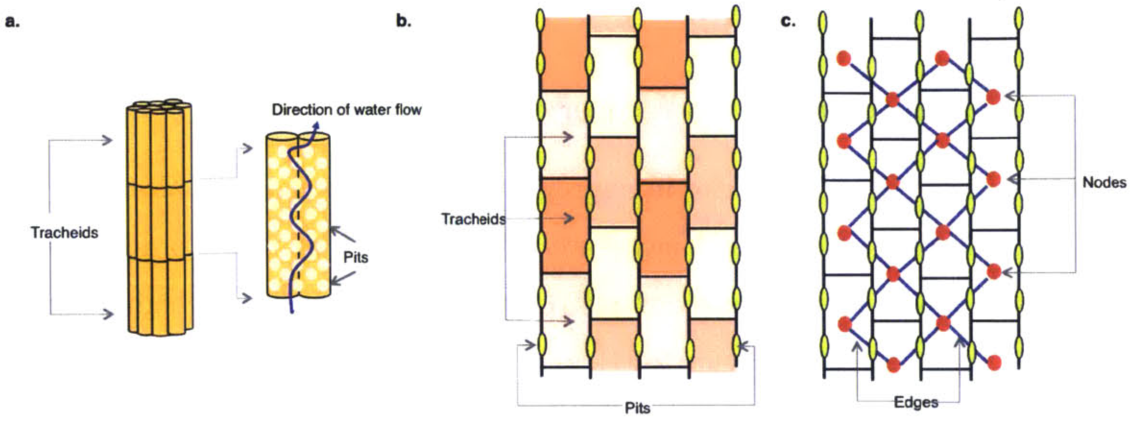


Figure 2-32: a) Interconnectivity between tracheids via pits b) 2D representation of interconnected tracheids c) Percolation model for pit-tracheid network

versus the length curve has a characteristic U-shape with the middle sections have lesser permeability than the ends (Figure 2-33).

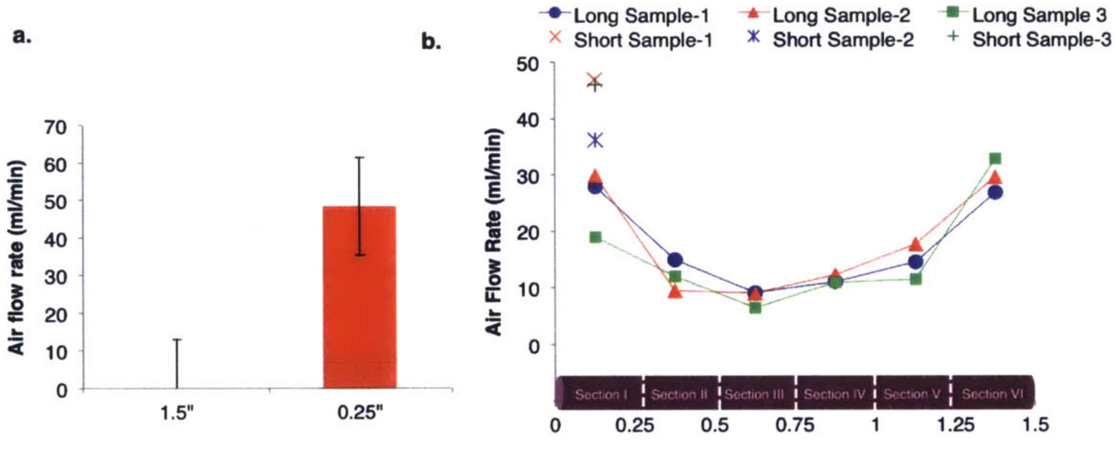


Figure 2-33: a) Comparison of air flow rates between 0.25 and 1.5 inch long samples b) Variation of air flow rates across 0.25 inch sections cut from samples that were 1.5 inch long.

Though percolation theory provides a basis for the strong dependence of permeability on sample length, it cannot be used to explain why the air flow through the middle sections is lower. This requires further investigation.

Experiments conducted did not show that evaporation rate, drying period and solute concentration affect recovery significantly. However, connectivity between pores wit increase in sample length did seem to play a critical role in determining permeability.

Effect of ethanol treatment on recovery of long samples

To check if the use of ethanol helps in improving recovery of longer samples, samples having a length of 1.0, 0.5 and 0.25 inches were flushed with 5 ml of 200 proof ethanol procured from VWR and completely dried. Water flow rate tests showed high recovery for ethanol-treated samples (Figure 2-34).

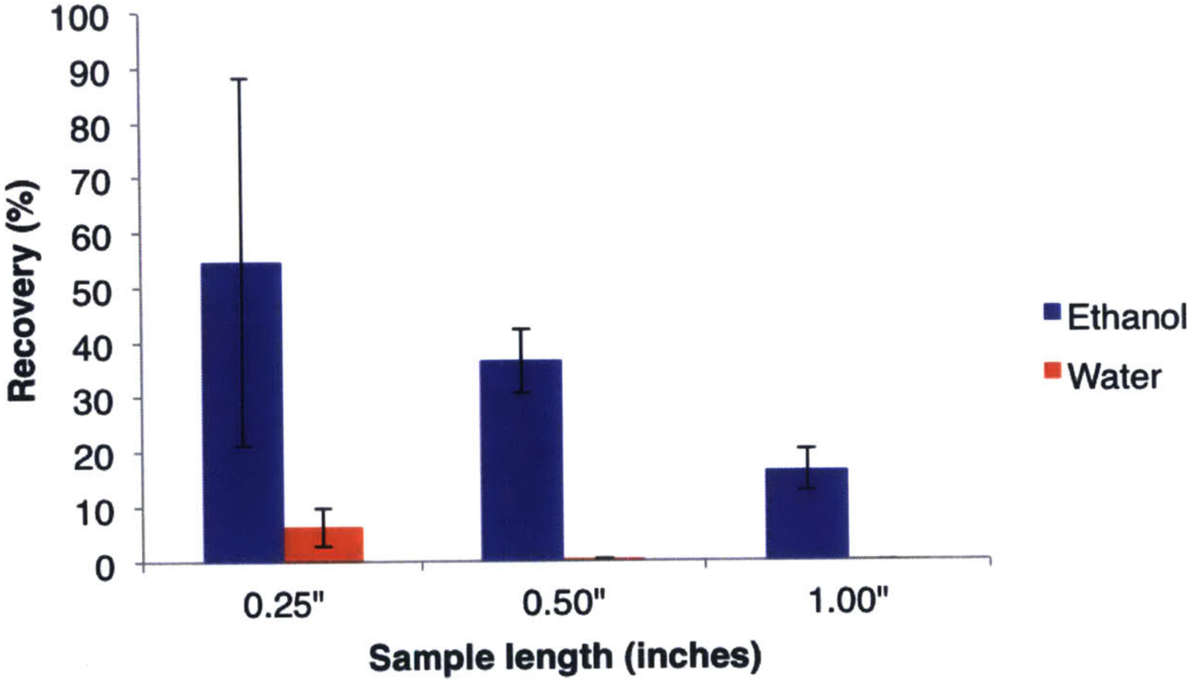


Figure 2-34: Effect of ethanol treatment on recovery for samples with different lengths

This observation can also be explained using percolation theory. Flushing samples with ethanol would increase the probability p of an edge existing between any two nodes resulting in a corresponding increase in the maximum cluster size S. If S happens to be greater than the sample lengths tested for, permeability will be observed. Further, experiments described in the previous section were repeated with 1.5 inch samples. Filters 1.5 inches long and 0.25 inches long were flushed with 200 proof ethanol (supplied by VWR) and completely dried at 45°C in the oven. This was followed by airflow rates measurements following which the 1.5 inch samples were cut into smaller sections of 0.25 inches. The airflow rate tests were repeated and the results were compared to control samples that were flushed with DI water and dried. Not only did the 1.5 inch samples flushed with ethanol show higher recovery, the 0.25 inch sections did not show any noticeable U-trend in permeability along the length as was observed in the previous case (Figure 2-35).

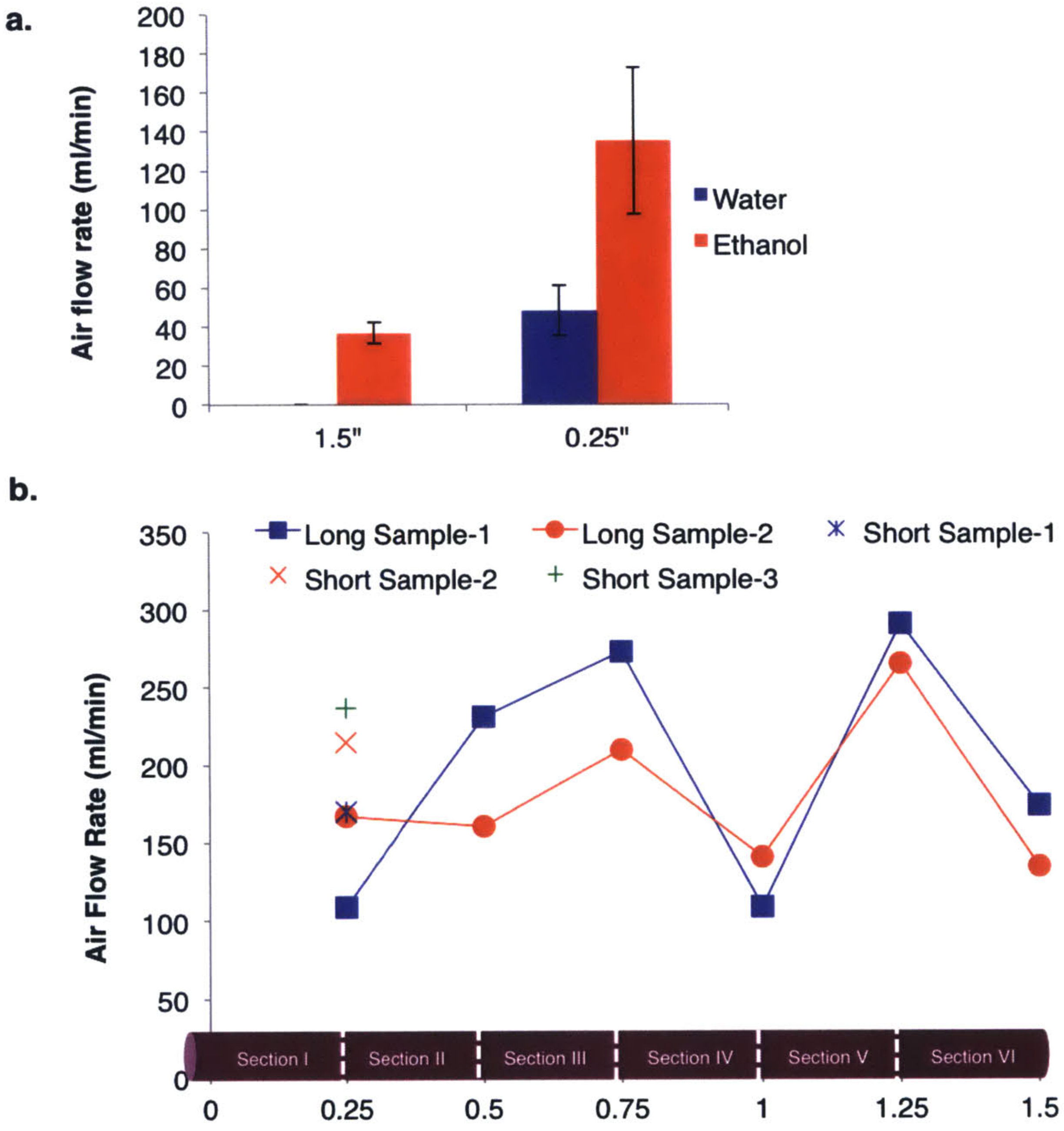


Figure 2-35: a) Comparison of air flow rates between ethanol-preserved and dried samples for different lengths b) Variation of air flow rates across 0.25 inch sections cut from ethanol-preserved samples that were 1.5 inch long.

Further, the conducting pathways in the 1.5 inch long samples were visualized with dye perfusion studies using Fluorescent Brightener 28 (FB28). The 1.5 inch samples were completely dried and flushed with the dye solution. Thin horizontal sections were cut along the length of the sample and visualized under a microscope. Figure 2-36 shows the cross sections of water-dried and ethanol-dried samples. There are hardly any pathways available for flow in the former case. The five sub images (Figure 2-36i-v) represent the sections extracted form the end through the dye was flown through.

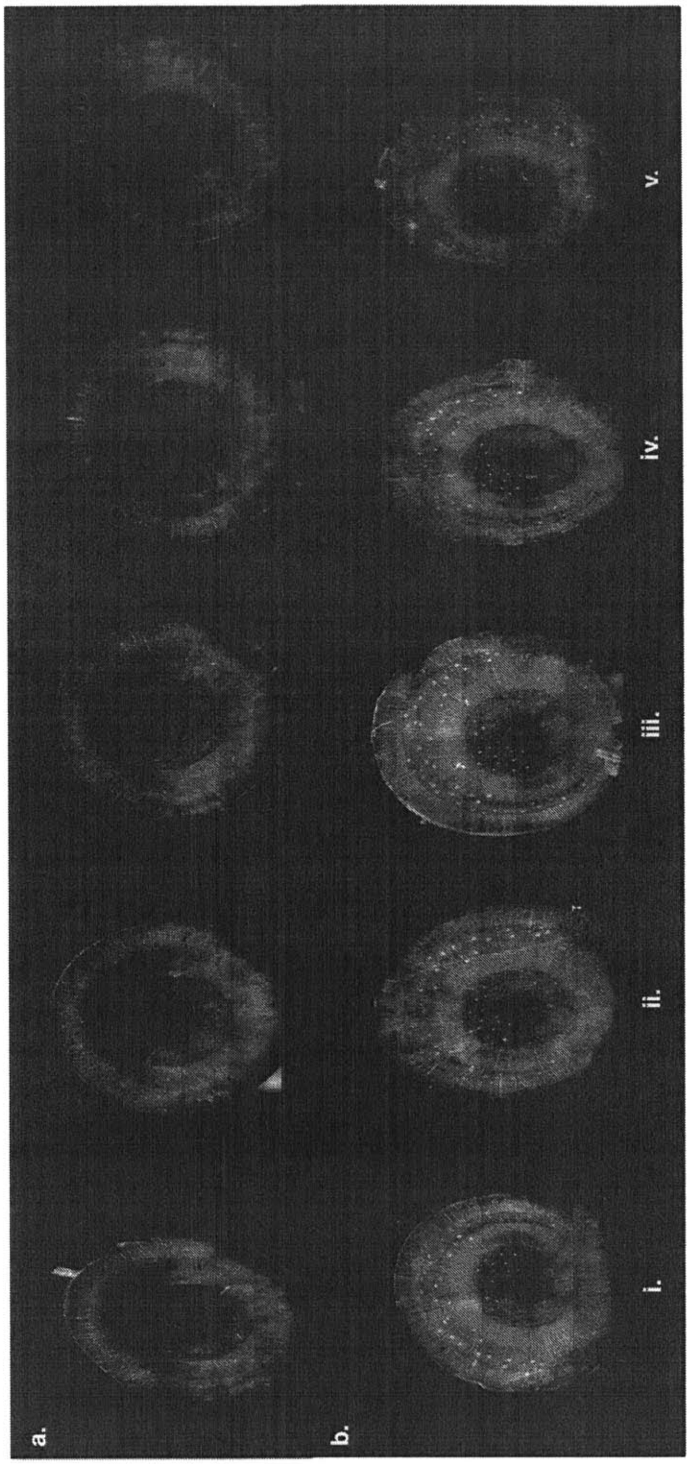


Figure 2-36: Visualization of conducting pathways using Fluorescent Brightener 28 (FB28) a) Cross sections of water-dried 1.5 inch long samples having a diameter of 0.5 inches b) Cross sections of ethanol-dried 1.5 long samples having a diameter of 0.5 inches. i-v represent sequential sections taken along the length starting from the end through which dye was flown through

Stacking filters to improve recovery and rejection

The longer samples have poor recovery in comparison to the shorter ones. However, reducing the length of the sample below that of the tracheids will result in poor rejection. Achieving a good filtration performance involves optimizing the length to balance these trade-offs. This can be done in two ways:

1. Use the tracheid length of the wood species (which can be found from literature or measured) as a benchmark and restrict the filter length to 2-3 times the tracheid length
2. Cut thin sections of the wood and stack them up while rotating them with respect to one another to make a xylem filter module. The thin sections would have high recovery and reorienting the filters will create a misalignment between the leakage pathways thereby preserving the rejection ability of the filter (Figure 2-37)

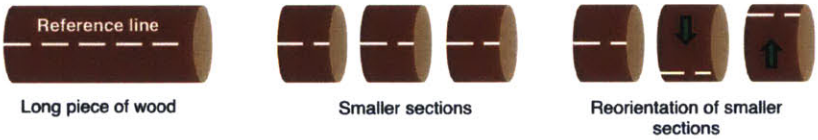


Figure 2-37: Schematic to illustrate stacking of shorter filters to get high recovery and rejection

The second method can serve as an alternative for ethanol treatment to improve permeability of xylem filters. Thin sections of the wood can be simply dried and stacked to achieve the desired rejection. Experiments were conducted to test the effectiveness of stacking on recovery and rejection with 0.25 inch and 0.5 inch samples in different configuration. The performance of the water-flushed and ethanol-flushed samples was compared against that of fresh samples. The results have been shown in Figure 2-38.

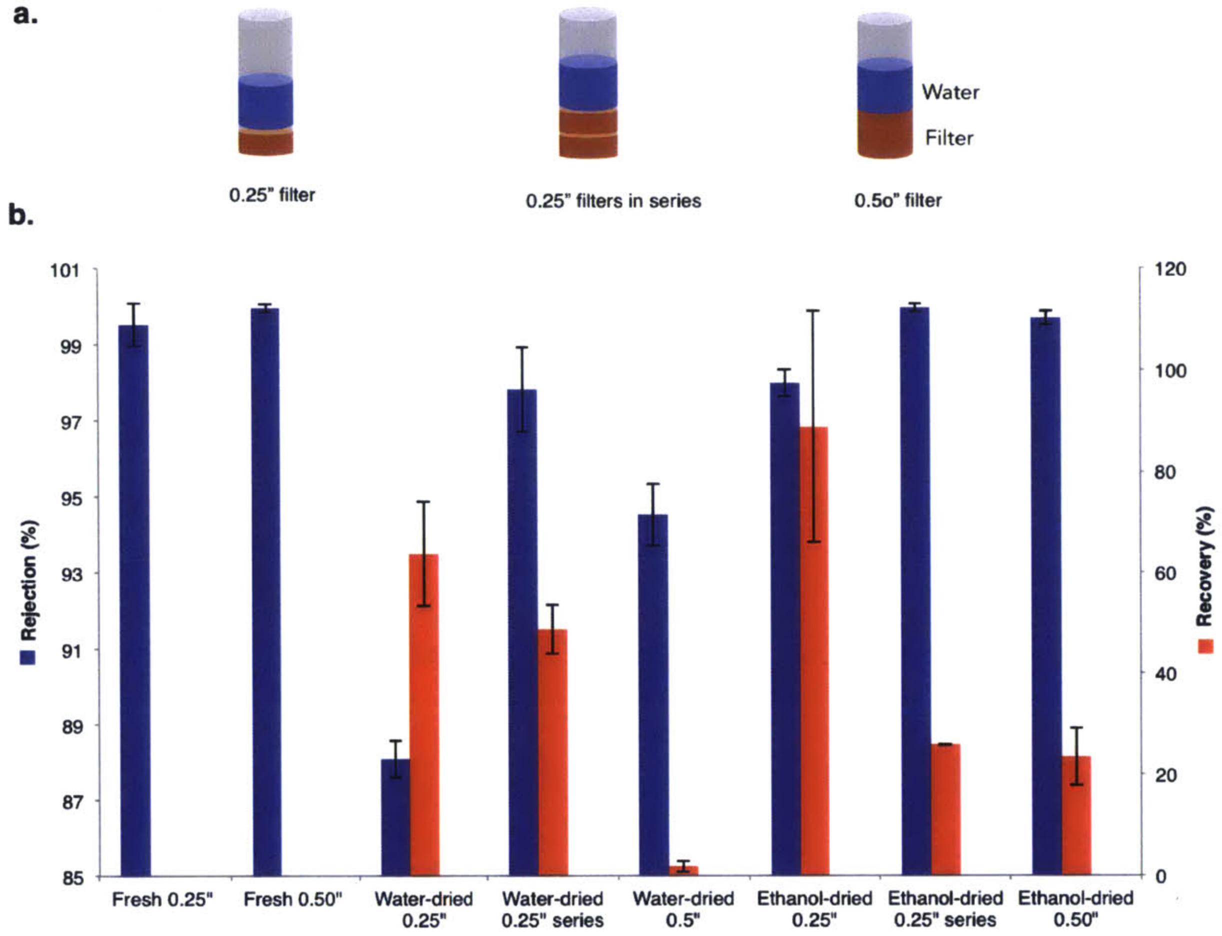


Figure 2-38: a) Schematic to illustrate different configurations tested b) Comparison of recovery and rejection for fresh, dried and ethanol-preserved samples in different configurations

The key takeaways from the results are:

1. Stacking two 0.25 inch samples in series improves the rejection considerably in comparison to a single 0.25 inch sample (especially in the case of water-dried samples)
2. Using two 0.25 inch filters in series is better than using a single 0.5 inch sample, both for recovery and rejection
3. The use of ethanol improves both, rejection and recovery, in all cases.

Conclusion

The filter permeability was found to have a strong dependence on length with an order of magnitude difference between the 0.5 inch and 0.25 inch long samples. In an attempt to explain this behavior, experiments were conducted to check for the role played by different physical parameters that would vary with length, such as evaporation rate, drying period and solute concentration. None of these was found to have a significant effect on permeability. It was hypothesized that the observed trend could be an outcome of a stochastic process where the manner in which the open pits align with one another plays a dominant role. Drawing references from percolation theory that is commonly used in the analysis of flow through porous media, the tracheids and pits in the xylem were modeled as nodes and edges respectively. Each edge was associated with a probability p of staying open during the drying process and the maximum number of interconnected nodes or the cluster size, S was a function of p. Percolation or flow through the sample would therefore be observed only when S was greater than sample length. It was proposed that the longer samples do not satisfy this criterion and hence, exhibit extremely low or no permeability. Experiments where smaller sections of impermeable long samples showed much higher flow rates lent further support to this hypothesis. However, the sections cut from the middle showed lesser permeability in comparison to the ends. Further investigation is required to explain this trend. As drying process proceeds from the end and adjacent tracheids affect one another, models that follow the drying and aspiration process are needed to provide insights into the mechanism. Treatment with ethanol helped achieve high flow recovery even for the longer samples. Choosing an appropriate length for the filter involves striking an optimal balance between rejection and recovery. Two approaches to achieve this objective were discussed, one where the filter length is restricted to two to three times that of the tracheid and the other where thin sections are reoriented and stacked one on top of the other in a series arrangement. A combination of ethanol treatment and filter stacking was found to have the best performance in terms of both rejection and performance.

2.4 Conclusion

It was found that the permeability and filtration characteristics of xylem filters can be preserved by treating them with ethanol before drying. It might also be possible to use other solvents like heptane, which have a similar surface tension like ethanol, to achieve high flow recovery.

Further, the recovery was found to have a strong dependence on filter length. The permeability after drying was found to drop abruptly to almost zero on increasing the filter length from 0.25 inches to 0.50 inches. While the exact reason underlying this observation is yet to be understood completely, there is evidence to indicate that this behavior can be partly explained using percolation theory where the nature of connectivity between the open pores is expected to govern permeability.

Nevertheless, this opens up the possibility to preserve the permeability xylem filters by cutting them into thin sections. To achieve improved rejection, these sections can be stacked on top of one another while rotating them relatively to create misalignment between leakage pathways.

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Chapter 3

Gravity-driven filtration through xylem

3.1 Introduction

One of the major costs associated with the commercially available filters in the market comes from manufacturing the membrane responsible for filtration. As the xylem filter relies on the use of a naturally available membrane, it is a promising candidate for the development of low cost filtration devices. However, to ensure that the operating costs are minimal, it is necessary to eliminate the use of utilities such as electricity or pumps to drive the flow through the filter. A gravity-based offline model is, therefore, the most suited design configuration for such filters. Experiments with xylem filters have been conducted at a laboratory scale using a pressure of 10 psi, which translates to a gravitational head of 7 meters. Unless there is a provision for installing overhead tanks on rooftops, realizing such high heads in practical applications is difficult. Reducing the head requirement to 1-3 meters (1.5-4.2 psi) can go a long way towards facilitating implementation of these filters in the real world.

However, if the filter dimensions and material properties are not varied, decreasing the pressure would lead to a reduction in flow rate by the same factor. Filters having a diameter of 1 cm and length of 0.25 inches have flow rates around 30 ml/min at 10 psi and can be expected to yield 9-12 ml/min with a head of 2-3 meters. This value is very low in comparison to other commercial filters that can filter water at 100-120 ml/min (based on inputs gathered during field surveys and discussions with companies involved in the water filtration segment).

One way to increase the flow rates is by increasing the area, or in other words, the diameter of the xylem filter. Attempts were made to study the interplay between filter diameter, pressure and flow rate to gain a good understanding of how these design parameters can be tailored to achieve the desired performance.

To do so, experiments were conducted to study the dependency of flow rate on gravity head and area. Further, as discussed in Chapter 2, it was found that the permeability and rejection ability of xylem filters with a diameter of 0.5 inches can be preserved by treating the filters with ethanol before drying. The efficacy of ethanol treatment for cases when the area is increased and the filter is operated under gravity was evaluated and a comparison was drawn between performance of filters that were operated under gas-pressure and gravity.

3.2 Materials and Methods

3.2.1 Sample Preparation

The method of sample preparation was similar to what has been described previously in 2.2.1.

Apart from branches with a diameter of 0.5 inches, xylem samples having a diameter of 2 inches were extracted from larger branches.

3.2.2 Testing procedure for flow rates using gas pressure

Gas pressure was used to conduct flow rate tests on samples having a diameter of 0.50 inches. The samples were mounted in a rubber tubing with an inner diameter of approximately half an inch. To maintain the natural direction of flow, the un-dotted end of the sample was loaded into the tube. The samples were secured in place using a hose clamp to prevent any leakage. The rubber tube was then filled with deionized

Figure 3-1: a) Corona pruner used for harvesting branches b) Steps involved in constructing a xylem filter [3]

water. Nitrogen gas was used to pressurize the water through the xylem sample at 10 psi (3-1). The time required for 5 ml of water to flow through the filter was recorded.

3.2.3 Testing procedure for flow rates using gravity

Gravity based flow rates were conducted for samples having diameters of 2 inches. The samples was mounted between two holders as depicted in Figure . The holders had slots to insert O-rings to prevent leakage (Figure 3-2). The entire arrangement was then connected to a carboy having a spigot placed on an elevated platform to achieve the desired head. The upper lid of the carboy was removed to operate the system at atmospheric pressure (Figure 3-3) .

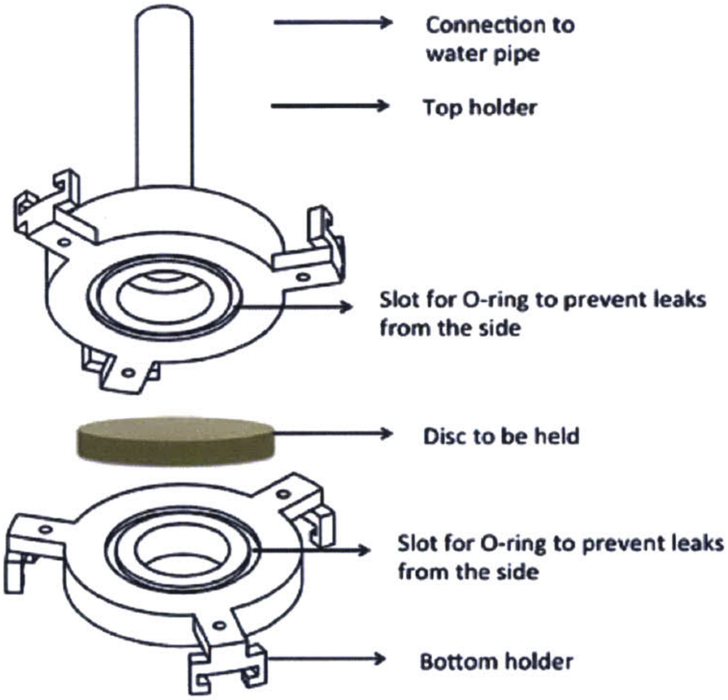


Figure 3-2: CAD model of holder used for gravity-driven flow rate tests

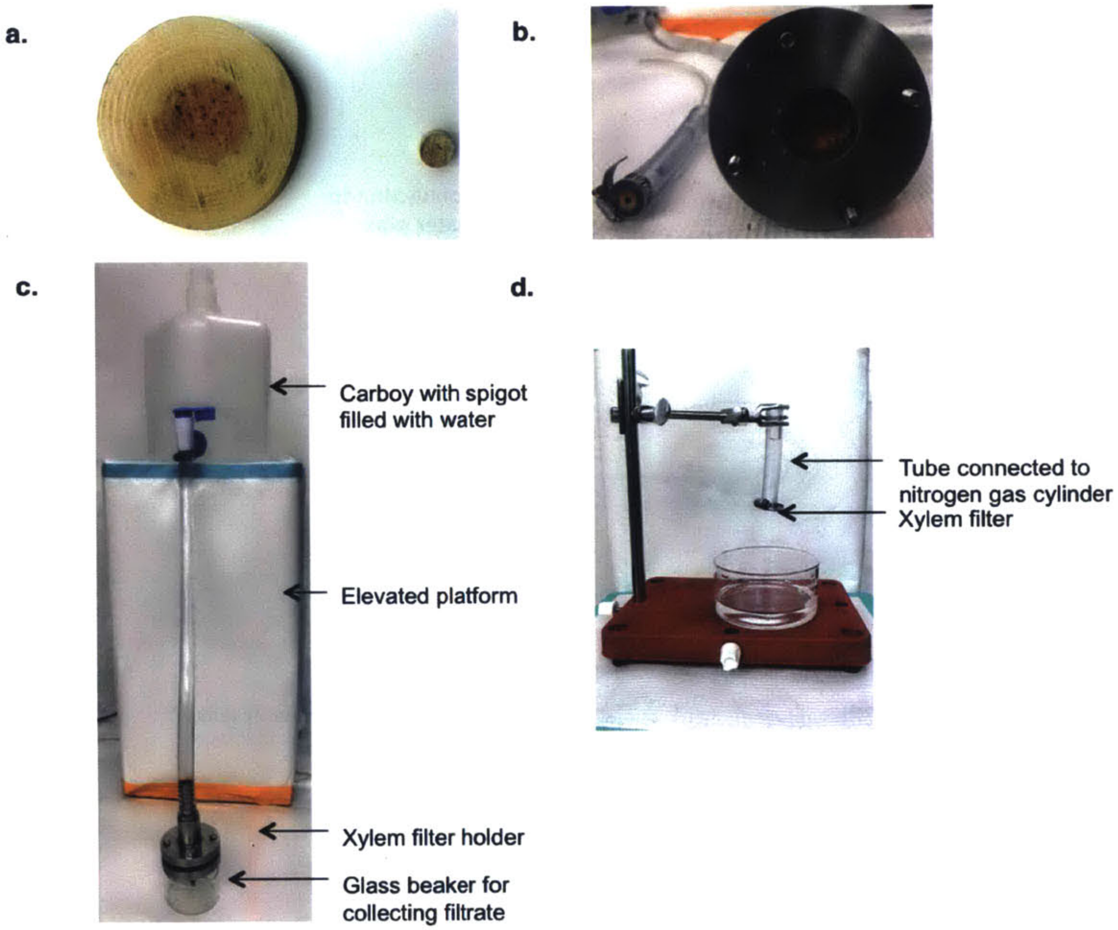


Figure 3-3: a)Xylem filters having diameter of 2 inches and 0.5 inches b) Sample holders c) Setup for testing gravity-driven flow through samples with 2 inch diameter d) Setup for testing gas-pressure driven flow through samples with 0.5 inch diameter

3.2.4 Treatment of xylem filters with ethanol

Xylem samples having a diameter of 0.5 inches were flushed with 5 ml of 200-proof ethanol procured from VWR at a pressure of 10 psi. Samples having a diameter of 2 inches were soaked in 200 proof ethanol for upto 2 weeks to allow enough time for diffusion. The ethanol was replaced every second day.

3.2.5 Drying of xylem samples

All samples were dried in a VWR vacuum oven at 45°C. The sample weights were continuously monitored with a Mettler Toldo AL104 scale until no further reduction in weight was observed.

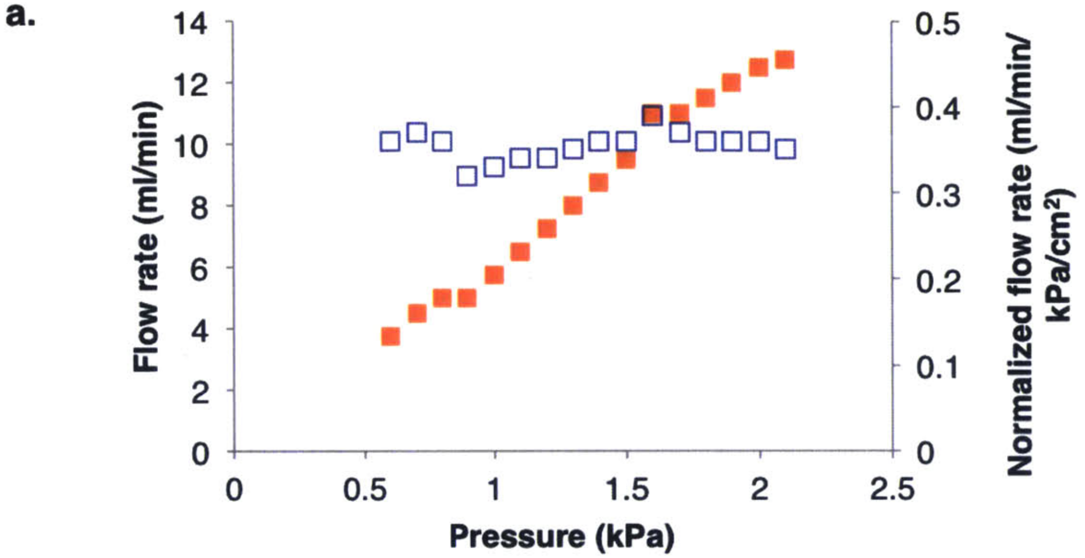
3.3 Results

3.3.1 Scaling of flow rate with pressure and area in gravity-driven flow

If the xylem filter is modeled as a porous medium, then according to Darcy's Law, the flow rate can be expected to scale linearly with area and pressure if the filter length, structure and material properties are not varied.

To verify this experimentally, flow rates through samples having a diameter of 2 inches were measured at different gravitational heads. The relationship between the two variables tested for was linear as anticipated. Figure 3-4b also shows that the normalized flow rate (which the flow rate per unit area per unit pressure applied) is more or less constant across all measurements.

Further, the normalized flow rates of samples having diameters 0.5 inches and 2 inches tested under gas-pressure and gravity respectively were also found to be in the same range (Figure 3-4b).



b.

	Small samples	Larger samples
Diameter (cm)	1.3	4.8
Area (cm^2)	1.3	17.8
Pressure (psi)	10	0.08-0.3
Flow rate (ml/min)	30-40	4-13
Flow rate/Area/Pressure (ml/min-cm^2-kPa)	0.35-0.45	0.32-0.39

Figure 3-4: a)Variation in flow rate with pressure head b) Comparison between different parameters used for gas-driven and gravity-driven flow

3.3.2 Preservation of filters using ethanol

Experiments were conducted to check if immersion in ethanol helps flow recovery by comparing flow rates of ethanol-preserved samples against ones that were fresh. The measurements were repeated at three different heights for filters with a diameter of 2 inches. The average flow recovery was found to be 67.68 ± 2.89 % (Figure 3-5a). Further, the flow rates through ethanol-preserved samples was measured at different pressure heads. As for the fresh samples, a linear dependence was found between the two parameters (Figure 3-5b).

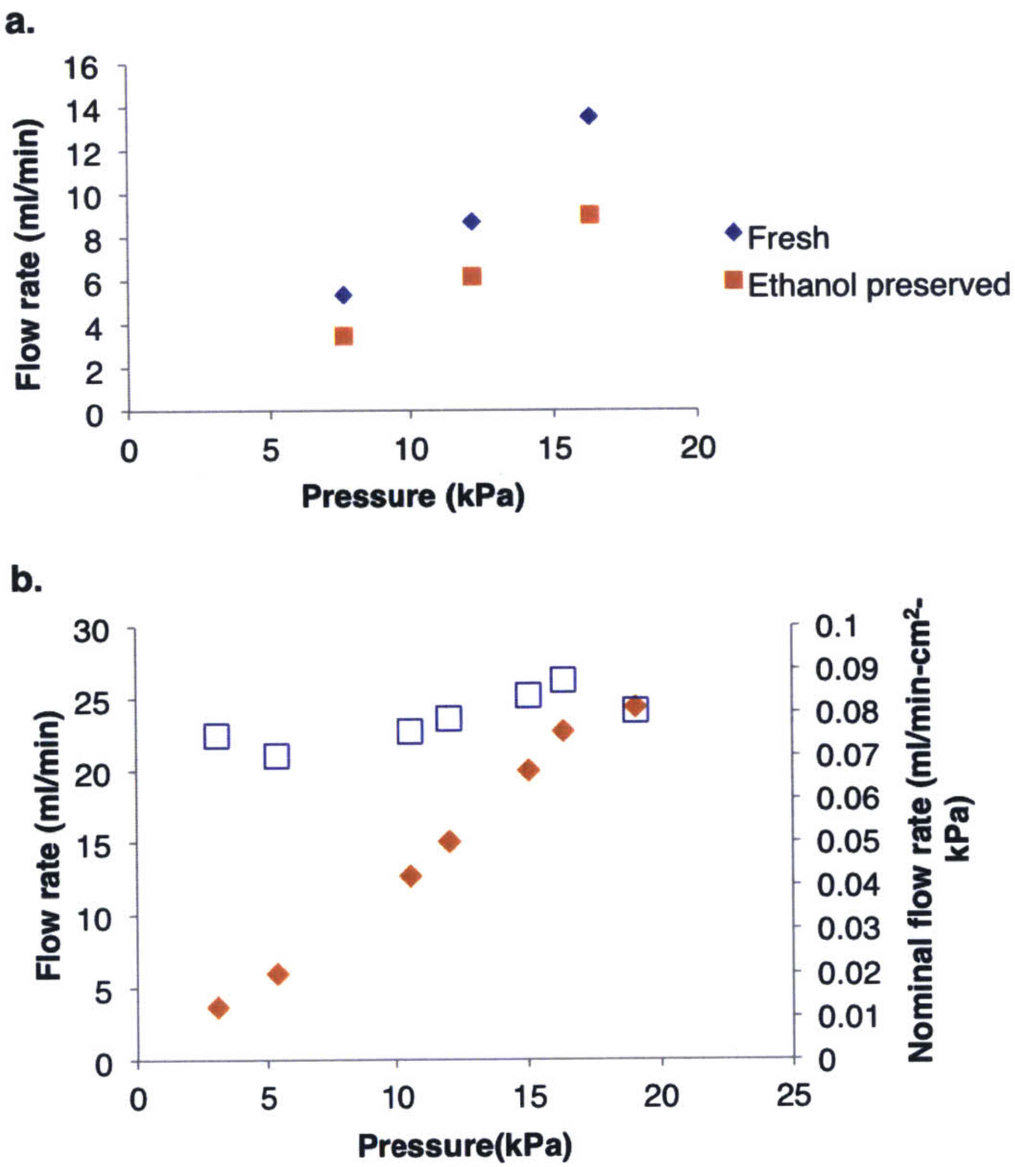


Figure 3-5: a) Comparison between flow rates of fresh samples and ethanol-preserved samples at different pressure heads b) Variation of flow rate through ethanol-preserved samples at different gravitational heads

3.4 Conclusion

Operation of xylem filters under gravity was achieved and a linear dependency between flow rate and pressure and flow rate and area was established. This opens up the possibility to explore different combinations of filter geometry and operation parameters to meet target flow rates that suit user expectations. Further, it was found that treatment with ethanol was as effective for xylem filters with a 2 inch diameter as it was for the ones with 0.5 inches. The predictable efficacy of ethanol treatment and the knowledge of interdependence between area, pressure and flow rate provide a simple way to control the design of xylem filters to achieve the desired performance.

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Chapter 4

Study of operation attributes of xylem filters

4.1 Introduction

The implementation of xylem filters on the field would be impossible without a thorough understanding of the operational attributes. It is imperative to develop a deep understanding of filter characteristics like the variation of flow rate with time or rate of fouling for different water qualities, expected filter lifetime and frequency of replacement. Further, as wood is biodegradable, there is a possibility that storing xylem filters in water might lead to deterioration in filtration ability due to degradation. As the use of xylem in the context of water filtration has not been explored before, attempts were made to study some of the aforementioned aspects and characterize the performance of xylem filters.

4.2 Materials and Methods

4.2.1 Sample Preparation

The method of sample preparation was similar to what has been described previously in Section 2.2.1

4.2.2 Testing procedure for flow rates in degradation studies

The samples were mounted in a rubber tubing with an inner diameter of approximately half an inch. To maintain the natural direction of flow, the un-dotted end of the sample was loaded into the tube. The samples were secured in place using a hose clamp to prevent any leakage. The rubber tube was then filled with DI water. Nitrogen gas was used to pressurize the water through the xylem sample at 10 psi. The time required for 5 ml of water to flow through the sample was recorded (Figure ??).

4.2.3 Preparation of test solutions

Test waters having different turbidities were prepared by mixing Arizona Test Dust in DI water in varying concentrations. The dust used was ISO-12103-1 Arizona A2 fine test dust and was procured from Powder Technology Inc. The test waters were prepared in batches of 6 liters. The turbidity of the final solution was measured. The quantity of test dust added to 6 liters of DI water and the resultant turbidity have been given in Table 4.1.

Table 4.1: Concentration and turbidity of test solutions

S. No.	Amount of dust added (in grams)	Turbidity (NTU)
1	5	540
2	2.5	250
3	1.25	44

4.2.4 Testing procedure for fouling studies

The fouling studies were conducted on samples having a diameter of 0.5 inches and length of 0.25 inches. Gravity was used to drive the flow through. The head used in all the experiments was 95 cm which corresponds to a pressure of 9.3 kPa or 1.35 psi. The test solution was stirred from time to time to prevent settling of dust particles.

4.2.5 Testing procedure for degradation studies

Degradation studies were also conducted on samples having a diameter and length of 0.5 inches and 0.25 inches. Samples were dipped in DI water and tap water in separate glass vials that were closed and stored in shade at room temperature. Flow rate and rejection tests were done after varying days of soaking.

4.2.6 Rejection tests using fluorescent microspheres

Rejection tests were conducted using 1 μm fluospheres. Fluorescently labeled yellow-green microspheres having a peak emission wavelength of 488 nm were purchased from LifeSciences. The fluosphere solution was sonicated for 1 minute using a VWR Ultrasonic Mixer to break down particle aggregates. The fluospheres solutions were prepared in batches of 50 ml. The required quantity of fluospheres was pipetted out and added to DI water to achieve concentrations of the order of 106 particles/ml. While the goal was to test for the maximum rejection capacity of the xylem, extremely high concentrations would have made counting difficult and time-consuming. Further, increased exposure time can cause bleaching of fluorescent particles leading to inaccuracy in counting. The mixture was then left on a VWR Ananlog Vortex Mixer for 2-3 minutes to ensure homogenous mixing. The input count was evaluated using a hemacytometer (a disposable Neubaeur-modified C-chip procured from InCyto). 10μL of the prepared solution was inserted into the C-chip which was mounted under a Nikon TE2000U inverted epifluorescence microscope and illuminated with FITC light. FITC has a wavelength in the range of 495-519 nm which is close to peak emission wavelength of the yellow green particles and would therefore cause these particles to fluoresce brightly facilitating detection. The particles were counted under a magnification of 30x. The number of particles in each of the four 1 mm^2 corner arrays of the hemacytometer were enumerated and the final concentration was obtained by averaging these numbers and multiplying by a factor of 10^4. The same protocol was followed to estimate the concentration of fluospheres in the filtrate as well.

4.3 Results

4.3.1 Wood degradation

Fresh samples were soaked in DI water and tap water and tested for flow rates and rejection of 1 μm fluospheres after different durations of soaking. All values have been averaged across three measurements. Both the flow rates and the rejection did not change significantly with time(Figure 4-1). Further, the solution in which the samples were stored did not show any change in color.

The same study was repeated for samples preserved using ethanol (Figure 4-2). Yet again, no significant variations were observed in flow rates and rejection.

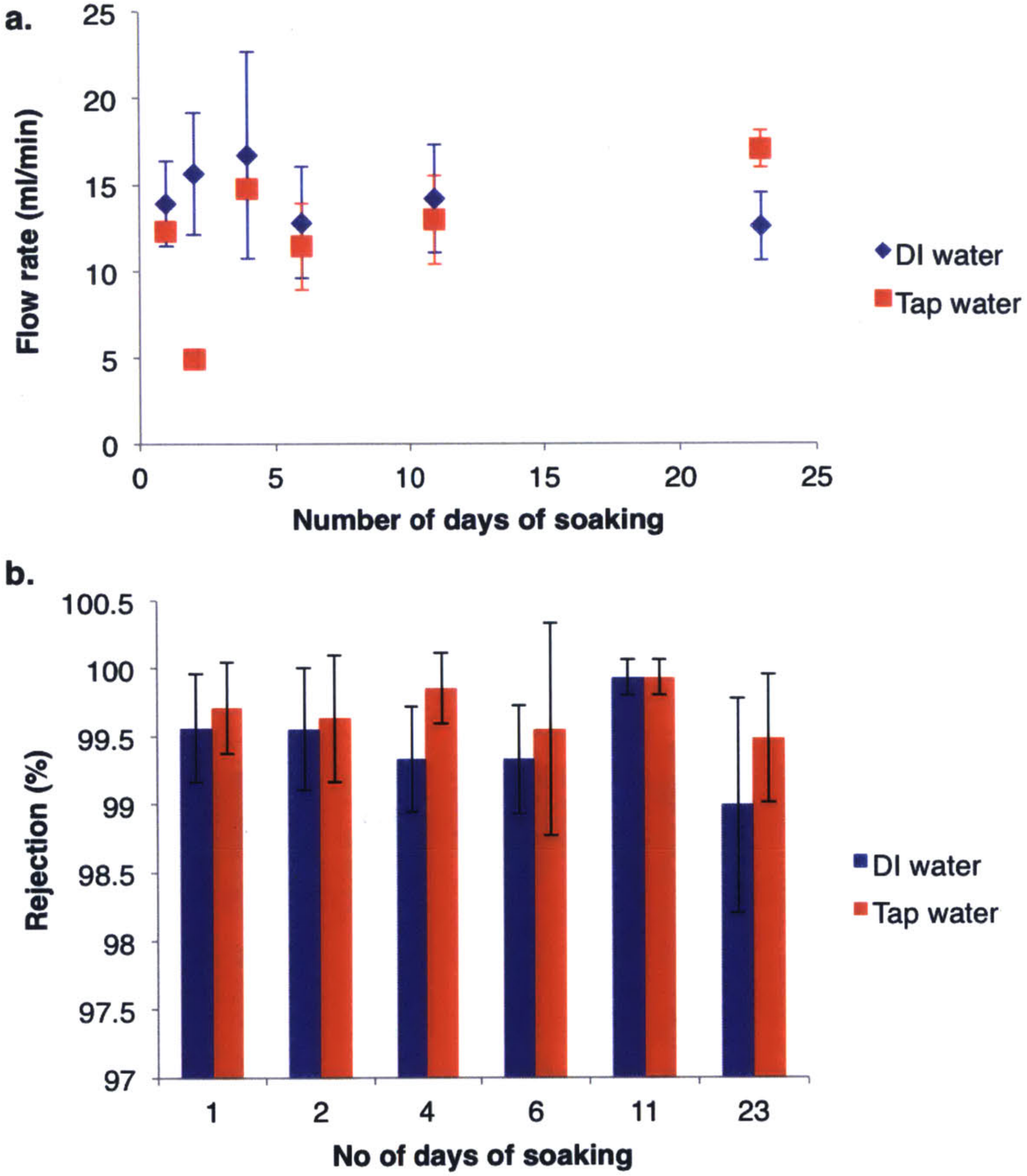

Figure 4-1: a) Variation of flow rate with number of days of soaking in DI water and tap water b) Variation of rejection of 1 μm fluospheres with number of days of soaking in DI water and tap water. Input concentration was 1.11 x 10^6 particles/ml

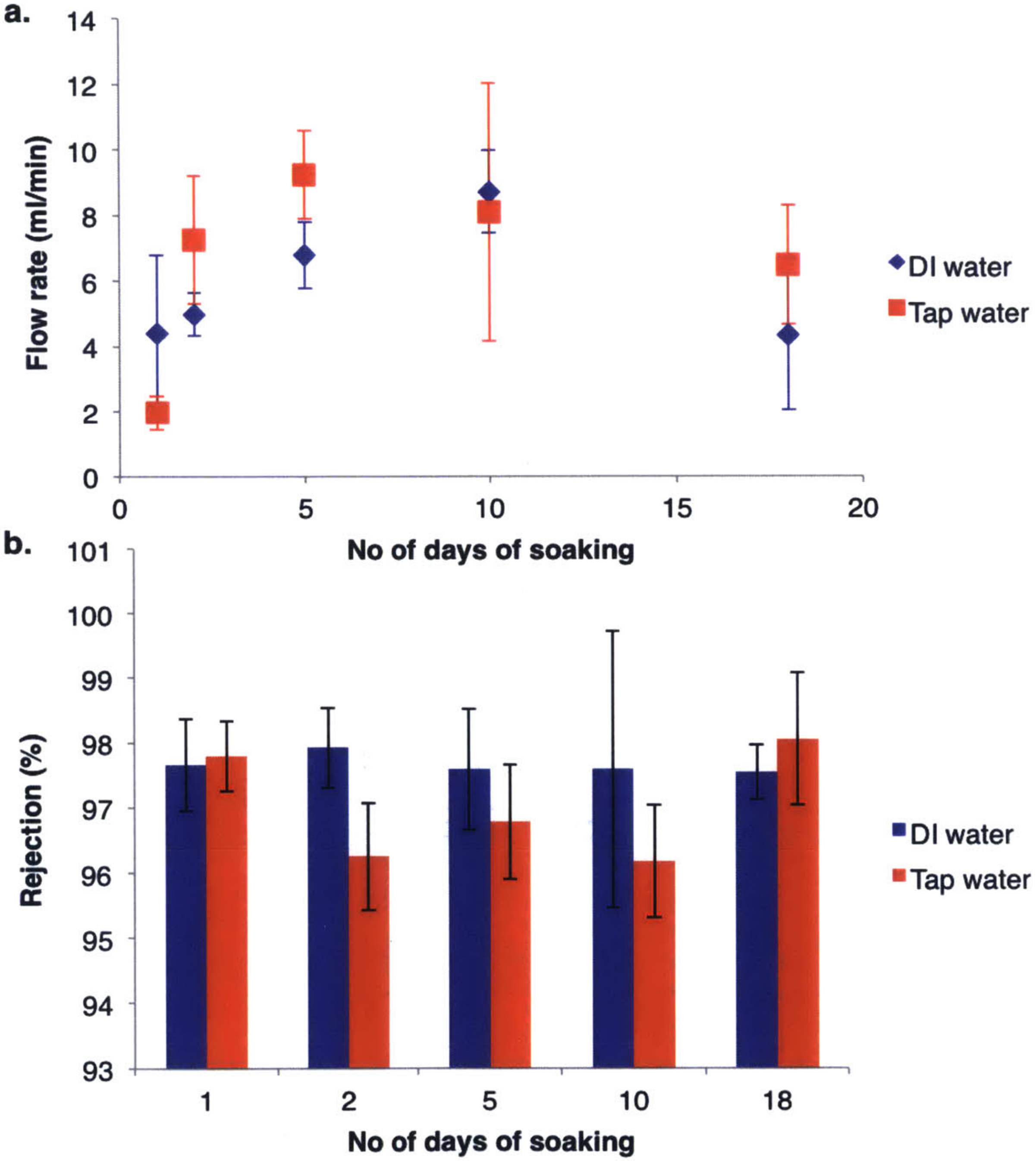

Figure 4-2: a) Variation of flow rate with number of days of soaking in DI water and tap water b) Variation of rejection of 1 μm fluospheres with number of days of soaking in DI water and tap water. Input concentration was 1.25 x 10^6 particles/ml

4.3.2 Fouling studies using fresh samples

Experiments were conducted with the following test solutions:

- Deionized water
- Charles river water
- Arizona A2 test dust solution with turbidity 540 NTU
- Arizona A2 test dust solution with turbidity 250 NTU
- Arizona A2 test dust solution with turbidity 44 NTU

Figure 4-3 shows the time taken for the flow rate to be reduced to half its initial value and the volume of filtrate collected until this time for each of the test solutions. While the former parameter did not show a strong variation with turbidity, the volume of filtrate collected significantly reduced with increasing turbidity.

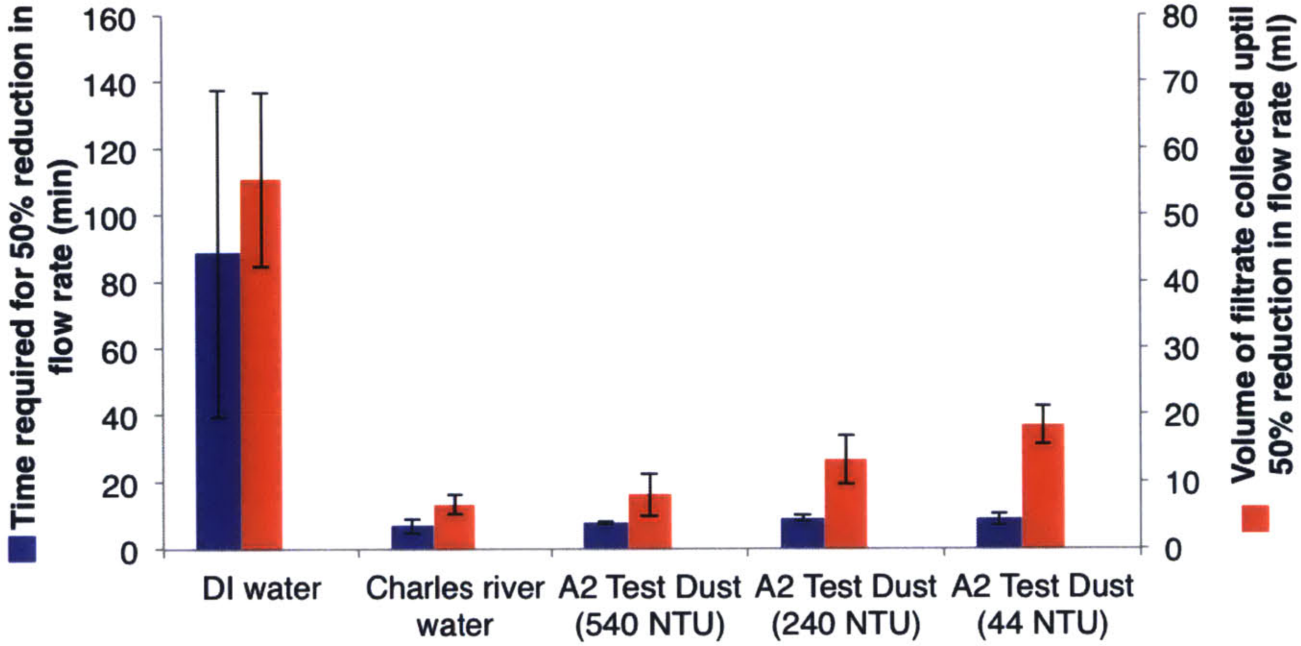

Figure 4-3: Variation of time required for 50% reduction until flow rate, t_50 and volume collected in time t_50 with different water qualities

Given the above results, the volume of water filtered per unit surface area in each of the above cases has been summarized in Table 4.2.

Table 4.2: Summary of fouling studies

Test Solution	Volume collected per unit area (ml/cm^2)	Normalized volume (ml/cm^2-kPa)
DI water	44.58	4.79
Charles river water	5.33	0.58
A2 test dust solution (540 NTU)	6.48	0.70
A2 test dust solution (250 NTU)	10.67	1.15
A2 test dust solution (44 NTU)	14.77	1.59

It was found the permeability drops even when DI water is flown through the samples. The underlying cause for this behavior is not understood and requires further research.

4.3.3 Fouling studies using ethanol-preserved samples

The experiments described in Section 4.3.2 were aimed at studying the variation in flow rate and volume of water processed with water quality and were not continued until the filter got completely blocked. As these experiments did not give a very good estimate of the total amount of water the xylem filters can process, filter lifetime studies were conducted where deionized water was flown through ethanol-preserved samples operated under gravity. The experiments were conducted at two different pressure heads of 1 meter and 0.20 meters. The variation of cumulative volume and flow rate with time has been shown in Figure 4-4. As can be seen, similar trends for flow rates were observed in both cases but the total volume that the filter could process was much greater at a higher head. The total capacity of the filter in terms of the volume of water processed for the two head has been summarized in Table 4.3.

Table 4.3: Summary of fouling studies for ethanol preserved samples

Head	Volume collected per unit area (ml/cm^2)	Normalized volume (ml/cm^2-kPa)
1.0 meter	2768	276.8
0.2 meters	389	194.5

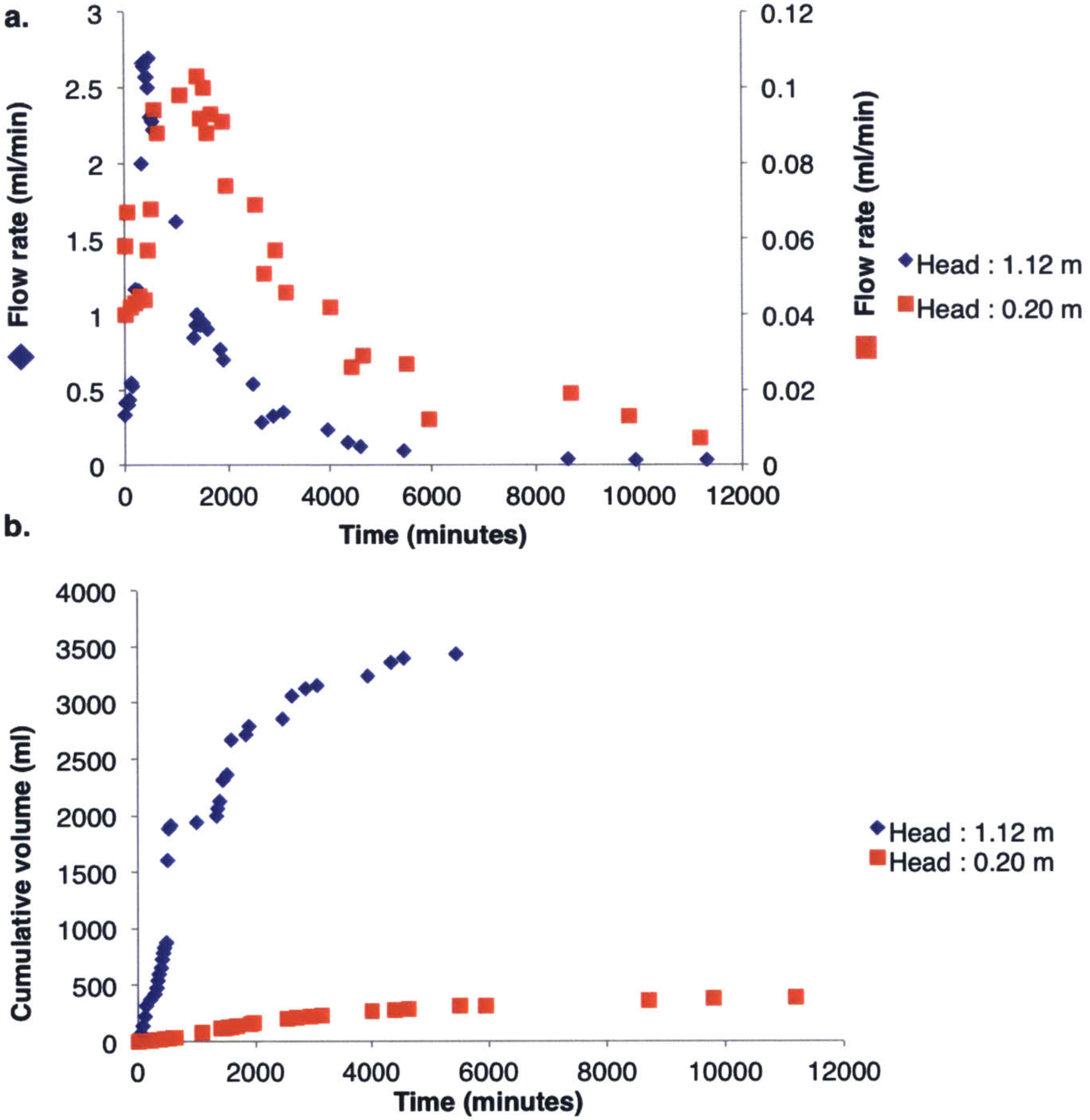

Figure 4-4: Fouling studies with ethanol-preserved samples a) Variation in flow rate with time. As flow rates at different heads varied considerably, they have been plotted on separate vertical axes b) Variation of cumulative volume passed through the filter with time

4.4 Conclusion

The preliminary fouling and degradation studies showed that storing xylem filters, both, fresh and ethanol-preserved, in DI water and tap water for up to 20 days does not affect their permeability and rejection ability. Filter lifetime studies show that increasing the turbidity and concentration of contaminants led to a sharp reduction in the filter time. Interestingly, it was found that the filters get clogged even when DI water is passed through them. Based on the results obtained, fouling seems to be a greater cause of concern over wood degradation and can be expected to play a major role in limiting filter lifetime. Further investigation into the mechanism of fouling and factors that lead to decline in permeability and methods to improve filter lifetime is required.

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Chapter 5

Improving the rejection ability of xylem filters

Apart from using the xylem as a stand-alone water filter, the use of xylem filters along with other technologies that have already been in use for water purification can allow for the filtration of wider variety of contaminants. Some ways in which the filter performance can be improved are listed below.

1. **Adsorption using sorbent materials:** There is a lot of literature available on the use of sorbents, especially iron oxide, for virus removal and inactivation [1, 2, 3], and for chemical removal. The negative charge on the virus allows it to bind to specific sites in the positively charged iron oxide. Iron oxide is commonly available in the form of rust on iron nails, iron wool or commercially, as a powder. Alternatively, attempts have been made to use iron oxide coated sand for water filtration also. The sorbent materials can be used with the xylem in the following ways:
   (a) As a separate module: The sorbent (e.g. iron oxide) particles can be compacted in the form of a porous filter capsule and added upstream of the xylem filter as a separate pre-filtration module. The water can be allowed to pass through this capsule first to remove viruses and then through the xylem. The size and the quantity of iron particles used would depend upon the efficacy of the treatment required. Porous pre-filters based on

activated carbon is another example.

   (b) Incorporating sorbent particles inside or on the xylem: Iron oxide powders are available in a wide variety of particle sizes. Depending upon the pore size of the xylem filter (which can be easily found using imaging techniques such as SEM), an iron oxide powder having particle sizes greater than that of the pore can be chosen. The powder can then be dissolved in a suitable solvent (such as water, ethanol) and then flushed through the xylem such that the iron oxide particles would be caught in the fibers of the pit membranes. As the solvent evaporates, the iron oxide would remain in the pores and serve to reduce the pore size thereby reducing the cut-off size of the filter and also act as a substrate for adsorption of viruses. Incorporating these particles in the membranes would also introduce increased resistance to the flow and reduce the flow rate. The size and the concentration of iron oxide particles in the xylem can be optimized to achieve a good rejection rate of viruses while ensuring that the flow rates are not very low. The sorbent particles may completely infiltrate into the xylem or form a layer on top of the xylem.

   (c) The xylem material itself may be functionalized with a sorbents through chemical modification of the xylem material with functional groups, polymers, or other materials that adsorb chemicals or viruses. Examples of these approaches are found in literature on modification of filter papers for adsorption of contaminants[4, 5]

   (d) Xylem filters may be inspected for defects (e.g. optical inspection for cracks), and the defects may be sealed using glue or other methods.

   (e) The xylem filter may be sterilized and packaged, or incorporated into a filtration device that may then be sterilized and packaged.

2. **Combination with coagulation or flocculation:** This method is widely used for wastewater and drinking water treatment. The process involves the addition of a coagulant to the water to be treated. The choice of the coagulant and dosage depends upon the nature of impurities, pH and desired water

quality. Electrostatic interactions cause the impurities to stick to the coagulant resulting in the formation of small suspended particles. The mixture is then stirred to allow these small suspensions to bond and form larger particles (referred to as flocculation). These particles then settle down at the bottom of the container due to their mass and the purified water on the top can be extracted for use [6, 7]. Some âĂÍof the common coagulants include natural clay such as bentonite and aluminum or iron based compounds [8, 9]. The use of clay for the removal of organic and inorganic compounds has been studied. Natural clay, such as bentonite, has been used to achieve 3-4 log virus reduction through flocculation [10]. Aluminum and iron salts have also been used to remove chemical compounds such as arsenic and fluoride and viruses based on this principle [6]. A combination of coagulation and flocculation with the xylem filter would therefore lead to significant improvement in the filtrate quality. Furthermore, coagulation by itself would be effective provided the coagulated particles are large enough to be filtered by the xylem filter.

3. **Use of metal ions with antimicrobial properties:** People have used metal ions like silver and copper for disinfection of water for centuries [11]. Numerous water purification technologies based on silver nanoparticles are currently available. Efforts to incorporate copper and silver nanoparticles into filter papers have also been made [4, 5]. While this technology is known to work very effectively against bacteria, a lot of studies have suggested that this can also be used fight off certain kinds of viruses [12]. Silver nanoparticles are commercially available in a wide range of sizes. These can be used in combination with the xylem filters in a manner similar to iron oxide, they can either be incorporated into the pit membranes themselves or can be added as a separate pre-filtration module. These materials may also be directly precipitated or coated on the xylem tracheids through varieties of surface coating methods well-established in the literature[4, 5].
4. **Use of zeolite:** Zeolite is very commonly used for the removal of metal ions and organic compounds. Some of the applications of zeolite include its usage in

swimming pools, wastewater treatment plants and flue gas cleaning. The pores in the xylem filter are not small enough to handle chemical contaminants but using them with zeolite might help them do so, where zeolites act as sorbents.

5. **Pre-loading of particles or polymers:** Xylem may be pre-loaded with particles with a distribution of sizes to control pore size (e.g. sand with a mixture of particle sizes from less than 100 nm to greater than 1 μm). For example, the particles can get caught in the xylem tissue due to the pit membranes and provide effective pore sizes that are smaller than the xylem filter size cutoff, improving rejection of smaller particles. Similar results may be obtained by use of long polymers (e.g. PVP) that get caught in the margo structures to decrease the effective size. These particles or polymers may also serve to control the resistance of the xylem filter to water flow, which may be useful for adsorption-based purification within the xylem, on the same particles, or in a pre- filter, or, for decreasing the propensity of the xylem to fouling.

Further, the xylem filter can also be combined with a pre-filtration module to improve the lifetime. Using a pre-filter such as muslin cloth, sieve or activated carbon black that can handle contaminants that are comparatively larger in size, would reduce the load handled by the xylem and allow it to last longer. Another method to increase the lifespan of the xylem filters is to use them with anti-microbial agents such as silver nanoparticles. This would prevent the degradation of wood due to growth of microbial colonies. Some forms in which the xylem filter can be made have been shown below in Figure 5-1

In Figure 5-1a, the device may be made from a flexible material such as thin polythene used for making bags. A hook or loop is provided to secure the device. A collection bag or cup may be provided, attached below the xylem Other filters may employ a separate vessel connected to the xylem filter by a tube that allows the xylem filter to be placed at a height lower than that of the water surface, which functions to increase the gravitational head to thereby increase the flow rate of water. In Figure 5-1b, the entire filter module consists of two components, a pre-filter that could be made of activated carbon black, sorbent materials, coagulants, metal ions, zeolite or

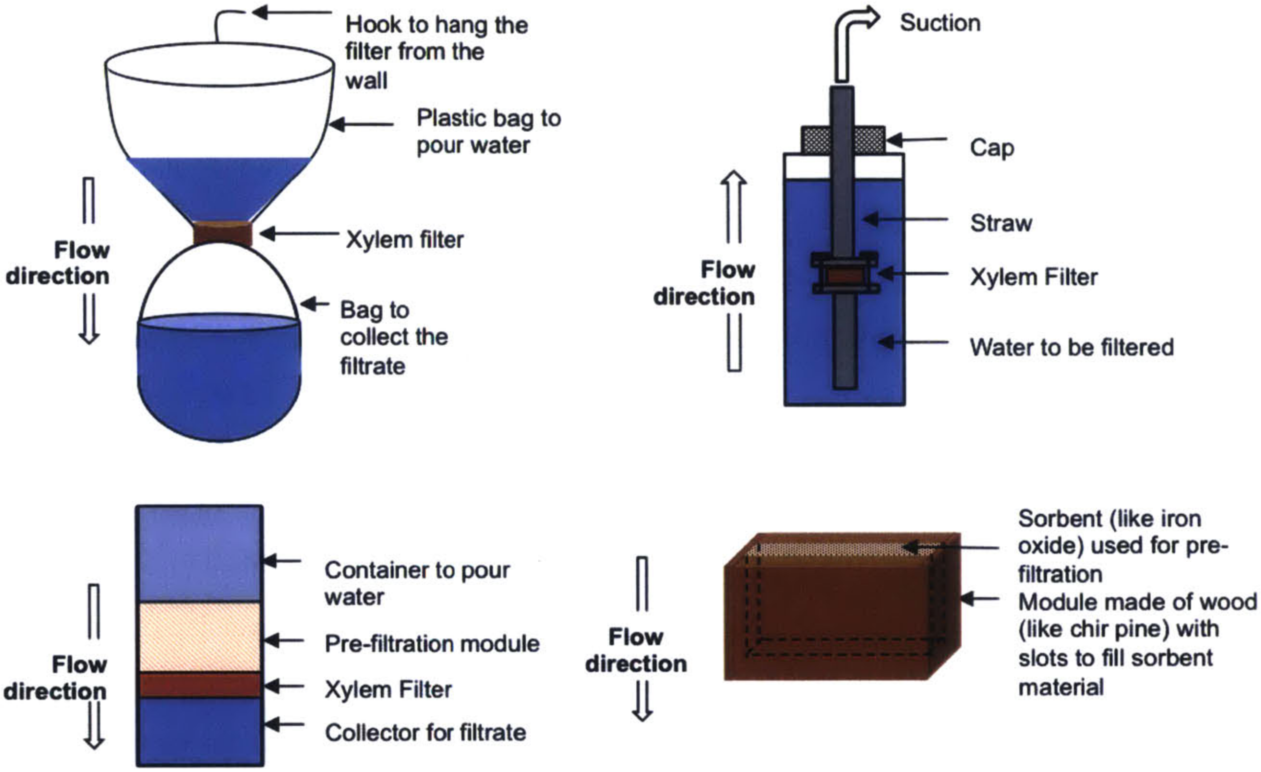

Figure 5-1: Some of the possible design configurations for the xylem filter a) Xylem filter being used as a standalone unit. The filter has a plastic bag on the top into which water can be poured. The filtrate can be collected either in a separate container placed below the xylem filter b) The xylem filter in combination with a pre-filtration unit. This unit can be a block of activated carbon black, a capsule containing silver nanoparticles, iron oxide powder, coagulants or zeolite, or could be a simple muslin cloth or sieve. c) The xylem filter can also be integrated with a straw and incorporated inside a bottle for drinking water. d) The filter could also be made as block of pine wood with slots cut into it for filling in sorbent material

could be as simple as muslin cloth or sieve and the xylem filter. The water can be poured upstream of the pre-filter and the filtrate can be collected in a compartment below the xylem. Figure 5-1c depicts the use of xylem filter in a bottle where the straw could be designed as a holder for the xylem filter. The suction pressure provided by the user may be used to draw the fluid up the straw. In Figure 5-1d, the filter has the form of a block made of pine wood. The block could have slots cut into it for filling in sorbent material like iron oxide. In the above designs, the xylem filter may be replaced with stacked xylem filters, or any combination of xylem filters and pre-filtration capsules or xylem filters impregnated with sorbents or modified as described above. The filters may employ 'U' tubes with xylem located near the bottom of the 'U', which functions to retain a certain amount of water to maintain the xylem filter wet and immersed in water. The filters may use xylem filters with diameters ranging from less than 1 cm to greater than 2 feet, which is limited by the diameter of the branch or trunk from which the xylem is obtained. Xylem filters may also be made by joining together several pieces of xylem.

Further, the mechanism underlying filtration in xylem is size-based exclusion. The filtration performance is therefore dependent on the structure of the pit membrane, which varies from one tree to the other. Though Eastern White Pine (*Pinus Strobus*) has been used for all the experiments described in previous sections, other species such as Eastern White Cedar (*Thuja occidentalis*) and Maidenhair Tree (*Gingko biloba*) have a tighter membrane (suggested by James K. Wheeler, University of California, Santa Cruz)and can reject even smaller particles. Figure shows a comparison between the pit structure and the rejection performance of these species. Choosing the appropriate tree can therefore go a long way towards achieving better rejection performance. (Figure 5-2)

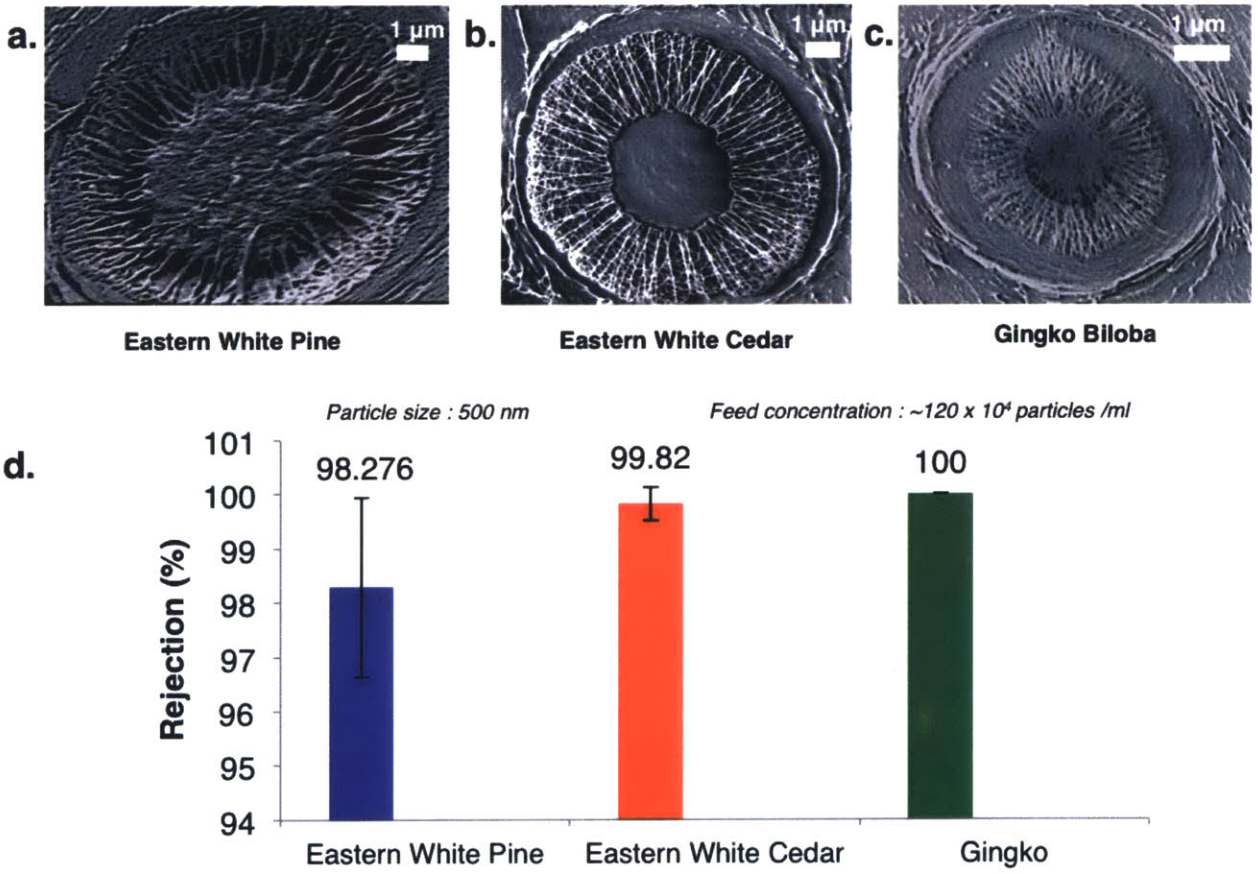

Figure 5-2: a) Apart from Eastern White Pine, other commonly available species like Eastern White Cedar and Gingko have a tighter margo, as can be seen from the SEM images of the pit membranes . b)The graph shows that Gingko and Eastern White Cedar can achieve better rejection in comparison to White Pine

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Chapter 6

Implementation of xylem filters in India

6.1 Introduction

Apart from focusing on the technological aspect of development of xylem filters, efforts are underway to implement this technology on field. Being funded the Tata Center of Technology and Design at MIT, the project has a special focus on India where the lack of clean drinking water continues to be a major problem. Although, the 2001 census report released by the Indian government states that 94% of the rural population and 91% of the people living in urban areas have access to safe drinking water, a report published by Nandi, a Public Charitable Trust, estimates that 37.7 million Indians are affected by water borne diseases annually and 1.5 million children die because of diarrhea alone [1]. Despite the presence of a wide array of commercial products and the prevalence of traditional and household methods to purify water, access to safe drinking water continues to be a major issue. Surveys across the consumer market indicate that while awareness is one of the key concerns, affordability is a primary barrier that deters people from purchasing products available in the market. The insights gained from the exploration of xylem as a filter material and knowledge gathered from field trips and interviews with stakeholders in India strongly indicate that xylem filters have real potential for large-scale impact in water filtration. To aid the process of product design and development, a thorough review of water quality issues, commercial products available in the market and case studies of products that have met with success and failure was done. Further, field trips to India were made during summer and IAP to find commercial partners that would help launch these filters on field. The key findings have been summarized in the sections below.

6.2 Literature/Prior Art Review

6.2.1 Water Resources

In order to design an effective water filter, it is essential to understand the source of drinking water, its availability and the nature of contaminants. A few pointers regarding the water sources and their utility in India are provided below:

- 85% of the population depends on groundwater (92% agricultural use, 5% industrial use and 3% domestic use) [2]
- Utilization of surface water also follows a similar trend with 89% consumed by the agricultural sector, and industrial and domestic sector consuming substantially less (2% and 9% respectively) [2]
- India has just 4% of the world‘s fresh water resources but 16% of the global population [3]
- Half of India‘s water supply in rural areas, where 70% of the country‘s population lives is routinely contaminated with toxic bacteria [3]
- Results consolidated by the Central Pollution Control Board from 1995-2009 indicate that organic and bacterial contamination of water is critical in the water bodies [4]. Figure 6-1 depicts the results.

As the common practice in a lot of villages is to fetch water from ponds, rivers and other local water bodies, there is no wonder that a huge percentage of the rural Indian population is affected by the biochemical quality of water. These trends demonstrate a necessity to tackle the issue of providing safe drinking water in rural areas, be it in the form of implementing existing solutions well (if taking the solutions to the field is

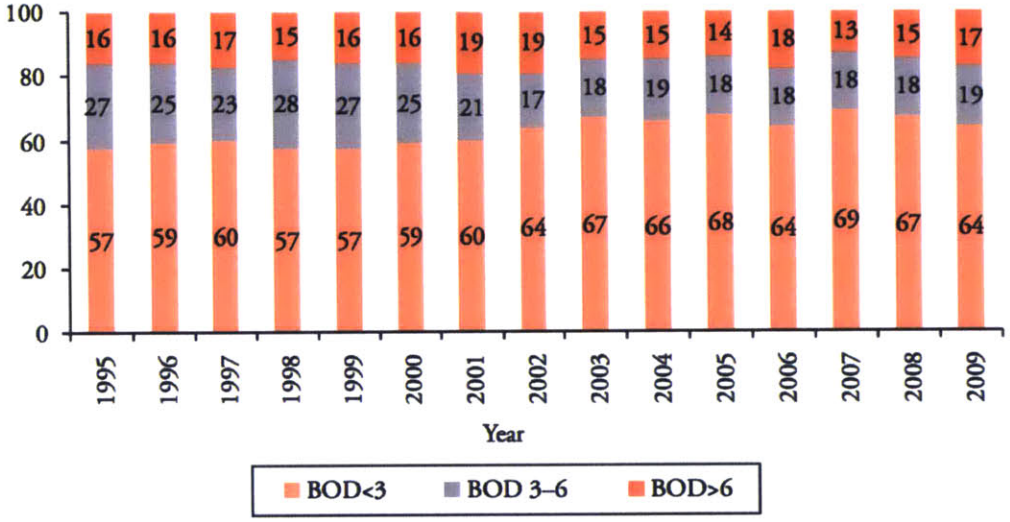


Figure 6-1: Trend of Biochemical Oxygen Demand, 1995-2009 [4]

an issue) or coming up with new solutions (if there is a lack of suitable alternatives).

6.2.2 Water Treatment Methods

Given that the need for water purification/filtration is too glaring to ignore, one would expect people to resort to some form of water treatment in an attempt to make the water potable. Some of the common low-cost household methods used for water purification are listed below [2, 1]:

- Boiling of water before consumption is very common. This helps kill bacteria and protozoa
- Kataka seeds (*Strychancs potatroum*) are used for removing turbidity from water
- Plant ashes, earth from termite hills, rice husks or the seed coats of cardamom are commonly used to improve water clarity
- Tulsi (*Osimum sanctum*) leaves are added to water because of their antibacterial properties
- Drumsticks are used to as a coagulant and to inhibit bacterial and fungal growth
- Water is often stored in earthen (copper or brass) to prevent bacterial growth
- Cloth or jail filters are some of the commonly available kinds of filters
- Water is also stored in clay pots

Apart from such household treatment methods, there are also a wide variety of commercial filters that are available in the market. The Comprehensive Initiative for Technology Evaluation (CITE) at MIT evaluated the household water filters in the market in Ahmedabad, India in 2014 and classified the water filters available in the market into three groups (Figure 6-2)[5]:

1. Reverse Osmosis filters ($98-233)
2. Gravity non-electric filters ($17-50)
3. Conventional particle filters like cloth or strainers ($0.5-1)

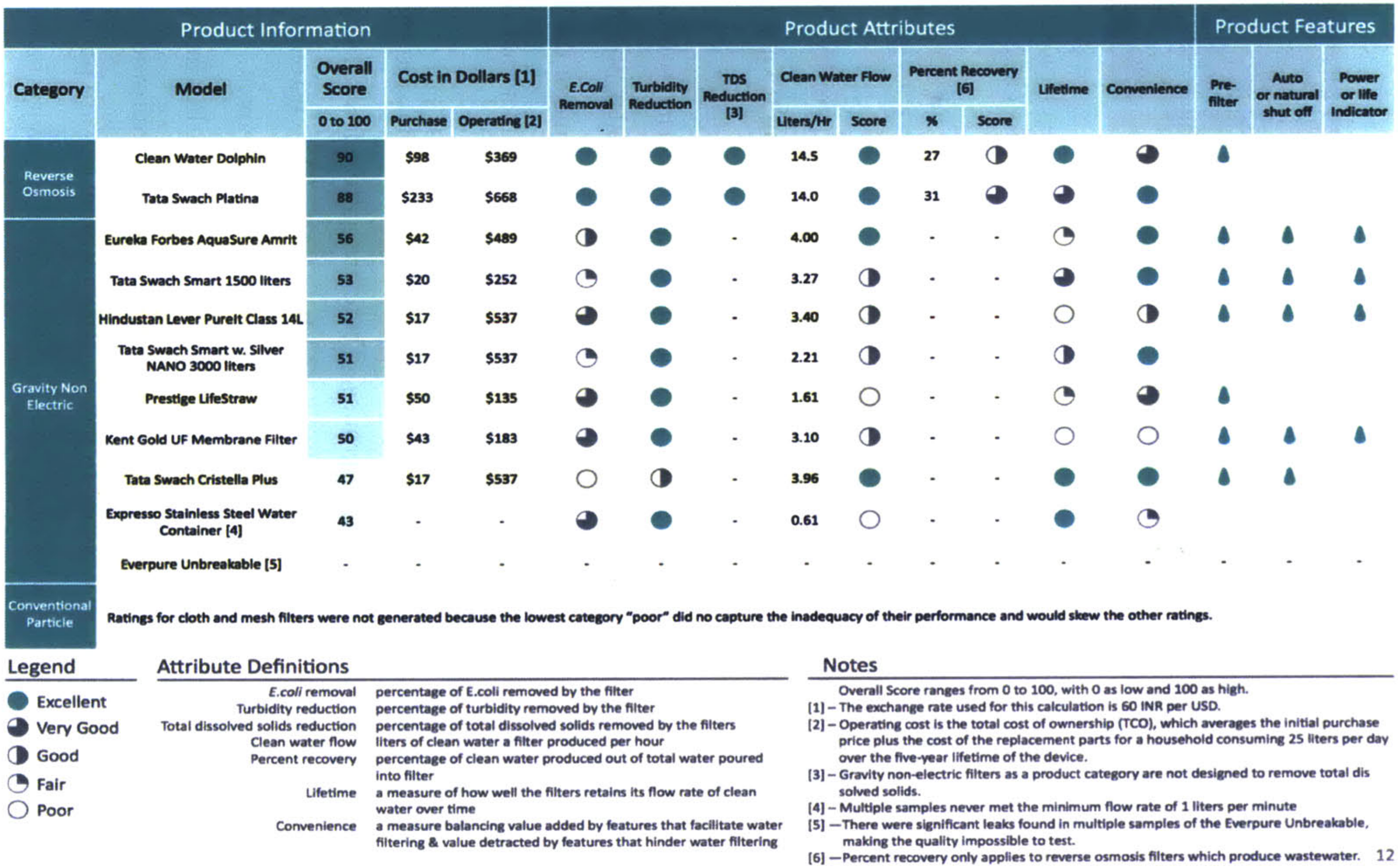
A comparison between the filters that are commonly used has been presented in Figure 6-3 [5]

Despite the availability of numerous alternatives to deal with the issue of drinking water quality, India is still trying hard to battle out water-borne diseases. A few reasons that are commonly cited as an explanation to this observation are:

Figure 6-2: Classification of water filters in Indian market a) Reverse Osmosis Filters, b) Gravity non-electric filters, c) Conventional particle filters such as cloth and *jali*

- Awareness about the importance of water quality continues to be a major impediment in the eradication of water-borne diseases.
- The efficacy of the household treatment methods is hard to quantify. Varying dosages are required depending upon the water quality making it very difficult to have standards on chlorination
- Numerous low cost alternatives such as chlorine tablets/chlorine solutions are also available in the market to make the water free from bacterial contamination. The use of these products is however not very widespread. Some of the reasons for the lack of popularity of these solutions has to do with the fact that chlorine often causes the water to taste distinctly different.
- As per the findings of the CITE survey, the RO filters fall outside the range of affordability for the low-income groups. There are quite a few gravity-based filters that fall within the affordability range but require the customer to pay the entire amount upfront and need regular cartridge replacement. Access to these filters could be greatly improved if schemes to pay monthly installments are put in place. Access to these filters could be greatly improved if better financing options such as schemes to pay monthly installments are put in place. A comparison of the conventional particle filters and the gravity based filters has been shown in Figure 6-4.

Given the above findings, an ideal product in this scenario would be a filter that is as cheap as the conventional particle filters but has a better performance.



Product Information					Product Attributes									Product Features		
Category	Model	Overall Score	Cost in Dollars [1]		*E.Coli* Removal	Turbidity Reduction	TDS Reduction [3]	Clean Water Flow		Percent Recovery [6]		Lifetime	Convenience	Pre-filter	Auto or natural shut off	Power or life Indicator
		0 to 100	Purchase	Operating [2]				Liters/Hr	Score	%	Score					
Reverse Osmosis	Clean Water Dolphin	90	$98	$369	●	●	●	14.5	●	27	◑	●	◕	💧		
	Tata Swach Platina	88	$233	$668	●	●	●	14.0	●	31	◕	◕	●			
Gravity Non Electric	Eureka Forbes AquaSure Amrit	56	$42	$489	◑	●	-	4.00	●	-	-	◔	●	💧	💧	💧
	Tata Swach Smart 1500 liters	53	$20	$252	◔	●	-	3.27	◑	-	-	◕	●	💧	💧	💧
	Hindustan Lever Pureit Class 14L	52	$17	$537	◕	●	-	3.40	◑	-	-	○	◑	💧	💧	💧
	Tata Swach Smart w. Silver NANO 3000 liters	51	$17	$537	◔	●	-	2.21	◑	-	-	◑	●			
	Prestige LifeStraw	51	$50	$135	◕	●	-	1.61	○	-	-	◔	◕	💧		
	Kent Gold UF Membrane Filter	50	$43	$183	◕	●	-	3.10	◑	-	-	○	○	💧	💧	💧
	Tata Swach Cristella Plus	47	$17	$537	○	◑	-	3.96	●	-	-	●	●	💧	💧	
	Expresso Stainless Steel Water Container [4]	43	-	-	◕	●	-	0.61	○	-	-	●	◔			
	Everpure Unbreakable [5]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Conventional Particle	Ratings for cloth and mesh filters were not generated because the lowest category "poor" did no capture the inadequacy of their performance and would skew the other ratings.															

Legend

- ● Excellent
- ◕ Very Good
- ◑ Good
- ◔ Fair
- ○ Poor

Attribute Definitions

E.coli removal	percentage of E.coli removed by the filter
Turbidity reduction	percentage of turbidity removed by the filter
Total dissolved solids reduction	percentage of total dissolved solids removed by the filters
Clean water flow	liters of clean water a filter produced per hour
Percent recovery	percentage of clean water produced out of total water poured into filter
Lifetime	a measure of how well the filters retains its flow rate of clean water over time
Convenience	a measure balancing value added by features that facilitate water filtering & value detracted by features that hinder water filtering

Notes

Overall Score ranges from 0 to 100, with 0 as low and 100 as high.

[1] – The exchange rate used for this calculation is 60 INR per USD.

[2] – Operating cost is the total cost of ownership (TCO), which averages the initial purchase price plus the cost of the replacement parts for a household consuming 25 liters per day over the five-year lifetime of the device.

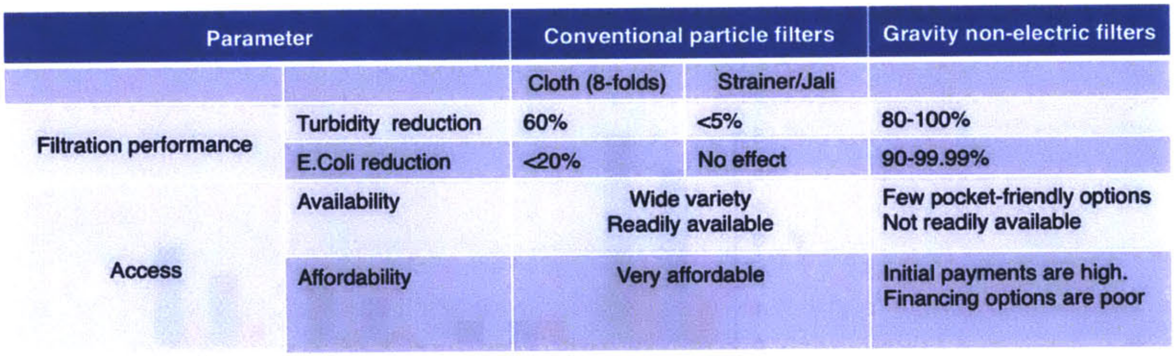
[3] – Gravity non-electric filters as a product category are not designed to remove total dis solved solids.

[4] – Multiple samples never met the minimum flow rate of 1 liters per minute

[5] —There were significant leaks found in multiple samples of the Everpure Unbreakable, making the quality impossible to test.

[6] —Percent recovery only applies to reverse osmosis filters which produce wastewater.

Figure 6-3: Comparison between commercial filters that are commonly used in India



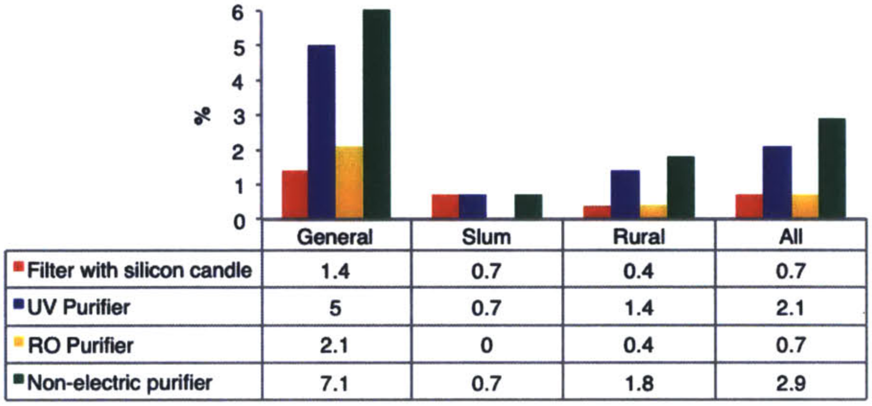
Parameter		Conventional particle filters		Gravity non-electric filters
		Cloth (8-folds)	Strainer/Jali	
Filtration performance	Turbidity reduction	60%	<5%	80-100%
	E.Coli reduction	<20%	No effect	90-99.99%
Access	Availability	Wide variety Readily available		Few pocket-friendly options Not readily available
	Affordability	Very affordable		Initial payments are high. Financing options are poor

Figure 6-4: Comparison between options available for the poorest of the poor in India

6.2.3 Trends in consumer behavior

Further, a report published by Grasp Analytique in 2011 captures some really interesting trends in the consumer market and provides a lot of valuable insights into the potential for water filtration products in the BOP market [6]. The survey was conducted mainly in North India (Uttar Pradesh, Punjab and Haryana) across 420 consumers living in rural and urban (general and slum) areas. The ratio of the people interviewed in each of these categories was proportional to the relative share of their contribution to the total population. A few graphs capturing relevant information are shown below:

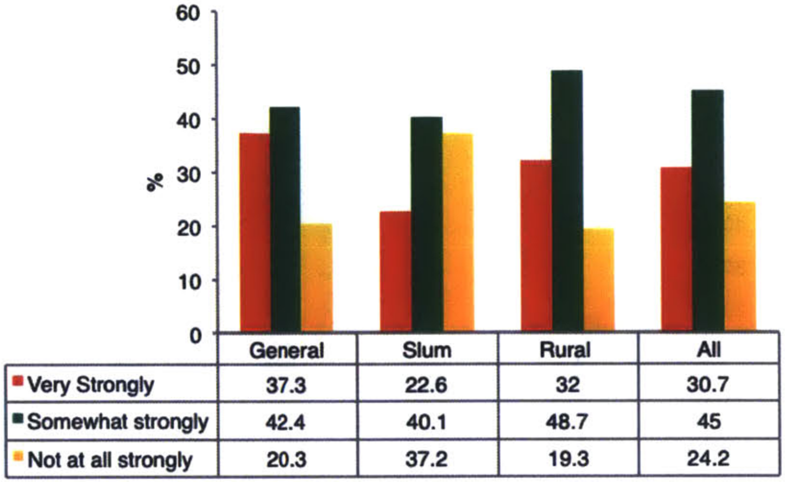
1. Only a meager 4% of the rural population used water purifiers. This is again a reflection of how poorly the water purification products have performed in the rural areas (Figure 6-5).



	General	Slum	Rural	All
🟥 Filter with silicon candle	1.4	0.7	0.4	0.7
🟦 UV Purifier	5	0.7	1.4	2.1
🟨 RO Purifier	2.1	0	0.4	0.7
🟩 Non-electric purifier	7.1	0.7	1.8	2.9

Figure 6-5: User-ship of water purifier by place of residence

2. Surprisingly, around 81% of the people living in rural areas felt the need for a water purifier (Figure 6-6).
3. About 60% of the respondents in urban as well as rural areas cite cost as the major barrier preventing them from buying a water filter. The survey also estimated that people were willing to pay between Rs.447 - Rs.573 for purchasing a water filter (Figure 6-7).



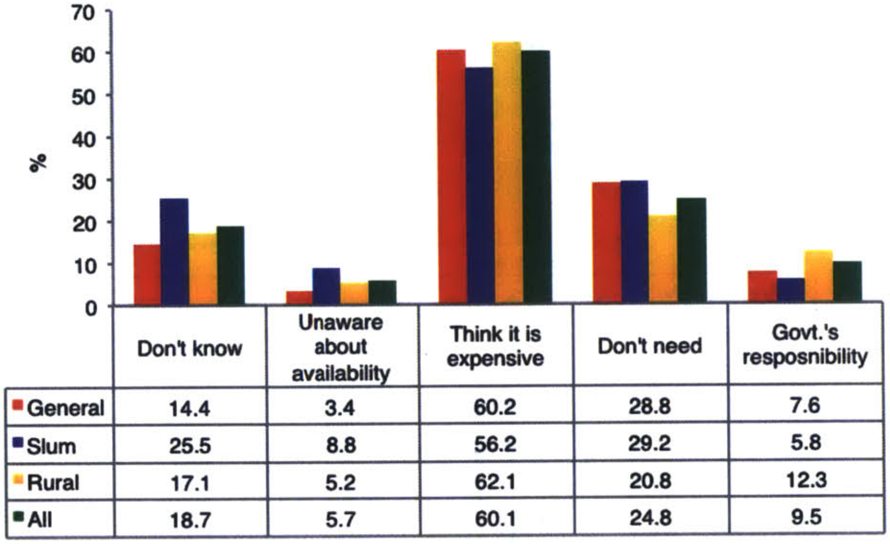
	General	Slum	Rural	All
🟥 Very Strongly	37.3	22.6	32	30.7
⬛ Somewhat strongly	42.4	40.1	48.7	45
🟧 Not at all strongly	20.3	37.2	19.3	24.2

Figure 6-6: Percentage of people who felt the need for a water purifier

4. Customers prefer offline systems that require low maintenance (Figure 6-8).

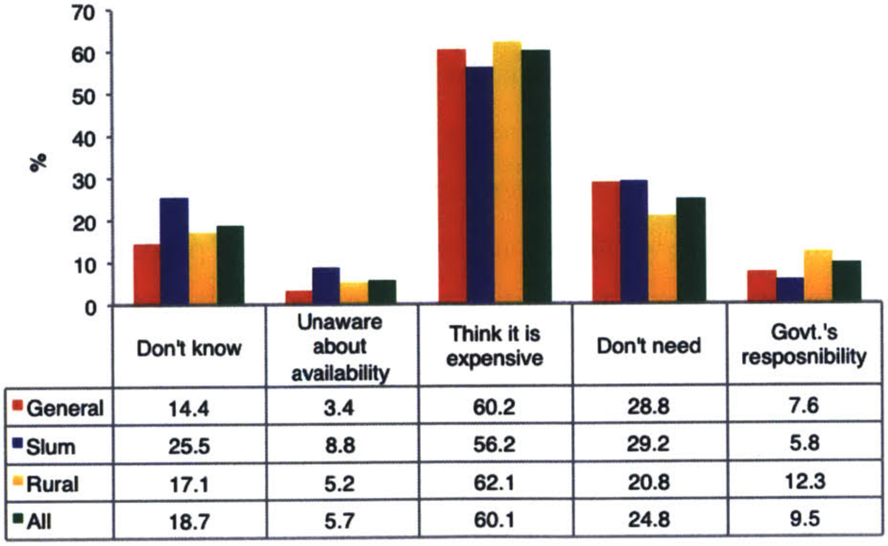
Based on the information presented above and other results presented in this report, the key conclusions that can be drawn are:

- The fact that only 16% of the people living in rural India are dissatisfied with the water quality but 81% of them would like to have a water purifier clearly demonstrates that people view water filters as good-to-haves and not a must-have. Under such circumstances, any product that is priced even slightly higher than the average affordability is likely to be received poorly in the market.
- Further, that fact that 60% of the people find the water filters to be expensive serves to strengthen the argument that the underlying factor behind the lack of market share for water filtration products in BOP is product pricing.
- With most of the rural population not being aware of any brand of water filter, marketing and advertising also seems to be an issue.



	Don't know	Unaware about availability	Think it is expensive	Don't need	Govt.'s resposnibility
🟥 General	14.4	3.4	60.2	28.8	7.6
🟦 Slum	25.5	8.8	56.2	29.2	5.8
🟨 Rural	17.1	5.2	62.1	20.8	12.3
🟩 All	18.7	5.7	60.1	24.8	9.5

Figure 6-7: Barriers for buying water purifiers



	Don't know	Unaware about availability	Think it is expensive	Don't need	Govt.'s resposnibility
🟥 General	14.4	3.4	60.2	28.8	7.6
🟦 Slum	25.5	8.8	56.2	29.2	5.8
🟨 Rural	17.1	5.2	62.1	20.8	12.3
🟩 All	18.7	5.7	60.1	24.8	9.5

Figure 6-8: Desired product attributes

6.2.4 Case studies of commercial products

There are numerous case studies of water filtration products that have met with success as well as failure in the market. Hindustan Unilever Limited (HUL) launched its water purifier PureIt in rural India. They made significant headway in the initial stages of the launch where they adopted a three pronged marketing strategy: door-to-door sales service, setting up kiosks and working with hospitals [7]. However, the sales growth was not as high once the door-to-door sales were discontinued. The company's strength also lay in laying down a super strong retail network and highly effective marketing strategies. To increase awareness and to sensitize people, HUL trained 'water experts' who could provide one-on-one demonstrations and educate customers on the importance of clean drinking water [8].

Further, through discussions with various stakeholders in IAP 2015, it was found that companies often design products at the lower end of the price spectrum but resort to differential marketing. In other words, they tend to market their high-end products more due to their higher profit margins. Hence, despite the presence of numerous low cost water filters in the market, these products might not do well commercially because they do not figure at the priority list when it comes to product promotions. Further, the cost of marketing, supply and distribution of such products in rural areas, which are often remotely located, far outweighs the revenues from sales.

Therefore, facilitating access to low cost solutions is critical to address the issues associated with drinking water quality.

6.3 Cost estimate of xylem filter

As the primary advantage of the xylem filter is its low cost, a preliminary analysis was done to estimate the cost of such a filter.

6.3.1 Methodology for calculating cost

In order to calculate the costs, the following assumptions were made: The following assumptions were made in order to come up with a cost estimate:

- Daily water requirement per person: 3 liters per day
- Filter size: Cylindrical disc with 5 cm diameter and 6 mm thickness
- Weight of each filter: 8 g
- Flow rate: 4.5-6 L/hr with a gravity head of 1 m
- Volume of ethanol required for each filter: 50 mL
- Average family size: 4 members
- Volume of water required for a family per week: 84 liters
- Filter lifetime (to satisfy minimum consumer expectations): 7 days

The costs involved in making the xylem filters can be broken into three categories:

- Raw material
- Wood processing
- Packaging
- Transportation and storage

Each of these categories except packaging have been discussed in detail in the following sections.

6.3.2 Raw material

Experiments on the utility of xylem as a water filter have so far been done using Eastern White Pine (*Pinus Strobus*). The most common variety of pine available in India is Chir Pine (*Pinus Roxburghii*) and is present in abundance in the Himalayan mountains in Himachal Pradesh and Uttarakhand. The trees are normally cut and stored in the form of logs by the government through Himachal Pradesh State Forest Development Corporation Limited. These logs are then auctioned off. Logs in customized sizes can also be obtained. The prices for various conifers per cubic meter have been listed in Table 6.1 below [9]:

As can be seen, chir pine is the cheapest wood available. If 8 g of wood is used in

Table 6.1: Prices of different woods

Wood	**Sawn sizes**		**Log forms**	
	USD/m^3	INR/m^3	USD/m^3	INR/m^3
Deodar	450	30000	330	22000
Kail	360	24000	240	16000
Partal	150	10000	90	6000
Chir Pine	105	7000	75	5000

each filter, the cost of wood for every filter comes out to Rs. 0.2 (0.3¢).

6.3.3 Processing

Immediately after the wood is cut from the tree, it has to be sliced into thin sections and flushed with/dipped in ethanol before it begins to dry. The details of ethanol treatment of fresh green wood are yet to be worked out. The costs involved here would be that of ethanol and labor. Ethanol costs Rs 50/liter and the volume of ethanol required is typically 4-5 times that of the wood to be preserved. If 50 ml of ethanol is required for every filter, then the cost of ethanol treatment comes to Rs 2.5 (4¢). Further, wooden logs would have to be sliced into sections having the desired filter length. If we assume a large scale setup to manufacture This process would require an automated saw and labor. The equipment cost has been estimated as follows:

- Cost of automated vertical saw : ~Rs. 50,000 ($750) [10]
- Warranty period : 5 years
- Duration of operation per day : 8 hours
- Duration of operation over warranty period (assuming 5 working days/week) : 10,400 hours
- Time taken to cut each filter : 30 sec
- Number of filters the saw can cut over its lifetime : 1.2×10^6
- Cost of saw per filter : Rs 0.04 (0.06¢)

The cost of labor can be estimated using the method below:

- Time taken to cut a filter : 30 seconds
- Number of hours of operation per day : 8 hours
- Number of filters that can be processed in a day : 960
- Labor charges for a day : ~Rs. 300 [11]
- Labor charge per filter : Rs. 0.3 (0.5¢)

The total processing cost for the filter is ~Rs. 3 (4.5¢).

6.3.4 Transportation

The location of the various depots from which transportation is provided for by the Himachal Pradesh State Forest Development Corporation Limited have been shown in Figure 6-9 below on the right hand side (marked with star).

All these depots provide transportation to a lot of destinations in Punjab and Haryana including Chandigarh. To estimate the cost, it was assumed that all the wood would be transported from the depots to Chandigarh and then to Delhi from where it would be routed to different cities. This was done because it was easy to obtain the freight charges for trucks from Delhi to other places. The freight charges (in INR) from Delhi to different cities per nine-tonne load have been listed in Table 6.2[12].

Using these numbers, if the volumes transported are large, the cost of transportation of each filter comes down to Rs. 1.08. While the inter-city transportation charges can be estimated, calculating the local distribution costs would require exact identification of the target locations and a thorough understanding of the supply chain for each of the customer segments. It can be assumed that the filters can be sent to these locations by post. Assuming no infrastructure for distribution, the cost of sending filters by government post across India is Rs.35-90 for a single filter and Rs.0.2-0.5 when sent as a package of 200.

Table 6.2: Freight charges from Delhi to different cities per nine-tonne load

Destination	Cost (INR)
Jaipur	13000
Hyderabad	52000
Chandigarh	14000
Vijayawada	57000
Ludhiana	15000
Bangalore	62000
Kanpur	16000
Chennai	63000
Ahmedabad	19000
Patna	22000
Gwalior	14000
Kolkata	28000
Guwahati	51000
Pune	26000

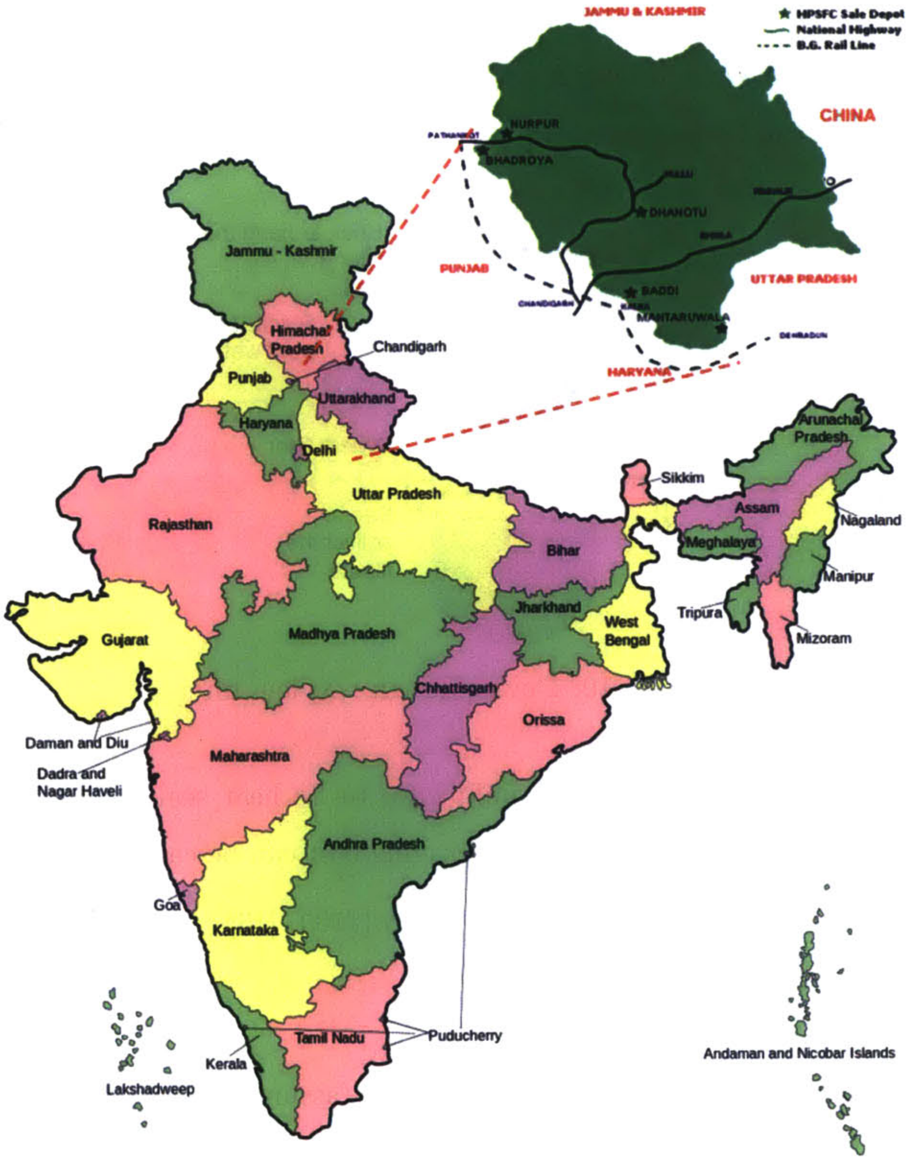


Figure 6-9: Location of transportation depots

6.3.5 Packaging

As the final product configuration of a xylem filter can very from an extremely simple design such as one where the filter is used as a stand-alone device to one where its integrated with a pre-filtration module, the preliminary cost analysis excludes the packaging cost. This offers the flexibility to alter the design of the filter to meet cost targets as well. If the xylem filter is used as a stand alone device, then in its simplest configuration, it can be housed in a plastic sleeve that is shown in Figure 6-10.

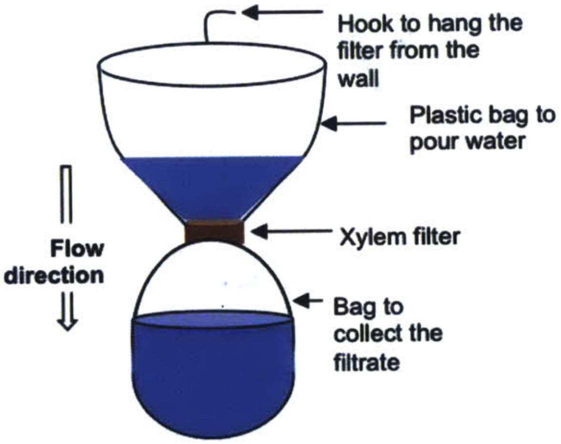


Figure 6-10: Device design : Xylem filter

In such a case, the plastic bag would have to be heat sealed around the filter requiring a heat sealing machine. The packaging cost can be calculated as follows:

- Cost of heat sealing machine : ~Rs. 7,000 ($100) [13]
- Warranty period : 1 year
- Duration of operation per day : 8 hours
- Duration of operation over warranty period (assuming 5 working days a week) : 2,080 hours
- Time taken to cut each filter : ~60 sec
- Number of filters the be packed over the lifetime of the product : 124,800
- Cost of the machine per filter : Rs 0.06 (0.09¢)

The cost of labor can be estimated using the method below:

- Time taken to pack a filter : 60 seconds

- Number of hours of operation per day : 8 hours
- Number of filters that can be processed in a day : 480
- Labor charges for a day : ~Rs. 300 [11]
- Labor charge per filter : Rs. 0.6 (1¢)

The total packaging cost of the filter comes to Rs. 1 (1.5¢).

6.3.6 Results

The methodology adopted to estimate the cost contribution from each of the four elements listed has been summarized in Figure 6-11

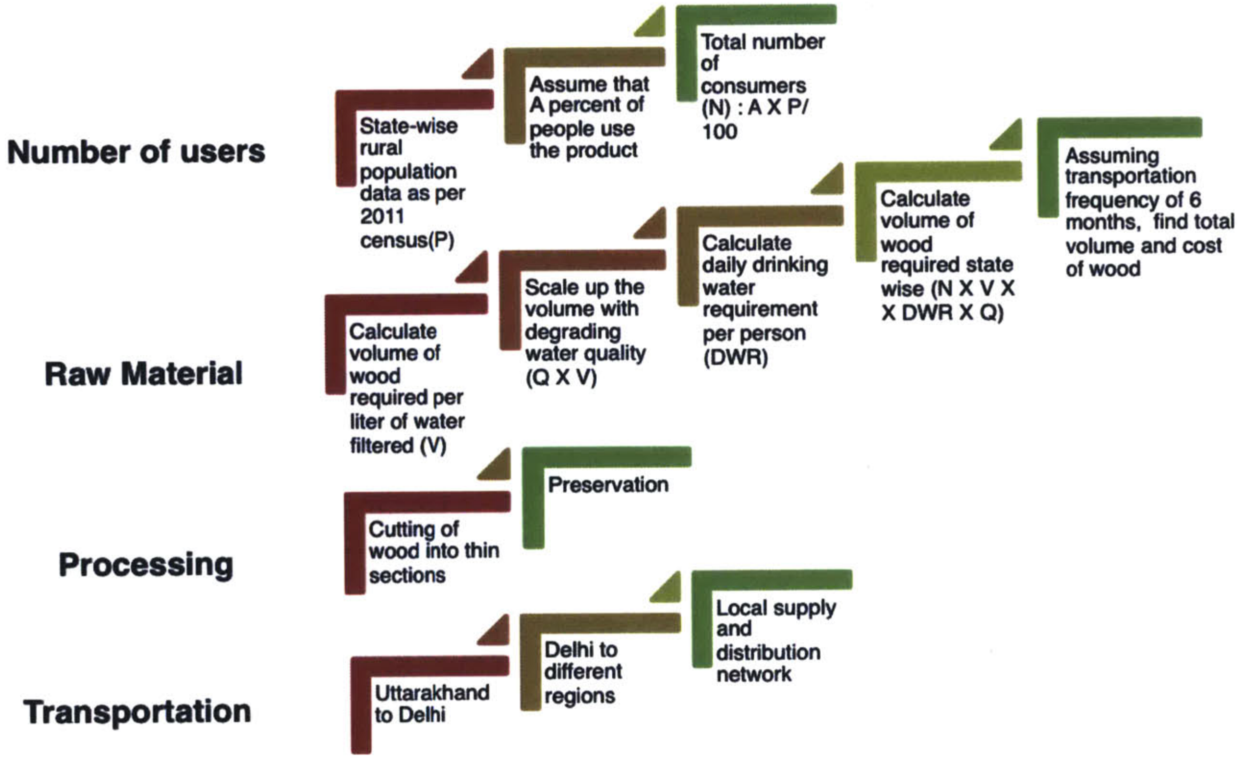


Figure 6-11: Steps involved in estimating cost of xylem filters

Based on the above calculations:

- Weight of each filter: 8 g
- Cost of filtration per household per week: ~Rs. 5.5 (8¢)
- Cost of each filter: ~Rs. 5.5 (8¢) (as each filter is expected to take care of the water filtration requirements of a household for atleast a week)

Depending upon the water quality, the lifetime of the filter or the total volume of water it can process would vary. This would increase the cost of filtration. The variation of the cost with the water handling capacity of the filter has been shown in Figure 6-12.

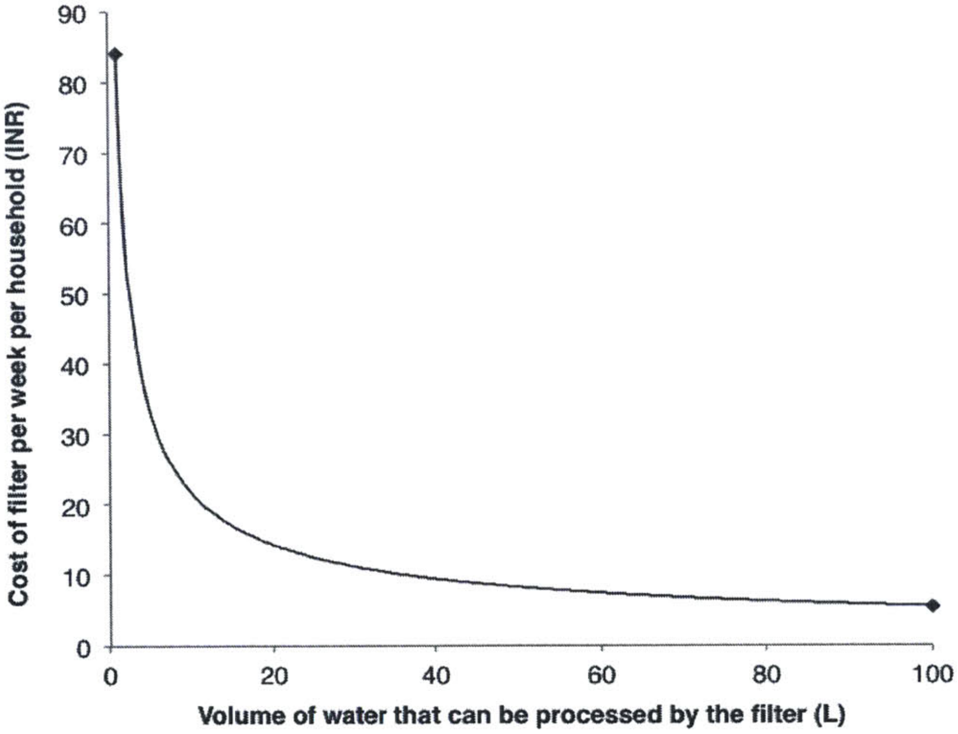


Figure 6-12: Variation in cost with total volume processed by the filter. While the cost is Rs. 4 if the filter processes 84 liters of water, it is Rs 84 if it can process just 1 liter

6.4 Identification of potential applications and customer segments

Filters made out of xylem can have many interesting applications in India:

- Household water filtration: Xylem filters can serve as a great alternative for the conventional particle filters (such as cloth or jali) that are commonly used by the poorest of poor in India. While the xylem filters have a cost that is comparable to the latter, they have a much better performance in terms of turbidity reduction and bacterial removal.

- Filters for emergency use: Xylem filters can be extremely useful in disasters and emergencies where access to drinking water is one of the major problems. Cans or bottles of filtered water are either transported via road or packets of filtered water are airdropped in affected regions, but connectivity via roads often gets severed due to disaster-related damage and the process is expensive as the volumes handled are large. Through meetings with the National Disaster Management Authority in India, we learnt that a product like the xylem filter, that is compact and easily distributable, would be highly desirable and well-suited for emergencies (e.g. a wallet-sized package weighing 100 g that has the filter inserted into a plastic sleeve bag that holds water and can provide >100 liters of water at >1 L/h at an estimated cost of <$0.5/device).
- Filters for analysis of bacteria in water: Membrane filtration is a major method in bacteria analysis to concentrate bacteria for better sensitivity. Here, 100 mL of water is filtered through a membrane, which is then placed in culture media. Bacterial growth results in colonies that are visually enumerated. However, these membranes cost approximately $1, and most labs in India do not use membrane filtration. Field-test kits could benefit enormously from low-cost membranes that improve their sensitivity. The cost of xylem as a filter material is 10-100x lower than membrane filters with similar pore size cutoff (which also have similar flow rates), which could greatly reduce the costs of these techniques for detection of bacteria in water.

6.5 Conclusion

Commercial technologies for water filtration that fall within the range of affordability of the poorest of poor in India are limited to a few gravity-based offline filters and conventional particle filters. While high upfront costs deter adoption of the former, the conventional particle filters are hardly effective at reducing turbidity and filtering bacteria. As of now, there is a need for technological solutions that are priced as low as the conventional particle filters but have a performance comparable to that of the gravity-based filters. Previous studies with the xylem have demonstrated their ability to reject 99.99% E.Coli and a preliminary cost analysis of the xylem filter shows that it has the potential to provide safe drinking water to a household of 4 at Rs. 5/week. Owing to their low cost, efficacy against bacteria and ease of manufacturing, xylem filters possess immense potential in making safe drinking water accessible to the low-income groups in India.

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Chapter 7

Conclusion

7.0.1 Summary of Thesis

The goal of the thesis was to develop methods for dry storage of xylem filters, to achieve gravity driven flow using xylem and advance the understanding of xylem as a filter material.

In Chapter 1, an overview of the problems associated with drinking water quality and a brief review of the current technologies in the market was presented. The need for technological solutions such as xylem filters that possess immense potential to address this issue by striking an optimal balance between performance and cost was established. Prior work done towards the use of xylem as a filtration material and the challenges involved in the implementation of this technology were discussed.

Chapter 2 focused on the development of methods for dry storage of xylem filters. It was found that the permeability and filtration characteristics of xylem filters can be preserved by treating them with ethanol before drying. It might also be possible to use other solvents like heptane, which have a similar surface tension like ethanol, to achieve high flow recovery. Further, the recovery was found to have a strong dependence on filter length. The permeability after drying was found to drop abruptly to almost zero on increasing the filter length from 0.25 inches to 0.50 inches. While the exact reason underlying this observation is yet to be understood completely, there is evidence to indicate that this behavior can be partly explained using percolation theory where the nature of connectivity between the open pores is expected to govern permeability.

In Chapter 3, gravity driven filtration through xylem filters was discussed. Operation of xylem filters under gravity was achieved and a linear dependency between flow rate and pressure and flow rate and area was established. This opens up the possibility to explore different combinations of filter geometry and operation parameters to meet target flow rates that suit user expectations. Further, it was found that treatment with ethanol was as effective for xylem filters with a 2 inch diameter as it was for the ones with 0.5 inches. The predictable efficacy of ethanol treatment and the knowledge of interdependence between area, pressure and flow rate provide a great way to control the design of xylem filters to achieve the desired performance.

Chapter 4 dealt with the study of operational attributes of xylem filters. The preliminary fouling and degradation studies showed that storing xylem filters, both, fresh and ethanol-preserved, in DI water and tap water for up to 20 days does not affect their permeability and rejection ability. Filter lifetime studies show that increasing the turbidity and concentration of contaminants led to a sharp reduction in filter time. Interestingly, it was found that the filters get clogged even when DI water is passed through them. Based on the results obtained, fouling seems to be a greater cause of concern over wood degradation and can be expected to play a major role in limiting filter lifetime. Further investigation into the mechanism of fouling and factors that lead to decline in permeability and methods to improve filter lifetime is required.

In Chapter 5, methods to enhance the performance characteristics of xylem were explored. The use of other trees such as Eastern White Cedar and Gingko Biloba which have a tighter margo can help in achieving better rejection. The use of xylem filters in conjunction with other materials such as flocculants/coagulants, sorbent materials (like iron oxide), silver and copper ions that have antibacterial properties or zeolite can enhance the rejection properties of the xylem and can allow for filtration of a wider variety of contaminants including viruses. A pre-filtration module can be added to improve the lifetime of xylem filters.

In Chapter 6, the relevance of xylem technology in making drinking water accessible to the poorest of the poor in India was explored.

7.0.2 Future Work

Although significant progress has been made to towards the use of xylem as a filtration device, significant potential exists for improvement and extension of the work. The following is a list of recommendations for future research based on the different topics covered in the thesis:

Dry storage of xylem filters

- Investigate the factors the responsible for strong dependence of permeability on length

Gravity-driven flow through xylem

- Study rejection characteristics of filters with large diameters

Study of operational attributes

- Understand the mechanism responsible for drop in permeability
- Explore methods to enhance fiter lifetime (such as use of pre-filtration module)
- Conduct lifetime studies with water containing biological and chemical contaminants
- Monitor filtrate quality to check for variations in filter performance with time
- Conduct wood degradation studies with water having different kinds (biological and chemical as well) and concentrations

Improving rejection performance of xylem filters

- Study flow recovery of species like Eastern White Cedar and Gingko Biloba that have better rejection characteristics and explore other such options
- Explore the use of xylem with iron oxide particles and test for virus rejection
- Design and build product prototypes

Implementation in India

- Find partners and collaborators to develop the xylem technology further and work towards the goal of commercialization of xylem filters
- Conduct field tests with product prototypes and use the feedback to guide the

process of product design and development