Identification and Evaluation of Techniques for Quality Control of Low-Cost Xylem Filters

by

Bettina K. Arkhurst

Submitted to the Department of Mechanical Engineering in Partial Fulfillment of the Requirements for the Degree of

Bachelor of Science in Mechanical Engineering

at the

Massachusetts Institute of Technology

June 2018

© 2018 Massachusetts Institute of Technology. All rights reserved.

Signature redacted

Signature of Author:

Department of Mechanical Engineering May 21, 2018

Signature redacted

Certified by:

Dr. Rohit Karnik Associate Professor of Mechanical Engineering Thesis Supervisor

Accepted by:



Rohit Karnik Associate Professor of Mechanical Engineering Undergraduate Officer



77 Massachusetts Avenue Cambridge, MA 02139 http://libraries.mit.edu/ask

DISCLAIMER NOTICE

Due to the condition of the original material, there are unavoidable flaws in this reproduction. We have made every effort possible to provide you with the best copy available.

Thank you.

The images contained in this document are of the best quality available.

Identification and Evaluation of Techniques for Quality Control of Low-Cost Xylem Filters

by

Bettina K. Arkhurst

Submitted to the Department of Mechanical Engineering on May 21, 2018 in Partial Fulfillment of the Requirements for the Degree of

Bachelor of Science in Mechanical Engineering

ABSTRACT

2.1 billion people worldwide, majority of whom are of the poorest income quintile, lack access to safe, readily available water in their homes. The need for affordable, decentralized methods of water filtration led to the development of a low-cost membrane filter produced from the xylem of coniferous trees. Due to xylem structure variation and the potential for improper filter processing during mass production, quality control protocols are a necessity. Manufacturers must ensure xylem filters are functional in terms of microbial rejection and adequate flow rates. Testing methods similar to those mentioned in this thesis can also be developed for other membrane filters.

The suitability of two fluids, water and air, were evaluated for use in the quality control process. For testing using water, turmeric and blue dye were used to create a visual indication test to detect a filter's major failures. We found that this method has the potential to detect both leaks and improperly prepared filters, but it lacks affordable, quantitative analysis for determining rejection percentages. Air was found to be a viable option for xylem filter testing at pressures of 6 psi and above, though presence of the xylem lowered the concentration of particles detected at the outlet by one-fourth. The substances found to be most suitable for testing the filter were Baker's yeast, jeweler's rouge, turmeric, and buttermilk given their affordability, particle/microbe size, and availability. Further exploration is required to determine the optimal particle to use in water and air testing and the equipment necessary for the quality control process to be implemented.

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

Acknowledgements

I would like to thank Professor Rohit Karnik for all of his guidance throughout this thesis journey. I would also like to thank Krithika Ramchander for her mentorship and the many hours she gave me throughout this process. To all members of the Microfluidics & Nanofluidics Research Laboratory, thank you for welcoming me into the lab and showing your willingness to help me when I needed it.

As I reflect on my time at MIT, I realize how much I owe to my mentors and supporters who have aided me throughout my time here. I'd like to thank my academic advisor, Professor Rohan Abeyaratne, for taking me in as an advisee and for his unceasing willingness to help me through my many academic and work-related existential crises. You have been one of the kindest people I have met since coming to MIT and I'm grateful for getting a chance to get to know you.

I'd also like to thank my family for their never-ending love and support, particularly my mother and sister. Mom, you have sacrificed so much for us and all of the opportunities I have been provided are due to your hard work. You have been my rock and my backbone throughout my life and it is a privilege to be called your daughter. Dianna, you have shown me what it truly means to be a light in the darkness. You have defied all odds and you are bound for greatness. You are a star that brightens up every life you touch. Congrats on degree #3, sis!

Finally, I'd like to thank my wonderful friends and members of my Course 2 family. After several nights filled with problem sets and studying, we made it, Class of 2018! Thank you for making this journey so much better.

3

Table of Contents

Abstract	2
Acknowledgements	3
Table of Contents	4
List of Figures	6
List of Tables	. 7
1. Introduction	8
1.1 Importance of the Xylem Filter	8
1.2 Xylem Water Filtration	9
1.2.1 Coniferous Tree Xylem Structure	9
1.2.2 Xylem Water Filtration Process	10
1.3 Xylem Filter Manufacturing Process	11
1.4 Xylem Filter Quality Control	13
2. Test Particle Selection	14
2.1 Particle Selection Criteria	14
2.2 Particle Options	14
2.3 Testing Particle Suggestions	18
3. Testing the Xylem Filter using Water	19
3.1 Laboratory Testing Protocol using Water	19
3.2 Issues with Quality Control using Water	19
3.3 Filtration Testing using Turmeric and Food Dye	20
3.3.1 Why Turmeric	20
3.3.2 Experimental Setup	20
3.3.3 Method	22
3.3.4 Water Experiment Results	22
3.4 Testing Water-Dried Filters	28
3.5 Turmeric Size Distribution	31
3.6 Concentration Optimization	33
3.6.1 Optimization Method	33

3.6.2 Optimization Results	34
3.7 Next Steps for Water Testing	36
4. Testing the Xylem Filter using Air	37
4.1 Benefits of Testing with Air	37
4.2 Testing Xylem for Air Filtration	37
4.2.1 Experimental Setup	37
4.2.2 Preliminary Air Experiment	38
4.2.3 Method	39
4.2.4 Air Experimental Results	40
4.3 Next steps for Air Testing	42
5. The Quality Control Process	43
5.1 Goals for Xylem Filter Quality Control	43
5.2 Quality Control using Water	43
5.3 Quality Control using Air	44
6. Conclusion	47
7. References	49
8. Appendix	52

List of Figures

Figure 1.1:	Xylem structure and rejection	9
Figure 1.2:	Size distribution of common water contaminants	9
Figure 1.3:	SEM Images of torus-margo pits of lodgepole pine stems	10
Figure 1.4:	Filtration points in angiosperm and gymnosperm xylem	11
Figure 1.5:	Structure and rejection of Eastern White Pine, Cedar and Gingko biloba	12
Figure 3.1:	Setup for testing the xylem filter	19
Figure 3.2:	Filtering of the aqueous turmeric-blue dye mixture	21
Figure 3.3:	Preliminary color indication test images	22
Figure 3.4:	Feed and filtrate of turmeric, dye, and turmeric-dye mixture	23
Figure 3.5:	Spectrum of the turmeric-only mixture in deionized water	24
Figure 3.6:	Spectrum of the blue dye solution feed and filtrate	25
Figure 3.7:	Spectrum of the blue dye and turmeric mixture feed and filtrate	25
Figure 3.8:	Normalized spectra of the blue dye and turmeric mixture feed and filtrate	26
Figure 3.9:	Combined spectrum of spectrums in Figure 3.5, 3.6, and 3.7	26
Figure 3.10:	Linearly superimposed dye and turmeric feed	27
Figure 3.11:	Linearly superimposed dye and turmeric filtrate	27
Figure 3.12:	Normalized spectra of filtrate in ethanol and water-dried filters	28
Figure 3.13:	Normalized filtrate spectra in ethanol and water-dried filters (100% Dye removal)	29
Figure 3.14:	Normalized filtrate spectra in ethanol and water-dried filters (90% Dye removal)	30
Figure 3.15:	Normalized filtrate spectra in ethanol and water-dried filters (50% Dye removal)	30
Figure 3.16:	Normalized filtrate spectra in ethanol and water-dried filters (10% Dye removal)	31
Figure 3.17:	Turmeric particles under a light microscope at x40 magnification	32
Figure 3.18:	Turmeric size distribution	32
Figure 3.19:	Turmeric and dye concentrations used for optimization testing	33
Figure 3.20:	Wells of varying concentrations of dyes, turmeric, and their combination	34
Figure 4.1:	PM sensor reading from preliminary air testing	38
Figure 4.2:	Schematic of xylem as an air filter	39
Figure 4.3:	Reading of the PM Sensor with no filter	40
Figure 4.4:	Reading of the PM Sensor with xylem filter	41
Figure 5.1:	CAD rendering of testing setup for quality control using water	43
Figure 5.2:	Typical pressurized inhaler showing mechanism of particle formation	44
Figure 5.3:	CAD renderings of inhaler-inspired air testing apparatus	45
Figure 5.4:	Labeled CAD rendering of inhaler-inspired air testing apparatus	45

List of Tables

Table 2.1 :	Particle options for use in air testing	15
Table 2.2 :	Particle options for water testing	16
Table 2.3 :	Particle options for use in air and water testing	17
Table 3.1 :	Turmeric and dye concentration optimization	35
Table 3.2 :	Turmeric concentration and corresponding flow rate	36
Table 4.1 :	Size distribution of Arizona Test Dust	37

1. Introduction

1.1 Importance of the Xylem Filter

2.1 billion people worldwide lack access to safe, readily available water in their homes [1]. About two dozen infectious diseases are related to water quality [2]. The main drinking-water risks in developing countries are associated with microbial pollution. Contaminated drinking water can transmit diseases such as diarrhea, cholera, dysentery, typhoid, and polio. According to the World Health Organization, contaminated drinking water is estimated to cause about 502,000 diarrheal deaths each year in which 361,000 are of children under the age of 5 years [3].

Children from developing country households in the poorest income quintile are significantly less likely to have access to improved drinking water sources according to UNICEF [4]. Those particularly in rural communities are situated farther away from centrally organized water resources and tend to be unable to exploit economies of scale for community-level water supply solutions [2]. Hunger, malnutrition and lack of safe drinking water contribute to at least half of total child mortalities and these incidences are highly concentrated among those in the poorer income quintiles. With about 80% of the world's population living on less than \$10 per day, affordability is not only a convenience, but a necessity when it comes to water filtration [5].

All of the aforementioned issues associated with the lack of potable water in developing countries calls for a cost-effective and convenient decentralized water cleaning system. Most water-quality issues are due to pathogens that can be completely retained by membrane filtration methods [2]. Generally, membrane processes are characterized by the use of a semi-permeable film or membrane and a driving force. Although there are currently thermal, UV, physical and chemical methods available to clean water, many of these methods are expensive, time-consuming, or both. In order to address these concerns, strides have been taken to create low-cost water cleaning systems such as the water filtration system created from the xylem of coniferous trees [6].

Xylem is a porous material that conducts fluid in plants, which has evolved to allow the ascent of sap with minimal resistance while maintaining small nanoscale pores to prevent cavitation (**Figure 1.1a**) [7]. In woody plants, the xylem tissue is called the sapwood, which often surrounds the heartwood, which is, in turn, surrounded by the bark. Coniferous trees, or conifers, were chosen as the tree from which to create theses filters due to their xylem structure consisting of larger cross section areas of conducting xylem tissue, and their rejection of small water contaminants [6]. Conifers are found in five continents: North America, Europe, Asia, South America, and Africa, making them widely available [8].

This gravity-driven membrane filter provides a novel, cheap method of removing particles and organisms of about 70 nm in characteristic length and larger from drinking water. By excluding particles and organisms greater than 0.07 microns in characteristic length, the xylem filter is able to filter common water contaminants such as viruses, bacteria, and visible particles (**Figure 1.1(b,c), Figure 1.2**) [6,9].



Figure 1.1: (a) Photograph of 1 cm diameter pine (*pinus strobus*) branch used in the 2014 Boutilier *et al* study that discovered the xylem filter. **(b)** Dependence of the rejection on the particle size estimated from filtration of a distribution of particles sizes. Boutilier *et al* used this data to suggest the xylem filter can be used to reject water contaminants >70 nm in size. **(c)** SEM images showing bacteria accumulated on the margo pit membranes of the xylem filter after filtration. [6]



Figure 1.2: The size distribution of common water contaminants. [9]

1.2 Xylem Water Filtration

1.2.1 Coniferous Tree Xylem Structure

The xylem structure of conifers consists of water-conducting cells called tracheids. Tracheids are overlapping single-celled hollow conduits, closed at both ends. More than 90 % of conifer xylem's cross section consists of tracheids [10]. Tracheid diameters usually vary between 5 and 80 μ m and tracheid lengths are usually less than 5 mm. Water moves through a tracheid's lumen and passes through a pit into the lumen of an adjacent tracheid. A coniferous pit is a circular perforation in the tracheid cell wall, which is paired with a coincident perforation on the adjacent tracheid – creating a pit pair. Coniferous pit pairs allow water movement from one cell to another, while protecting the cells from cavitation due to air entry from a gas-filled adjacent tracheid.

In the center of the pit membrane is a thickened, approximately circular, and water-impermeable torus that is surrounded by a porous membrane region called the margo, which provides passage for water. This structure is known as the torus-margo pit membrane. The margo acts as the main water filtration site in the xylem filter (Figure 1.1c). Membrane pits are largely confined to the radial walls, as shown in Figure 1.3, suggesting relative ease of water-sharing in the tangential direction around a stem.



Figure 1.3: "Scanning electron microscopy images showing torus-margo pits of lodgepole pine (*Pinus contorta*) stems. Samples were taken from open-grown (**a**-**c**) and shaded (**d**) trees. These images represent a continuum of margo porosities. Some pits have a dense margo with small pores, particularly near the edge of the membrane (**a**) while other pits have few margo 'spokes' and large pores between the margo fibrils (**d**)." – Hacke *et al* [10,11]

1.2.2 Xylem Water Filtration Process

Conifers are good candidates for the xylem filters because their xylem structure, as in other gymnosperms, contain more points of water filtration. The pits have membranes with nanoscale pores that serve as a means for preventing cavitation bubbles from crossing over from one conduit to another. These nanoscale pores also act as "filtration points" in the xylem filters and gymnosperms, which includes conifers, have more than angiosperms, or flowering plants [6] (**Figure 1.4**). These filtration points act as membrane filters of contaminants greater than 70 nm in size. This size-based exclusion that occurs in the filter is seen in **Figure 1.1c**.



Figure 1.4: The path of water flow through the xylem of angiosperms (left) and gymnosperms (right) with the filtration points, also known as pit pairs, marked. Gymnosperms, which include conifers, act as better filters due to their large number of filtration points when compared to angiosperms. [6]

1.3 Xylem Filter Manufacturing Process

The function of the aforementioned pits, or pores, form the basis of xylem filtration; therefore, their preservation is a key concern within the manufacturing process. Initially, the xylem would have to be kept in water to keep the pores of the severed portion of the tree from closing and restricting water flow through the filter [6]. With that limitation, filters could only be created using freshly collected portions of the tree before drying out. Given the impracticality of transporting and selling xylem filters while they are wet, a method to store these filters in their dry state was developed [12]. This method consisted of the following protocol:

A portion of the tree is collected, the filters are cut out of the wood and the bark is peeled off. The filters are then soaked in deionized water at 60°C for 1 hour, soaked in ethanol and then dried for distribution.

After determining the process to keep the xylem pores open, the search for the tree that generated the most effective xylem filter proceeded. When evaluating the most effective conifer to use in filter production, the rejection of the filter was taken into account [12]. The rejection is the percentage of contaminants in an artificially-contaminated water feed that does not appear in the filtrate (**Eq. 1**). The Environmental Protection Agency (EPA) calls for at least a 4 log reduction in water contaminants, which is a rejection of 99.99% (**Eq. 2**) [13].

Eq. 1.1
$$Rejection = \left(\frac{Input - Output}{Input}\right) \times 100$$

Eq. 1.2 $Rejection_{EPA} \ge 99.99\%$

Ginko biloba was found to have the capability to obtain a rejection that meets the 4-log reduction criterion (99.99%) while the Eastern White Pine and Eastern White Cedar trees did not (**Figure 1.5**) [12].



Figure 1.5: (**a**,**b**,**c**) SEM images of the pits of Eastern White Pine, Eastern White Cedar and *Gingko Biloba* trees. *Gingko Biloba* has a tighter margo and is able to more effectively reject contaminants. (**d**) The rejection of the *Gingko Biloba* is higher than both the Easter While Pine and Cedar- making it a more suitable choice for filter production. [12]

Given the fact that these are naturally produced filters, the structure and quality of the filters may vary from species to species, within a species from one tree to another, or even from branch to branch. The cell types that make up xylem tissue show great variability across different plant groups and even within the same plant [14]. Variation in the xylem filters has the propensity to induce varying filter performances. The structure and density of the tracheids and the pit membranes within the xylem differ from one tree to another, which has the potential to change the flow rates and filtration efficacy in the xylem filters [12,15].

Along with the inherent variation amongst conifers, other points of filter failure manifest in cases when ethanol soaking is not done properly or the wood used for filter production is already partially dried before preparation [12]. The propensity for natural variation and improper filter processing calls for an implementation of quality control protocols for large scale filter production. Before xylem filters can be distributed to the public, a procedure must be implemented within the manufacturing process to ensure each filter has met a standard of function. Many filter manufacturer use the EPA 4-log reduction criterion [13].

1.4 Xylem Filter Quality Control

The exploration of quality control methods for mass production of xylem filters is the focus of this thesis. For the purposes of this study, methods for detecting serious failures within the xylem filter were explored. Particles were dispersed within fluids, passed through the filter and the filtrate was observed and analyzed. We strove to create filter testing methods that not only allow manufacturers to test their filters, but also empower the customer to test the filter, as well. Two fluids, water and air, were considered when selecting the medium of choice to use for quality control testing. We sought to create a quality control process that was cost-effective, repeatable, and capable of being implemented simply. The experiments and information in this thesis are provided to inform xylem filter manufacturers and consumers alike on methods, tools and materials capable of testing the filter's efficacy.

The next chapter of this thesis focuses on the criteria and selection process for choosing particles to use for testing the xylem filter. Chapter 3 focuses on the process, equipment, and protocol for testing the xylem filter with water. Chapter 4 explore testing filters using air and the xylem filter's potential to act as an air filter. Chapter 5 focuses on proposed methods and equipment designs for xylem filter testing and Chapter 6 concludes the thesis and describes suggested future work in this area.

2. Test Particle Selection

2.1 Particle Selection Criteria

In addition to selecting the testing fluid, choosing the test particle is an important aspect of determining the overall quality control process during xylem filters manufacturing. There are four essential criteria the particles used for testing should meet in order to be chosen for these purposes. The criteria are as follows:

- 1. **Purchasable/Manufacturable in bulk**: Given the large volume of xylem filters that will require testing, the particles should be available in bulk regardless of whether they are purchased or made at the manufacturing facility. Along with this requirement, the particles should be available in bulk within the region of manufacturing.
- 2. Affordable: Given the cost-constrained nature of this filter's applications, the particles used for testing must be affordable, particularly when purchased in bulk.
- **3.** Safe: The particles and/or the process in which the particles are used must be safe for the person carrying out the quality control tests and those in the vicinity.
- 4. Particle Size and Size Distribution: The substance used should include 1-micron particles because this size is around that of the microbes the filter must reject (Figure 1.2). Ideally, a batch of testing particles will not only include 1 micron particles, but also have a relatively small particle size distribution.

When evaluating the priority of each criterion, contingencies were taken into account. For example, the fact that personal protective equipment can be worn to mitigate the effects of a potentially hazardous substance was taken into account when evaluating each particle. Compromises with respect to the criteria can be made as long as there is no obstruction to the purposes of creating an effective testing system for the xylem filter. Given the small target size, in the case of air tests, it is expected that the apparatus used will need to be air-tight and/or the person carrying out the tests will need to wear a mask or some other form of personal protective equipment to avoid the hazards of inhaling particulate matter [16].

2.2 Particle Options

Using the aforementioned criteria, 16 particles were evaluated on their suitability for use in the filter quality control process. Five of these particles can only be used for air testing, three can only be used for water testing, and eight can be used for testing in both fluids.

The particles that can only be used for testing in air are displayed in **Table 2.1** along with their specifications, pros, and cons. These particles are generated from incense, pollen, chalk powder,

wood smoke, and Holi powder. Particles considered to only be useful in air testing tend to be those that are commonly found in air, those that can easily be seeded in air, or those that are water soluble, and thus, are not useful in water filter rejection testing. The pros and cons listed in the table were created using the criteria discussed in Section 2.1.

The most suitable particles of those listed for testing in air were Holi powder and chalk powder. The ubiquity and affordability of Holi and chalk powder in the region (India) where the filter will first be introduced were the main positive aspects of these two substances, though both powders also have large particle size distributions and tend to be larger than the 1 micron target size.

Particles	Specifications	Pros	Cons
Incense	1) Tends to be <1 micron (0.32– 0.56 μm) in characteristic length [17]	 Widely available Affordable product 	 Smaller than target size of 1 micron May be harmful if not
			contained properly
Pollen	 Tends to be >6 microns (usually 20 - 60 μm) in characteristic length [18] 	1) Can provide information on the pollen-resistance of the xylem filter	1) Smallest is 6 microns in size and most are much larger
		2) A lot of studies on its dispersal in air are available for	2) Hard to find in its pure form
		use as work is done to figure out methods to test filters using air	3) May not allow the filter to be used again due to potential allergy issues
			4) Large particle size distribution
Chalk powder	1) Size: ~2 - 100 microns [19]	1) Widely available	1) Large particle size distribution
		2) Alloldable	2) Would need to produce
Wood Smoke	1) Size: 0.1 - 0.2 microns [20]	 Widely available Affordable Can burn failed filters to produce 	1) Potentially harmful to the manufacturer if the smoke is not well- contained or the facility is not well-ventilated
			2) Air pollution in surrounding areas
			3) Particle size is ~10x smaller than target
Holi powder	1)~40% of particles are between	1) Widely available in regions of	1) Large size variation
	0.7 microns and 10 microns, but they manufactured with intent of being >10um	anterest	2) Made with the intention
		2) Can be used as a visual/color	[21]
		indicator	

Table 2.1: Particle options for use in air testing along with their pros and cons.

Though particles such as pollen and those in wood smoke were not the most suitable for the purpose of quality control, they can be used to provide information about the efficacy of the xylem filter as an air filter given that pollen is a common allergen and smoke is a common pollutant.

The testing particles that can only be used in water are those that are commonly found in aqueous solutions or environments. These are a combination of particles and microbes. They are bacterial cultures created from *E. coli* and buttermilk, and emulsions created using a mortar and pestle, acacia, gum powder, oil and water as seen in **Table 2.2**. The most promising substance to use as a substitute contaminant in water testing amongst the aforementioned particles and microbes is the bacterial culture created from buttermilk (*Lactobacillus*) due to the fact that it is an affordable and commonly available substance.

1) Can potentially be 2 – 4 microns [22]	1) Affordable	1) Since the mixture is
	 2) Made from commonly available materials 3) All items are edible: acacia gum powder, oil and water [22] 	crushed by hand, there will be particle size variation 2) Does not get down to the particle size target of 1
		3) Not commercially available in final form
an a		4) Susceptible to clumping
1) 2-6 microns in size [23]	1) Initial <i>E.coli</i> culture can be replicated – cutting down future	1) Incubation takes time and training
	costs on the bacteria	2) Not the safest option for manufacturers
		3) Requires equipment for incubation
		4) Larger than target size of 1 micron
1) Size of Lactobacillus bacteria	1) Commonly available	1) Minimum is twice the
ranges from $\sim 2 - 9 \ \mu m \ [24]$	2) Affordable	size of target particle size
	3) Naturally produced	2) Will require a method to tag/locate bacteria
		3) Orientation of bacteria matters since the diameter tends to be $<1 \mu m$ while the length varies from 2-9 μm [24]
	 2-6 microns in size [23] Size of <i>Lactobacillus</i> bacteria ranges from ~ 2 – 9 μm [24] 	 available materials 3) All items are edible: acacia gum powder, oil and water [22] 1) 2-6 microns in size [23] 1) Initial <i>E.coli</i> culture can be replicated – cutting down future costs on the bacteria 1) Size of <i>Lactobacillus</i> bacteria ranges from ~ 2 - 9 μm [24] 1) Commonly available 2) Affordable 3) Naturally produced

Table 2.2: Testing particle options with their relevant specifications, pros, and cons for water testing.

The final, and largest, category of particles are those capable of being used in either an air or waterbased testing system. These eight substances are displayed in **Table 2.3** along with their specifications, pros, and cons. They include 1 micron polystyrene microspheres, powdered milk protein, particulate dye pigment, Baker's yeast, turmeric, jeweler's rouge, Arizona test dust, and chloroplasts. Of the particles listed, the Baker's yeast, jeweler's rouge, turmeric, and Arizona test dust appear to be the ones with the most potential for use in both air and water testing of the xylem filter. All of the aforementioned particles are available for purchase. Arizona test dust is a substance typically used in a laboratory setting. Though its particle size distribution is provided, it is costly relative to the other particles.

Particles	Specifications	Pros	Cons
1 micron polystyrene microspheres	 1) 1 micron diameter 2) Made using polymers or ceramics 	 1) Tightly controlled particle size distribution 2) Fluorescent colors available 3) Previously used in lab to test xylem filter efficacy 	 Very expensive Inedible Requires special equipment to view
Powdered Milk Protein	1) The size is > 8 microns and can go into the hundreds of microns range [25]	 Safe to work with and consume Available for purchase Common 	 Large particle distribution Minimum size is 8 times the target test particle size
Particulate Dye Pigment	1) The dye is ~ 100 μm in size [26]	 Commonly available Affordable Safe to consume 	 Clogs filter and reduces the flow rate by ~5 times Dyes water, so cannot use as a visual indicator on its own Large particle size distribution
Baker's/Brewer's yeast and yeast spores	1) The size ranges between 2 and 10 μm [27]	 Readily available Safe to consume Affordable 	 1) The particles are larger than the target 2) Must be stored in a coo and dry location
Turmeric	1) Size: Between 20 and 200 microns Fat soluble, not water soluble - people warm up the water to make it easier to dissolve turmeric [28]	 Affordable Common in region of interest Curcumin particles on scale of nanometers Allows for visual indication Edible 	 Size Variation Particles larger than target size
Jeweler's Rouge	1) There is no set size for jeweler's rouge – it can range from a few hundred nanometers to several microns depending on its synthesis [29,30]	 Available for purchase Allows for a visual/color indication test 	 Can generate air-borne particulate matter Large particle size distribution

 Table 2.3: Testing particle options with their relevant specifications, pros, and cons.

Arizona Test Dust	1) Sizes of ~1 to 176 μm available [31]	 Available for purchase Known Distribution 	 1) Large particle size distribution 2) Relatively expensive
Chloroplast	 Size: Chloroplast: 3-10 microns [32] Chlorophyll a: ~1-2 nm [33] 	 Safe Allows for color indication testing 	 Not commonly available, therefore, will need an isolation kit Isolation kits tend to be hundreds of dollars Larger than our target

2.3 Testing Particle Suggestions

Of the 16 particles mentioned in **Table 2.1**, **2.2**, and **2.3**, two were deemed to be viable options for testing with only air (Holi and chalk powder), one was found to be a possible option in testing with only water (Buttermilk), and four were found to be viable options for testing in either air or water (Baker's yeast, jeweler's rouge, turmeric, and Arizona test dust). Along with particles that are useful for verification of xylem as a water filter, some of the particles discussed in Section **2.2** can be used to test the xylem filter's ability to act as an air filter. There is particular interest in observing the filter's rejection of pollen, smoke, and dust particles given that these are common allergens and respiratory irritants.

3. Testing the Xylem Filter using Water

3.1 Laboratory Testing Protocol using Water

The xylem filter's reduction capability was evaluated using water as the testing fluid of choice in the laboratory setting. A typical experiment for testing the filter consists of pressurizing a known mixture of dispersed microspheres and deionized water through a plastic tube containing a xylem filter (**Figure 3.1**). A hose clamp is used to prevent leakage around the filter. As the water and microsphere mixture passed through the filter, the filtrate was collected and analyzed to measure the particle reduction and, thus, the filter's rejection. For measuring the particle size distributions, dynamic light scattering measurements were performed using a Malvern Zetasizer Nano-ZS [6].



Figure 3.1: The setup for testing the xylem filter. [6,12]

3.2 Issues with Quality Control using Water

For mass production, the existing protocol would be ineffective for a multitude of reasons. The laboratory equipment currently used for deionizing water and measuring the particle reduction are relatively expensive to purchase, and tend to be difficult and costly to repair. Machinery such as the Malvern Zetasizer Nano-ZS also requires training to use and must be kept in a sterile environment to avoid contamination of specimens and incorrect readings. Even if the manufacturer were to utilize manual particle counting methods instead of purchasing expensive laboratory equipment, it would be a method too susceptible to counting error and too time consuming for it to be included in a practical quality control protocol in larger-scale manufacturing. Furthermore, the artificially produced microspheres are not cost effective as testing particles for quality control in mass production and are, therefore, not a sustainable option.

Aside from the expenses, effort, and time that go into the traditional method of testing the xylem filters using water, this method will prevent the manufacturer from selling the filters that are tested.

Using water to test the filter will wet the xylem and wet filters cannot be preserved, thus rendering them unsellable. This issue begets the following two effects:

1) All filters cannot be tested due to the fact that testing will be destructive. This means that a representative sample would have to be selected from each batch of filters and act as a means to evaluate all of the filters in said batch.

2) The filters that are tested cannot be sold, which reduces the manufacturers' potential profits. Though non-destructive testing models are gaining more popularity, they oftentimes require testing equipment that will be too expensive and inaccessible for the purpose of manufacturing these low-cost filters [34].

3.3 Filtration Testing using Turmeric and Food Dye

3.3.1 Why Turmeric

Turmeric is a spice from the turmeric plant that is commonly found in South Asian and Middle Eastern cooking. It can also be found in cosmetics and medicine [35]. This relatively cheap spice is ubiquitous and safe to consume. It is insoluble in water and contains particles from a few microns in characteristic length to a couple hundred microns, which is discussed in Section 2.2 [28]. The spice's affordability, accessibility, and distinct bright yellow hue makes it possible for both manufacturers and consumers alike to use turmeric as a visual indicator of whether a xylem filter is functioning as it should.

3.3.2 Experimental Setup

In order to test the propensity for manufacturers and clients to use turmeric and water to test the xylem filter, a processed xylem filter of a 1-cm diameter and a 0.25-inch thickness was placed into a plastic tube as shown in **Figure 3.1**. Turmeric was mixed into deionized water with blue food dye. This mixture was agitated by hand to disperse the turmeric and dissolve the dye. The turmeric and blue food dye mixture took on a green color as illustrated in **Figure 3.2**. For filtration testing, this green mixture was then pressurized through the xylem filter and the water and dye solution was collected from the outlet.



Figure 3.2: The setup for filtering an aqueous turmeric-blue dye mixture through the processed *Ginkgo* biloba xylem filter.

Before carrying out the experiment, two color combinations were created to identify the combination that is the most effective as a visual indicator. **Figure 3.4a** shows the blue filtrate on the left and the green feed (turmeric and blue dye) second to the left as well as the orange feed (turmeric and red dye) on the right with the red filtrate second to the right. The green feed and blue filtrate appeared to be a more distinct color indicator and was thus chosen as the color scheme for these experiments.

As a proof-of-concept, three mixtures were made for filtration. These mixture were: turmeric in deionized water, blue particulate food dye in deionized water, and turmeric and blue particulate food dye in deionized water (**Figure 3.3b**). These mixtures were each filtered through a processed xylem filter and the resulting filtrates are displayed in **Figure 3.3c**. The particulate blue food dye was later replaced with McCormick® liquid food dye due to the relatively slow flow rate the particulate food dye solution (4.58 mL/min at 10.76 psi) when compared to the flow rate of the liquid food dye solution was over 5 higher than that of the particulate food dye. Given the quantity of filters that will be tested during mass production, efficient testing, which requires a higher flow rate, is a necessity.



Figure 3.3: (a) Two dye colors (blue and red) compared as visual indicators of turmeric filtration. A more distinct color change was found in the filtrate of a blue dye and turmeric mixture, which transformed from a green feed to a blue filtrate (left). The red dye and turmeric appeared as an orange feed and transformed into a red filtrate (right), which was not as clearly different from the hue of the feed as the aforementioned combination. (b) The turmeric only, dye only, and combination of the two feed mixtures - all of which were mixed into deionized water. (c) The feeds found in (b) and their filtrates to the right of the respective feed. The mixtures with the blue food dye remained blue while the filtrate of the mixture with only turmeric became clear. (d) The xylem filter after the turmeric and particulate dye mixture passed through it.

3.3.3 Method

The turmeric feed mixture consisted of 11.5 μ g of turmeric and 15 mL of water. The dye feed solution consisted of 10 μ L of dye in 15 mL of water. The combination feed consisted of 11.7 μ g of turmeric and 10 μ L of McCormick® liquid blue food dye in 15 mL of deionized water. Each mixture was agitated by hand to disperse the turmeric and blue dye in the deionized water. The turmeric suspended in the blue food dye solution took on a green color as illustrated in **Figure 3.2** and seen in **Figure 3.3(b,c)**. Each of the aforementioned mixtures were filtered and the results are discussed in Section **3.3.4**.

3.3.4 Water Experiment Results

The filtrate from the feed that contained only turmeric suspended in deionized water did not retain the bright yellow hue of the feed. The filtrate looked clear to the naked eye as seen in **Figure 3.4a**, suggesting the turmeric was filtered out. The flow rate of the turmeric mixture was 2.44 mL/min

at 10.43 psi. The blue liquid dye solution passed through the filter and retained its color, suggesting the dye dissolved in the water and, thus, remained unfiltered as expected. The flow rate of this solution was 4.58 mL/min at 10.41 psi – about twice as large as the turmeric-only mixture's flow rate.

As suggested by the first experiments, the turmeric and dye mixture began with a green hue due to the mixture of the yellow turmeric and blue liquid dye and its filtrate only remained blue. This blue liquid appeared to the naked eye to be similar in color to that of the blue dye feed and filtrate. The flow rate of the filtration of the turmeric-dye mixture was 2.43 mL/min at 10.42 psi, which was similar to the filtration flow rate of the turmeric mixture of 2.44 mL/min at 10.43 psi. This similarity in flow rates demonstrated that the flow rate of a mixture only containing turmeric would indicate the flow rate of an aqueous mixture that contains liquid food dye and the same amount of turmeric.



Figure 3.4: (a) The feed mixtures and their resulting filtrates. From left to right, the tubes contain: turmeric and deionized water, the filtrate of this turmeric-only mixture, blue dye and deionized water, the filtrate of the dye-only filtrate, the turmeric in blue dye solution feed, and the filtrate of the turmeric and dye mixture. The xylem filtered the turmeric while allowing the water and the dissolved liquid blue dye to pass through. **(b)** The three xylem filters used to filter out the feeds above them.

In order to qualitatively assess how well the filters were functioning, the Agilent Technologies Cary 60 UV-Vis Probe was used to observe the absorbance of the aforementioned feeds and their respective filtrates. The device works by measuring the amount of light that gets absorbed by a liquid sample placed in a cuvette. For these tests, 100 μ L of the dye solution was placed into a cuvette for scanning. The probe was set to scan between wavelengths of 200 and 800 nm. Though the results of the UV-Vis probe are qualitative in nature, they are able to provide information on whether there are leaks or other major issues in a filter.

Deionized (DI) water (black line in **Figure 3.5**) was used as a reference of the absorbance spectrum of clear, filtered water. The turmeric filtrate follows a similar path to the deionized water with no distinct peaks in the data. The spectrum from the turmeric-only mixture shows a peak of 0.115 in the turmeric feed at a wavelength of 416.01 nm, which is not present in the filtrate. The filtrate spectrum more closely resembles that of the deionized (DI) water, suggesting effective turmeric filtration.



Figure 3.5: Spectrum of the turmeric-only mixture in deionized water. The feed is defined as the mixture before filtration and the filtrate represents the mixture after filtration.

The spectrum of the dye-only solution showed peaks in the same locations for both the feed and filtration (Figure 3.6). The peaks occur around wavelengths of 630 nm and 408 nm. The absorbance of the dye feed is about 0.880 at 630 nm and 0.166 at 408 nm. The absorbance of the dye filtrate is about 1.0395 at 630 nm and also 0.166 at 408 nm. Once the two spectra were normalized by their peak absorbances, the spectra revealed close alignment as seen in the spectrum on the right in Figure 3.6.



Figure 3.6: Spectrum of the blue dye solution feed and filtrate along with deionized water on the left and the normalized dye feed and filtrate spectra overlain on the right.

The spectra associated with the feed and filtrate of the combined mixture of dye and turmeric are shown in **Figure 3.7**. The peaks in the feed occur around wavelengths of 630 nm and 408 nm as they tend to in the dye solutions, but the influence of the turmeric can be seen in the upward slope of the absorbance that occurs between wavelengths of 400 and 500 nm. This slope is present in the turmeric feed shown in **Figure 3.5**, but is not present in the deionized water and blue dye solution spectrums. This gradual upward slope in the spectrum is not present in the dye-turmeric filtrate, which shows a local minimum around 442 nm before a steep upward slope to the peak at 408 nm. The filtrate shows a peak of about 1.353 at 628 nm and 0.225 at a wavelength of 408 nm. The peaks and changes in absorbance in this spectrum align with that of the dye-only solution feed and filtrate spectrums as seen in **Figure 3.8**, but the dye-turmeric feed has a higher absorbance at the location of the 408 nm peak.



Figure 3.7: Spectra of the blue dye and turmeric mixture feed and filtrate along with deionized water with the spectrum normalized by peak absorbance on the right.

The spectrum of the dye-turmeric mixture filtrate produces a spectrum that follows that of the dye feed as observed in **Figure 3.8**, indicating filtration of the turmeric particles.



Figure 3.8: Normalized spectra of the blue dye and turmeric mixture feed and filtrate along with deionized water. The spectra closely align, suggesting the complete filtration of turmeric particles.

In order to show the relationship amongst the spectrums containing the feed and filtrate of the turmeric-only aqueous mixture, dye-only solution, and the turmeric and dye mixture, all of them were plotted in **Figure 3.9**. The turmeric in the dye-turmeric feed shows an ascension as the turmeric ascends around 440nm while its filtrate, as well as the blue dye feed and filtrate approach local minima around 440 nm.



Figure 3.9: Combined spectrum of spectrums mentioned in Figures 3.5, 3.6 and 3.7.

The turmeric-only feed and dye solution feeds were linearly superimposed and plotted with the dye-turmeric feed spectrum. Figures 3.10 demonstrates the dye-turmeric feed consists of a linear

superposition of the dye feed and the turmeric feed due to the alignment of the respective spectrums.



Figure 3.10: Spectrum of the dye-turmeric feed and the linearly superimposed dye and turmeric feed spectra.

Figure 3.11 illustrates the peaks of the filtrates of the dye solution filtrate and the turmeric mixture filtrate spectrums match the structure of the dye-turmeric filtrate when linearly superimposed.



Figure 3.11: Spectrum of the dye-turmeric filtrate and the linearly superimposed dye and turmeric filtrate spectra.

During testing, we found that turmeric had a tendency to settle and aggregate at the bottom of the container while in water. A concern with using the UV-Vis Probe with turmeric is that it may not

get an accurate reading due to the alteration of the spectrum as turmeric settles with time after it is initially agitated.

In the field, a UV-Vis machine can be used to assess major failures or leaks in the filter or its enclosure. The experiments described in this section create clear visual indicators of filtration. The manufacturer or client can use the fact that their turmeric-dye mixture goes from green to blue to indicate the xylem is filtering as it should, but this process lacks quantitative analysis due to the inability for one to obtain a turmeric mixture calibration curve and any clear way to enumerate the particles rejected.

3.4 Testing Water-Dried Filters

Along with ensuring water contaminants are rejected, the quality control process is designed to guarantee a filter is manufactured correctly. Previous research suggests there is better rejection in xylem filters processed through the ethanol-drying method described in **Section 1.3** when compared to filters made using water [12]. Xylem filters produced using the latter method are referred to as "water-dried" filters. The water-dried filters have been found to have lower flow rates along with poorer rejection rates. A robust quality control system should be able to detect the difference between an ethanol and water-dried filter; therefore, the spectrum of dye-turmeric mixture filtrate from the ethanol-dried *Ginkgo biloba* filter was compared to water-dried white pine and *Ginkgo biloba* filters.

Once normalized, the spectrums of the water-dried samples were compared to that of the ethanoldried sample (**Figure 3.12**). Around the peaks related to blue dye, the water and ethanol-dried filtered showed similar spectra. The water-dried filters showed slightly higher absorbances between 350 and 450 nm. The peak found in the turmeric mixtures tends to be close to be between 416 and 440 nm; therefore, this higher absorbance suggests turmeric that does not pass through the ethanol-dried filter is present in small quantities in water-dried filter filtrate.



Figure 3.12: Spectrum of dye and turmeric mixture filtrate normalized at the peak around 630 nm. The water-dried filter show higher absorbances around wavelengths between 350 and 550 nm where the peak associated with an aqueous turmeric is located.

Given that the spectrum of the dye-turmeric aqueous mixture is merely the turmeric-only feed and dye solution feeds linearly superimposed, by subtracting the dye spectrum from these spectrums, one is left with only the portions of the spectrum that relate to turmeric. This method was used to produce the spectra found in **Figure 3.13**. The spectra were first normalized using their peak value found at a wavelength of about 630 nm, then the dye feed spectrum data was subtracted from the normalized spectra.



Figure 3.13: Normalized spectra of ethanol-dried and water-dried filters with 100% of the blue dye spectrum removed between wavelengths of 350 and 600 nm.

The filtrate that had an absorbance value at the wavelength of 416 nm was that of the waterdried *Ginkgo* filter, suggesting this water-dried filter did not successfully reject the turmeric particles. The water-dried pine filter is also shown to have small fractions of normalize absorbance once the dye spectrum is removed while the ethanol- preserved filter appears to reject the turmeric particles given that the filtrate of the filter when normalized consisted only of the spectrum related to the dye.

Along with identifying the reminants of turmeric, partially removing the dye spectrum from other spectra can model dilution of the dye. The initial concentration of the blue dye in deionized water was 10 μ L of McCormick blue dye in 15 mL of deionized water. **Figures 3.14**, **3.15**, and, **3.16** demonstrate the expected spectra if the original dye solution were to be diluted by 90%, 50%, and 10%. This dilution allows for more clear detection of turmeric in the 420-nm region. **Figure 3.14** more clearly demonstrated the filtrate of both of the water-dried filters potentially contain turmeric given their absorbances near the region in which the turmeric approaches its peak.



Figure 3.14: Spectrum of dye-turmeric filtrate normalized at the peak around 630 nm with 90% of the spectrum related to dye removed. Both the water-dried white pine and *Ginkgo* filters display poorer rejections than the ethanol-dried filter.

As less dye is removed, the spectra approach that observed in **Figure 3.12**. Half the dye is removed in **Figure 3.15** and 10% is removed in **Figure 3.16** resulting in maximum peaks that are half and 90% that found in a spectrum of no dye removal, respectively.



Figure 3.15: Spectrum of dye-turmeric filtrate normalized at the peak around 630 nm with 50% of the spectrum related to dye removed.

The spectra in which the dye is modeled to be diluted by only 10% follows a similar form of the spectra in which no dye is removed as seen in **Figure 3.16**.



Figure 3.16: Spectrum of dye-turmeric filtrate normalized at the peak around 630 nm with 10% of the spectrum related to dye removed. Spectrum appears to take the same form as if no dye were removed but the maximum absorbance is 90% of the original normalized spectrum.

As seen in this section's spectra, UV-Vis is capable of detecting trace amounts of turmeric in filters processed incorrectly, but the system does not provide quantitative results regarding the particle count. UV-Vis data also allows the user to model dilution of a liquid and the ways in which that dilution can impact spectra readings. In the case of the aforementioned spectra, the turmeric in the filtrate was most easily detected under fairly low blue dye concentrations, which is an aspect to keep in mind when determining the optimal concentrations of dye and turmeric to carry out these quality control tests.

3.5 Turmeric Size Distribution

Though turmeric is a commonly available and affordable spice, it may not be the best option for testing xylem filters in a manufacturing setting. From observing the particle size distribution, we found that most turmeric particles are larger than the target testing particle size of 1 micron and have a tendency to aggregate while in water. **Figure 3.17** displays two images of turmeric particles from the batch we used for testing. The images are taken on a light microscope at a magnification of 40 times.



Figure 3.17: (a,b) Turmeric particles under a light microscope at x40 magnification.

A sample of the turmeric used in the aforementioned water-based rejection experiments were manually measured under a microscope and the size distribution is found in **Figure 3.18a**. The longest side of the turmeric particle was used as the particle's characteristic length. The particle size distribution and sample size of this manual size analysis contains turmeric particles significantly smaller in characteristic diameter to those found in literature (**Figure 3.18b**) [28]. This size disparity may be due to different types of turmeric or different methods of turmeric production. This variation must be acknowledged as the particle for water testing is chosen.



Figure 3.18: (a) The particle size distribution of the turmeric used in the water testing experiments. n=45 **(b)** The weight distribution of turmeric particles by size from cryogenic and conventional grinding processes carried out by Manohar *et al* [28].

3.6 Concentration Optimization

As briefly discussed in Section **3.4**, the concentration of turmeric and blue dye is a crucial aspect of testing using water. Preliminary optimization experiments were carried out for the purposes of identifying which concentrations of turmeric and dye make this process the most intuitive visual indicator.

The turmeric and dye were measured before being placed in separate 15 mL of deionized water. The amounts of blue dye placed in 15 mL of deionized water were: 5 μ L, 10 μ L, 20 μ L, 40 μ L, and 50 μ L. The amounts of turmeric mixed into 15 mL of deionized water were: 5 mg, 10 mg, 20 mg, 40 mg, and 50 mg as pictured in **Figure 3.19**.



Figure 3.19: Varying turmeric and dye concentrations used for optimization testing. All indicated measurements were placed into 15 mL of deionized water.

3.6.1 Optimization Method

Equal amounts of the turmeric mixture and dye solution were placed into wells as shown in **Figure 3.20**. 500 μ L were added to wells with only dye or only turmeric, and 1000 μ L of fluid was added to the wells with both dye and turmeric. The optimal concentrations of turmeric and dye for visual indication were defined as the concentrations that had the greatest visual difference between the dye-only solution and the turmeric and dye mixture given that these represented the filtrate and feed, respectively.

For a given dye concentration, the colors of each well were analyzed and the red green blue (RGB) values of each pixel were averaged used to represent the color of the given concentration of dye to

account for aspects of the image that may alter the color of the well, such as a reflection or shadow. For a given dye-turmeric mixture, the pixel color values were averaged over the well.



Figure 3.20: The wells containing varying concentrations of dyes, turmeric, and a combination of both used in optimization analysis. The well plate in the top left corner contains dye solutions of 5 and 10 μ L of dye in 15 mL of deionized water. The top right plate contains dye solutions of 10 and 20 μ Lin 15 mL of water. The bottom left well plate contains dye solutions of 40 and 50 μ Lin 15 mL of water while the last well plate only contains a well with 50 μ L of water in 15 mL of water.

In order to identify the difference in colors between the feed and filtrate, the images in **Figure 3.20** were analyzed using the Image Processing tool in MATLAB. The red green blue (RGB) values of each pixel were gathered. A color's RGB value provides its red green and blue intensity. These RGB values were then averaged and displayed in **Table 3.1**. The MATLAB avg_RGB function code used for this analysis can be found in the Appendix (Chapter 8) of this thesis.

3.6.2 Optimization Results

The averaged RGB values and their corresponding colors are found in **Table 3.1**. The mean of the absolute value of the difference between the averaged RGB feed and filtrate values were then taken to act as a representative value of the color differences. Upon visual inspection, the color of solutions that contained 40 and 50 μ L of blue dye in 15 mL showed less of a visual difference

than those with lower concentrations of dye; therefore, only the solutions containing 5, 10, and 20 μ L of dye were analyzed in **Table 3.1**.

The largest mean differences correspond to the concentrations that provide the most distinct color differences, and thus have the potential to serve as the effective color indicators. The concentrations that begot the largest mean differences were 50 mg and 10 μ L, 40 mg and 5 μ L, and 50 mg and 5 μ L in 30 mL of water with mean differences of 61.95, 54.96, and 47.83, respectively.

Table 3.1: Table showing the various dye filtrate presentations and turmeric-dye mixture feeds from given quantities of turmeric and blue dye in 15 mL of deionized water. The Mean Difference is found under the corresponding cell pairs. RGB colors analyzed and averaged using the image processing tool in MATLAB and MATLAB function found in the Appendix of this thesis. RGB colors were converted into their respective colors in the table using w3schools.com/colors/colors_converter.asp.

		Blue Dye Concentrations (in 15 mL of deionized water)		
		5 μL	10 μL	20 μL
water)	5 mg	$\begin{array}{c c} R = 112 & R = 86 \\ G = 194 & G = 180 \\ B = 207 & B = 186 \end{array}$	R = 7352.7250G =160158.3125B = 178166.4750	$\begin{array}{ccc} R = 25 & 21.3364 \\ G = 116 & 113.3455 \\ B = 155 & 135.1727 \end{array}$
		Mean Difference: 20.45	Mean Difference: 11.55	Mean Difference: 8.75
ionized	10 mg	$\begin{array}{c c} R = 112 & R = 89 \\ G = 194 & G = 174 \\ B = 207 & B = 162 \end{array}$	R = 7352.8764G =160151.0787B = 178154.4270	$\begin{array}{ccc} R = 25 & 21.0818 \\ G = 116 & 100.8182 \\ B = 155 & 126.3000 \end{array}$
. of de		Mean Difference: 29.58	Mean Difference: 17.93	Mean Difference: 15.97
ons (in 15 mL	20 mg	R = 11289.7200G = 194168.6560B = 207143.6480	R = 7394.0000G =160180.2544B = 178164.9561	$\begin{array}{ccc} R = 25 & 31.7327 \\ G = 116 & 103.8218 \\ B = 155 & 123.9208 \end{array}$
		Mean Difference: 37.39	Mean Difference: 17.96	Mean Difference: 16.83
centrati	40 mg	R = 112100.5891 $G = 194$ 151.9302 $B = 207$ 96.7907	R = 7363.2273G = 160133.7386B = 17879.2727	R = 2540.9775 $G = 116$ 96.3933 $B = 155$ 84.2697
c Con		Mean Difference: 54.96	Mean Difference: 45.31	Mean Difference: 35.60
urmeri	50 mg	R = 112108.3049 $G = 194$ 163.4756 $B = 207$ 98.9146	R = 7359.1010G =160115.3939B = 17851.8384	R = 2542.6000 $G = 116$ 94.7333 $B = 155$ 74.6444
F		Mean Difference: 47.83	Mean Difference: 61.95	Mean Difference: 39.90

Based on color indication alone, it appears that the 50 mg and 10 μ L pairing in 30 mL will produce the optimal color pairing. It is important to note that this analysis process lends itself to potential mean difference variations based on the fact that the averaged color intensities include image shadows and reflections; therefore, for future tests, multiple images of a well should be used for analysis.

Another useful metric when considering the optimal concentrations for water testing using turmeric is the flow rate. Lower flow rates can slow down testing, which can be cumbersome - particularly in the manufacturing process. Given that the flow rate tends to depend heavily on the concentration of particulate matter in the aqueous mixture as discussed in Section 3.3.4, the turmeric concentration was compared to the flow rate in **Table 3.2**.

Table 3.2: Table showing turmeric concentrations used in optimization testing and their respective flow
rates at a pressure of 10.10 psi. The quantities in round brackets represent the amount of turmeric added to
15 mL of deionized water.

Turmeric Concentration [mg/mL]	Flow Rate [mL/sec]
0.33 (5 mg)	0.067
0.67 (10 mg)	0.047
1.33 (20 mg)	0.022
2.67 (40 mg)	0.016
3.33 (50 mg)	0.015

Though high concentrations of turmeric lead to more distinct color indications of filtration, the higher turmeric concentrations also result in lower flow rates. Between 0.33 and 1.33 mg/mL, there is a decrease in flow rate of about 67%, yet the increase in mean difference of color from 5 to 20 mg of turmeric in 15 mL of water increases by 82.8% in 5 μ L of blue dye and 55.5% in 10 μ L of blue dye. This relationship between the increase in flow rate, the change in mean color difference and their dependence on dye concentration must be taken into account as manufacturers seek to optimize the testing parameters for their needs.

3.7 Next Steps for Water Testing

In order to definitively determine the process for testing xylem filters with water during mass production, the optimal testing particle must be determined. This optimal particle should be cheap, safe to use, and easily acquired in the manufacturing region. Particle options are described in Chapter 2. Once this particle is selected, optimization methods to determine the concentrations for the manufacturer's applications such as those found in Section **3.6** must be carried out. Along with the optimal particle, the equipment used must be able to withstand use in the climate in which the filter is manufactured. Finally, a method and the corresponding equipment to quantitatively measure the particles remaining in the filtrate must be identified because UV-Vis cannot provide the quantitative results a manufacturer may desire.

4. Testing the Xylem Filter using Air

4.1 Benefits of Testing with Air

Using air to test the xylem filter allows the filter to still be sold after testing; therefore, more filters, or all of the filters, can be tested before they are sold. Use of Air can avoid the destructive testing that the use of water engenders. Another benefit of testing using air is that a particulate matter (PM) sensor can be obtained by local manufacturers at affordable prices and this sensor provides quantitative means to measure the xylem filter's rejection. Because particles can be directly seeded into air, the overall air testing process may also be faster than water testing.

4.2 Testing Xylem for Air Filtration

4.2.1 Experimental Setup

The testing setup was similar to that shown in **Figure 3.1**, but instead of water as the fluid medium and turmeric as the testing particle, nitrogen gas was the fluid medium in which Arizona test dust particles were dispersed. The flow rate was measured using a Sensirion® flow meter, and the Nova® SDS011 PM sensor was used to detect dust particles passing through the xylem filter. The distribution of the Arizona test dust particles used in this experiment is displayed in **Table 4.1**.

ISO 12103-1 ARIZONA TEST DUST CONTAMINANTS A2 FINE GRADES				
Iso Test Dust Particle Siz	Iso Test Dust Particle Size Distributions by Volume %			
Size Micrometer	ISO 12103-1, A2 Fine Test Dust % Less Than			
0.97	4.5 – 5.5			
1.38	8.0 - 9.5			
2.75	21.3 - 23.3			
5.50	39.5 - 42.5			
11.00	57.0 – 59.5			
22.00	73.5 - 76.0			
44.00	89.5 – 91.5			
88.00	97.9 – 98.9			
124.50	99.0 – 100.0			
176.00	100.0			

Table 4.1: The size distribution of fine grade Arizona Test Dust [31].

4.2.2 Preliminary Air Experiment

As a proof-of-concept, a xylem filter of 1-cm diameter and 0.635-cm thickness was tested and found to have a flow rate of 3.6 ± 0.1 slm at 8 psi, 0.29 ± 0.01 slm at 0.93 psi, and 0.28 ± 0.02 slm at 0.9 psi. The Reynold's number was found to be 3898 at 8 psi. In order to avoid compressibility affects, experiments after the preliminary air experiments were carried out in the laminar regions [36]. The Reynold's number (R_e) was calculated using equations 4.1, 4.2, and 4.3 in which ρ is the density of the fluid, V is the velocity of the fluid, μ is the viscosity of the fluid, d is the diameter of the tracheid, A is the area of the tracheid, Q is the volumetric flow rate, and ϑ is the kinematic viscosity.

Eq. 4. 1
$$R_e = \frac{\rho V d}{\mu}$$

Eq. 4. 2 $V = \frac{Q}{A}$
Eq. 4. 3 \therefore $R_e = \frac{\rho Q d}{\mu A} = \frac{Q d}{A\vartheta}$

The nitrogen gas was seeded with Arizona test dust particles at time t = 0 and no particulate matter was detected by the sensor. Around 75 seconds from the initiation of testing, the filter was removed and dust was allowed to flow to the PM sensor to validate the sensor was functioning properly. Around 125 seconds, more dust was dispersed using a compressed air duster. After allowing the dust to settle for 100 seconds, the tube with the filter was connected to the PM sensor and dust was dispersed into the inlet around a time of 225 and no particulate matter was detected by the PM sensor.



Figure 4.1: PM sensor reading from preliminary air testing in which Arizona test dust was dispersed with a xylem filter at the inlet at time 0 and 225 and dust was dispersed without the xylem filter around time 75 and 125. When the dust is dispersed without the filter, peaks in particulate matter concentration are observed while no such peaks are observed when the dust is dispersed through the xylem filter indicating air filtration.

The results from the preliminary experiments suggest the xylem filter has the potential to filter fine Arizona test dust particles. A schematic of this potential air filtration seen in the preliminary findings is shown in **Figure 4.2**. The pressurized nitrogen continues to flow through the filter while the filter excludes the dust particles.



Figure 4.2: Arizona test dust particles are captured by the xylem filter and only the filtered air passes through the xylem.

4.2.3 Method

The nitrogen gas was set to a pressure of ~ 0.21 psi when first entering the filter. The flow rate measured without the filter was 0.697 cm³/sec and the flow rate out of the filter was 0.674 cm³/sec at this pressure. In order to further identify whether xylem could be tested using air as the fluid of choice, a xylem filter of a 1 cm diameter and 0.635 cm thickness was placed in a tube, and fastened using a hose clamp as shown in **Figure 3.1**. Given that xylem tracheids are about 5 mm in length, the filter was cut to ensure the length would not allow proper filtration [10]. By using xylem too short to act as a filter, we ensured that if particulate matter were to flow through the filter, air could be considered a suitable medium for testing xylem filters because the particles would not have provided a false result of filtration. On the other hand, if the particles seeded in the air were attracted to the walls of the xylem's tracheids due to electrostatic forces, air would not be considered a viable fluid for use in the quality control process. In the case of the latter scenario,

though air could not be used for testing, it evokes the possibility that the xylem filter may be able to act as an air filter as shown in **Figure 4.2**.

As pressurized nitrogen gas flowed through the xylem filter, the flow rate before the filter was inserted at pressure of 0.21 psi was 0.697 cm³/sec begetting a Reynold's number of 734.3. The volumetric flow rate had to be kept below 2 cm³/sec to avoid exiting the laminar flow regime. 0.1 grams of fine Arizona test dust was dispersed in the nitrogen gas and the particulate matter concentration was measured using the Nova® SDS011 PM sensor.

4.2.4 Air Experimental Results

We began by observing the PM sensor reading when 0.1 grams of Arizona test dust were dispersed in the system with no filter about 30 seconds from the initiation of the experiment (**Figure 4.3**). The lag time of reading was estimated to be about 0.16 seconds given the fact that the inlet of the tube is about 18 cm from the PM sensor inlet and the system was estimated to have a particle velocity of about 110.9 cm/s from the volumetric flow rate of 0.697 cm³/s.



Figure 4.3: Reading of the SDS011 PM Sensor when Arizona test dust particles are introduced at a time of 30 seconds and reached a maximum PM10 particulate matter concentration reading of 2300 μ g/m³ and the maximum PM2.5 reading of 621.6 μ g/m³.

The xylem filter was then placed between the tube inlet and the PM sensor as shown in **Figure 4.2**. The resulting PM sensor readings are displayed in **Figure 4.4**. 0.1 grams of Arizona test dust were introduced when the reading first started (time = 0) under a pressure of 0.21 psi. Under these conditions, the xylem appeared to act as an air filter as observed in the preliminary experiment described in Section **4.2.2**. After 100 seconds the dust in the system, most of which rested on top of the filter, were re-agitated using a compressed air duster. The duster provided a maximum



pressure of 6 psi. Once the compressed air duster was introduced, particles were detected by the PM sensor as seen in **Figure 4.4**.

Figure 4.4: Reading of the SDS011 PM Sensor when Arizona test dust particles are introduced at a time of 0 seconds the pressure was increased by ~5.5 psi 100 seconds into the reading. The PM10 then reached a maximum particulate matter concentration reading of 628.5 μ g/m³ and the maximum PM2.5 reading was 147.2 μ g/m³ at this time.

Due to the fact that it would take the dust particles ~0.16 seconds to travel the 18 centimeter distance from the particle inlet to the inlet of the PM sensor, it does not appear that the peak in the reading is due to a time delay, but rather to the increase in pressure that was applied at a time of about 100 seconds. There was a drastic decrease in the concentration of particles that passed through the filter when the reading in **Figure 4.4** is compared to that found in **Figure 4.3**. The PM10 reached a maximum particulate matter concentration reading of 628.5 μ g/m³ and the maximum PM2.5 reading was 147.2 μ g/m³ in **Figure 4.4**, while **Figure 4.3** showed a maximum PM10 particulate matter concentration reading of 2300 μ g/m³ and the maximum PM2.5 reading dropped by about a fourth for both PM2.5 and PM10. This drop may have been even more drastic for PM10 readings, due to the fact that the maximum concentration reading of the PM sensor was 2300 μ g/m³.

Though the xylem filter initially displayed potential to act as an air filter, at higher pressures, the filter allows particles to pass through. This phenomenon at higher pressures could be due to a multiple factors. The electrostatic forces between the particles and the xylem tracheid walls may not be strong enough to withstand high pressure gradients, there may be leakage around the sides of the filter that allows the dust to flow around the filter, or the disturbance of settled dust particles

may create a better arrangement for the dust to pass through the filter. More exploration into this subject matter is required to verify these results.

4.3 Next steps for Air Testing

At higher pressures, air is shown to act as a viable medium for testing xylem filters in a manufacturing setting. More experimentation is required to verify this claim, but from initial experimentation, it appears that the xylem filter can be tested using both air and water. If air is to be used, work will need to be done in order to identify the calibrations necessary to translate results from air tests to their implications regarding the xylem's function as a water filter.

The results also suggest coniferous xylem has the propensity to act as an air filter in low pressure situations. In the future, the xylem filter should be tested using pollen and smoke particles to detect whether it can act as a filter for these common air particles.

5. The Quality Control Process

5.1 Goals for Xylem Filter Quality Control

This chapter explores possible testing apparatuses for use in quality control processes that either utilize water or air. Testing equipment should be affordable to acquire and, if applicable, build. The equipment should, preferably, allow manual actuation and require little to no electrical power to operate. The testing setup should allow the manufacturer to carry out quality control tests that are repeatable, simple, and, if possible, quantitative. The quality control protocol must also be susceptible to scaling. Finally, the quality control equipment must be safe and functional in the subtropical climate where it will be used. Previous case studies have been carried out in Uttarakhand in northern India, but given the presence of coniferous trees worldwide, the manufacturing process should be implementable in several regions.

5.2 Quality Control using Water

If the filter were to be tested using water, a setup would consist of these parts: a large syringeinspired pump, a leak-proof xylem filter connector and holder, and a container used to collect the filtrate, as portrayed in **Figure 5.1**.



Figure 5.1: CAD rendering of a xylem filter testing setup for quality control using water. The system includes a syringe used for introducing water with particulate "contaminants" into the xylem filter, a syringe holder, two filter connectors, and a container to collect the filtrate.

The particle that will act as the water contaminant used to verify the filter's rejection as well as the equipment to detect said particle will need to be selected. As described in Chapter 2, the optimal particles for testing are near 1 micron in size, have small particle size distributions, and are affordable and easily acquired in bulk. A visual indication system, such as the test described in Section **3.3**, can easily be used to test for major failures in the xylem filter. A device to identify filter leaks qualitatively, such as a UV-Vis probe, will suffice for this method. Though this method can be fast and easily interpreted, the process lacks quantitative verification. The addition of a particle-counting device will make this process more robust.

5.3 Quality Control using Air

If air is considered to be a viable medium for testing the xylem filter, an inhaler-inspired design can be implemented as a testing apparatus. The system will deliver air to a filter placed a distance of about 10 cm away to counteract some of the aggregation effects the particles experience straight out of the nozzle as seen in **Figure 5.2**. This inhaler design is meant to deliver a pre-determined concentration of particles seeded in the air. The particle-delivery portion of the system will take the form of the inhaler in **Figures 5.3** and **5.4**. All components of the apparatus that interact with the air introduced to the filter will have to be air-tight to avoid particle leakage.



Figure 5.2: "Diagram of a typical pressurized metered dose inhaler showing mechanism of particle formation. MMAD = Mass Median Aerodynamic Diameter." [37]

This testing apparatus design is comprised of a canister containing pressurized air and test particles, an inhaler-holding device that interfaces between the canister and the xylem filter, a

particulate matter (PM) sensor, and a platform that allows the manufacturer to slide components of the rig in order to test filters of various lengths (**Figure 5.3** and **Figure 5.4**). The aforementioned apparatus for air testing can also be used to test xylem as an air filter, if it is deemed to be appropriate for that application.



Figure 5.3: CAD renderings of inhaler-inspired air testing apparatus.



Figure 5.4: Labeled exploded view of CAD rendering of inhaler-inspired air testing apparatus.

Portions of the deign are available for purchase. Refillable inhaler canisters can be purchased for use in this system as can the PM sensor. The slotted base can be made using a mill or the base design can be altered so that 80/20 T-slotted aluminum beams can be used, instead. The canister holder can be machined, injection molded, or purchased in standard inhaler sizes. The filter-sensor connector can be machined or injection molded.

6. Conclusion

This thesis is meant to set the foundation as researchers attempt to create the manufacturing protocol for the xylem filter. The work done in this thesis can be used to inform manufacturers and consumers, alike, on potential methods to test their membrane filters. The manufacturing process, that will first be implemented in India, will require a quality control process that can be implemented locally. In order to develop this process, cost, simplicity, and the potential for scaling were taken into account. Water and air were explored as the potential fluids with which to test the xylem filters.

From initial experimentation, both water and air can be chosen as the fluid of choice for filter testing, yet more experiments should be carried out using air to further explore its ability to work as a testing fluid. In order to improve and implement the work discussed in this thesis, the first step will be to select the fluid to be used for quality control. Though water is commonly used in laboratory evaluations of filters, water testing destroys the evaluated xylem filter. Testing using air would allow each filter to be tested before sale, but requires further exploration and calibration data.

Air testing allows the filter to be sold after testing; therefore, more filters, or all of the filters, can be tested before they are sold. Testing using air seems to be available at pressures of about 6 psi and higher. If air is found to, in fact, be a viable option for xylem filter testing, calibration testing to identify the relationship between water and air flow rates, filter permeability, and rejection will be necessary. These calibrations will be used to translate the results from air testing to the xylem's function as a water filter.

Because air testing would allow a filter to be tested before sale, the particles used in air testing must be safe for the user to consume, otherwise, the testing would be considered destructive. In order to ultimately make the decision on the particle to be used for quality control testing, the process of obtaining, using, and analyzing the particles must be taken into account. This process must be simple to understand and repeatable. If the particles are affordable in bulk, safe to use, and within the size range of common water contaminants, then they have the potential to be effective in xylem filter testing.

For quality control using water and air, the particles suggested in Section **4.3** should all be tested to identify which is the most effective in terms of the criteria lain out. Once the test particle is determined, the measurement method for determining the particles remaining in the filtrate must also be identified. This step will allow for more quantitative results to definitively state a xylem filter is reaching the 4-log reduction criterion [13]. The optimization tests mentioned in Section **3.6** can provide a color-based system for determining turmeric concentration ranges, but this system would not be able to employ the accuracy needed for detailing the filters' particle rejection, particularly when it comes to reaching 4-log reduction.

Finally, the manufacturing process should take sustainability into account. Lean manufacturing methodologies should be applied throughout the whole process, and particularly in quality control in which there lies potential for a large amount of the items to become waste. Another concern in this manufacturing process is local deforestation due to xylem filter creation. More research is

needed to determine whether all portions of the tree can be used. Currently, the branches and the trunk are usually used to create filters, but an important question to keep in mind is how we can utilize more, or even all, of the tree. With all of these issues in mind, the manufacturing and quality control process can become efficient, affordable, and sustainable.

7. References

- [1] "WHO | 2.1 Billion People Lack Safe Drinking Water at Home, More than Twice as Many Lack Safe Sanitation," WHO [Online]. Available: http://www.who.int/mediacentre/news/releases/2017/water-sanitation-hygiene/en/. [Accessed: 12-Apr-2018].
- [2] Peter-Varbanets M, Zurbru "gg C, Swartz C, Wouter Pronk W, 2009, "Decentralized systems for potable water and the potential of membrane technology," Water Research, 43, pp.245– 265.
- [3] "Drinking-Water," World Health Organ. [Online]. Available: http://www.who.int/news-room/fact-sheets/detail/drinking-water. [Accessed: 25-Apr-2018].
- [4] Ortiz, I., Cummins, M., 2011 "Global Inequality: Beyond the Bottom Billion A Rapid Review of Income Distribution in 141 Countries"
- [5] Ravallion, M., Chen, S., and Sangraula, P., 2008, *Dollar a Day Revisited*, WPS4620, The World Bank.
- [6] Boutilier, M. S. H., Lee, J., Chambers, V., Venkatesh, V., and Karnik, R., 2014, "Water Filtration Using Plant Xylem," PLOS ONE, 9(2), p. e89934.
- [7] Sperry, J. S., 2003, "Evolution of Water Transport and Xylem Structure," Int. J. Plant Sci., 164(S3), pp. S115–S127.
- [8] "Temperate Coniferous Forests Ecology Oxford Bibliographies Obo" [Online]. Available: http://www.oxfordbibliographies.com/view/document/obo-9780199830060/obo-9780199830060-0162.xml. [Accessed: 26-Apr-2018].
- [9] Rogan, W. J., Brady, M. T., 2009, "Drinking Water From Private Wells and Risks to Children," Pediatrics, 123(6), p. e1123 e1137.
- [10] Hacke, U. G., ed., 2015, "The Hydraulic Architecture of Conifers," *Functional and Ecological Xylem Anatomy*, Springer International Publishing, Switzerland, pp. 39–75.
- [11] Schoonmaker, A. L., Hacke, U. G., Landhäusser, S. M., Lieffers, V. J., and Tyree, M. T., 2010, "Hydraulic Acclimation to Shading in Boreal Conifers of Varying Shade Tolerance," Plant Cell Environ., 33(3), pp. 382–393.
- [12] Ramchander, K., 2016, "Development of Xylem-Based Water Filters," Thesis, Massachusetts Institute of Technology.
- [13] US EPA, O., 2015, "Ground Water Rule," US EPA [Online]. Available: https://www.epa.gov/dwreginfo/ground-water-rule. [Accessed: 01-May-2018].
- [14] Myburg, A. A., and Sederoff, R. R., 2001, "Xylem Structure and Function," Encycl. Life Sci., 20, pp. 1–9.
- [15] Little, S. A., Jacobs, B., McKechnie, S. J., Cooper, R. L., Christianson, M. L., and Jernstedt, J. A., 2013, "Branch Architecture in Ginkgo Biloba: Wood Anatomy and Long Shoot-short Shoot Interactions," Am. J. Bot., **100**(10), pp. 1923–1935.
- [16] US EPA, O., 2016, "Health and Environmental Effects of Particulate Matter (PM)," US EPA [Online]. Available: https://www.epa.gov/pm-pollution/health-and-environmentaleffects-particulate-matter-pm. [Accessed: 20-May-2018].
- [17] Yang, C.-R., Lin, T.-C., and Chang, F.-H., 2007, "Particle Size Distribution and PAH Concentrations of Incense Smoke in a Combustion Chamber," Environ. Pollut., 145(2), pp. 606–615.
- [18] Niklas, K. J., 1985, "The Aerodynamics of Wind Pollination," Bot. Rev., 51(3), p. 328.

- [19] Majumdar, D., and William, S. P. M. P., 2009, "Chalk Dustfall during Classroom Teaching: Particle Size Distribution and Morphological Characteristics," Environ. Monit. Assess., 148(1–4), pp. 343–351.
- [20] Kleeman, M. J., Schauer, J. J., and Cass, G. R., 1999, "Size and Composition Distribution of Fine Particulate Matter Emitted from Wood Burning, Meat Charbroiling, and Cigarettes," Environ. Sci. Technol., 33(20), pp. 3516–3523.
- [21] Bossmann, K., Bach, S., Höflich, C., Valtanen, K., Heinze, R., Neumann, A., Straff, W., and Süring, K., 2016, "Holi Colours Contain PM10 and Can Induce Pro-Inflammatory Responses," J. Occup. Med. Toxicol. Lond. Engl., 11(1).
- [22] Briggs, T. R., DuCassé, F. R., and Clark, L. H., 1919, "Experiments on Emulsions. IV," J. Phys. Chem., 24(2), pp. 147–160.
- [23] Cullum, J., and Vicente, M., 1978, "Cell Growth and Length Distribution in Escherichia Coli.," J. Bacteriol., 134(1), pp. 330–337.
- [24] Kokkinosa, A., Fasseas, C., Eliopoulos, E., and Kalantzopoulos, G., 1998, "Cell Size of Various Lactic Acid Bacteria as Determined by Scanning Electron Microscope and Image Analysis," Le Lait, 78(5), pp. 491–500.
- [25] J.c, B., S, C., and B.p, L., 2017, "Particle Size of Milk Protein Concentrate Powder Affects the Texture of High-Protein Nutrition Bars During Storage," J. Food Sci., 82(4), pp. 913– 921.
- [26] Piccin, J. S., Dotto, G. L., Vieira, M. L. G., and Pinto, L. A. A., 2011, "Kinetics and Mechanism of the Food Dye FD&C Red 40 Adsorption onto Chitosan," J. Chem. Eng. Data, 56(10), pp. 3759–3765.
- [27] Kauffman, G. B., 1999, "McGraw-Hill Encyclopedia of Science and Technology, 8th Edition (Ed. Parker, Sybil P.)," J. Chem. Educ., 76(3), p. 324.
- [28] Manohar, B., and Sridhar, B. S., 2001, "Size and Shape Characterization of Conventionally and Cryogenically Ground Turmeric (Curcuma Domestica) Particles," Powder Technol., 120(3), pp. 292–297.
- [29] Kang, Y. S., Risbud, S., Rabolt, J. F., and Stroeve, P., 1996, "Synthesis and Characterization of Nanometer-Size Fe3O4 and γ-Fe2O3 Particles," Chem. Mater., **8**(9), pp. 2209–2211.
- [30] Andersen, H. L., Jensen, K. M. Ø., Tyrsted, C., Bøjesen, E. D., and Christensen, M., 2014, "Size and Size Distribution Control of γ-Fe2O3 Nanocrystallites: An in Situ Study," Cryst. Growth Des., 14(3), pp. 1307–1313.
- [31] "ISO 12103-1, A2 Fine Test Dust," Powder Technol. Inc [Online]. Available: http://www.powdertechnologyinc.com/product/iso-12103-1-a2-fine-test-dust/. [Accessed: 04-May-2018].
- [32] Flindt, R., 2006, Amazing Numbers in Biology, Springer-Verlag, Berlin Heidelberg.
- [33] Strebeyko, P., 2000, "Size and Power of Chlorophyll α Molecule," Photosynthetica, 38(1), pp. 159–160.
- [34] Cawley, P., 2001, "Non-Destructive Testing—current Capabilities and Future Directions," Proc. Inst. Mech. Eng. Part J. Mater. Des. Appl., 215(4), pp. 213–223.
- [35] Prasad, S., and Aggarwal, B. B., 2011, "Turmeric, the Golden Spice: From Traditional Medicine to Modern Medicine," *Herbal Medicine: Biomolecular and Clinical Aspects*, I.F.F. Benzie, and S. Wachtel-Galor, eds., CRC Press/Taylor & Francis, Boca Raton (FL).
- [36] Modesti, D., and Pirozzoli, S., 2016, "Reynolds and Mach Number Effects in Compressible Turbulent Channel Flow," Int. J. Heat Fluid Flow, **59**, pp. 33–49.

[37] Terzano, C., 1999, "Metered Dose Inhalers and Spacer Devices," Eur. Rev. Med. Pharmacol. Sci., 3(4), pp. 159–169.

8. Appendix

```
function [avg_RGB] = areapixelavg(image, region)
    % function returns the averaged RGB color values of a specified region in
a specified image
    % input image file as a string
    % input region of analysis as [xmin ymin width height] -> from imtool
    % output is a vector that is the averaged RGB color values of a specified
region in a specified image
    width = region(1,3);
    height = region(1,4);
   xmin = region(1,1);
   ymin = region(1,2);
   col = zeros(1,round(width));
   row = zeros(1, round (height));
   I = imread(image);
   col(1,1) = round(xmin);
   row(1,1) = round(ymin);
    for c = 2: round(width)
        col(1,c) = round(col(1,1)+ (c/width)*round(width));
    end
    for r = 2:round(height)
       row(1,r) = round(row(1,1) + (r/height)*round(height));
    end
    if width <= height
       total_pixels = impixel(I,col(1, 1:round(width)),row(1,
l:round(width)));
   else
       total_pixels = impixel(I,col(1,1:round(height)),row(1,
1:round(height)));
   end
   avg RGB = mean(total pixels);
```

end