NOVEL TECHNIQUES FOR WATER TREATMENT

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Abstract

A rapid decline in the quality of drinking water sources across the world has emerged as a considerable threat to human life. Maintaining a continuous supply of clean drinking water is a challenging task in rural and marginalized communities, where conventional methods of monitoring and treatment cannot be implemented due to a lack of appropriate infrastructure and skilled personnel. Hence, the main focus of the present thesis is to develop better water quality monitoring and purification techniques targeted towards portable point-of-use systems. The approach towards better water quality monitoring involves the development of a rapid and portable concentrating device which can be used in conjunction with microfluidics based biosensor platforms to detect pathogens in contaminated water. The solution to the problem of water treatment involves the assessment of a naturally available antimicrobial agent, the *Moringa oleifera* seed extract, followed by its integration into a novel and truly sustainable device capable of removing bacterial loads from contaminated water.

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Nomenclature and Abbreviations

N	Normalized bacteria count
τ	Normalized time
a	Coefficient of the decay equation
b	Exponent of the decay equation
TGS	Top Glucose Strip
BGS	Bottom Glucose Strip
MS	*Moringa* Strip
NS	Normal paper Strip

Chapter 1

Introduction

1.1 Background and Motivation

Water is one of the most fundamental resource required for the preservation of planetary bio-diversity. The availability of clean water is essential for human survival and directly impacts the quality of human life across the planet. Nearly 97.5% of water on earth is constituted by salty ocean waters which cannot be put to use without expensive and energy intensive desalination techniques. Glaciers and ice caps are huge repositories of fresh water comprising almost 2.15% of all the water on earth but are not easily accessible for immediate application. Only a small amount of water (0.65%) in the form of rivers, lakes and underground aquifers is available to us for direct utilization[1]. The global demand for freshwater has increased at an alarming rate, primarily due to the exponential rise in population, which has led to aggressive agricultural and industrial expansions. Freshwater withdrawals have increased globally at 1% per year since 1980 to cater to the growing needs of the society. The irrigated agriculture sector accounts for most of the freshwater withdrawal (70%) and use followed by the energy (15%)and industrial manufacturing (4%) sectors[2]. Such over-exploitation combined with unabated deforestation has led to the disruption

of carefully balanced ecosystems, whereby natural means of replenishment of freshwater sources have been hampered. Almost 38% of irrigated areas globally, still depend on groundwater[2]. Aggravated by poor irrigation techniques, this has accelerated the decline of water quality in most communities. In addition, due to a lack of strict policies, untreated and unregulated agricultural and industrial runoffs are deteriorating the conditions of existing surface and groundwater sources through further contamination. The entire scenario has been exacerbated by climate change, which has disrupted weather patterns across the world causing irregularities in the hydrological cycle and threatening the collapse of major ecosystems[3].

The uneven distribution of freshwater resources across the world has had the most impact in the developing countries of Africa, Asia and the Middle-East, where inadequate access to clean and safe drinking water has turned into the most potent threat to the quality of life. Nearly two thirds of the global population inhabit areas associated with economic and physical water scarcity. Recent reports indicate that an estimated 750 million people lack access to improved drinking water sources while nearly 1.8 billion others use a source of water which is fecally contaminated[4]. 82% of those who lack access to improved drinking water sources reside in rural areas or in minimal resource settings. Ingestion of such contaminated water has led to the prevalence of waterborne diseases like diarrhea and cholera causing approximately 2 million deaths annually with a majority of them being children under the age of five[5]. Naturally, proactive efforts are necessary to mitigate the challenges of contaminated drinking water sources in limited resource settings through water treatment and water quality monitoring techniques. The challenges are further elevated due to the dearth of appropriate infrastructure and connectivity in such communities to carry out traditional purification and monitoring processes. Hence, an active branch of research has delved into the development of effective and sustain-

able point-of-use devices to improve the access to clean and safe drinking water and consequently improve the quality of life in these marginalized communities. This thesis delves on the broader scientific approach to improve point-of-use water quality monitoring and provide a sustainable nature-inspired solution to the water treatment problem .

Microbial contamination of drinking water sources is one of the leading causes of waterborne diseases, which begs the need of developing efficient water monitoring systems to improve worldwide living standards. Even in low concentrations, the pathogens in water are detrimental to the well-being of the human population. Hence, there is an urgent need for developing efficient water monitoring systems that will improve global living standards. Conventional methods of pathogen detection[6, 7, 8, 9] are efficient and provide an accurate estimation of contaminants in water. However, these methods are limited by prolonged detection times (24 to 48 hours), requirement of trained personnel, along with expensive laboratory setup which hinder the effective monitoring of water quality at the point of use. A practical approach towards overcoming these shortcomings is the use of miniaturized systems such as microfluidics based detection platforms. Microfluidics based detection methods [10, 11] are rapid, portable and highly sensitive with the added advantage of being simple and cost-effective. However, the use of these devices is constrained by their inability to handle large sample volumes which is essential for the accurate estimation of microbial contaminants. Often, these microfluidics or miniaturized devices are able to handle only μL or picoliters of sample (water), which may not have enough pathogens (like *E. coli*) to make it a meaningful water quality monitoring system. Thus, an important challenge lies in developing an efficient concentrator capable of reducing the sample volume to a few hundred microliters yet retaining viable concentration of target pathogens in water to accommodate the miniaturized detection platforms. Currently available concentration procedures

are inconvenient with regards to portability, cost, energy requirements and procedural inadequacy which are important for point-of-use devices. Hence, there is a need for a concentrating process which can be properly integrated with microfluidics based devices for water quality monitoring.

Moreover, both developing and industrialized countries are challenged with a wide range of water contaminants ranging from traditional compounds such as heavy metals [12], fluorides [13] and deadly waterborne pathogens like *Escherichia coli* (*E.coli*) [14, 15, 16] to emerging micro-pollutants such as endocrine disrupters [17] and nitrosoamines [18]. Extensive research in this field has brought about a wide variety of effective processes to treat contaminated water ranging from chemical disinfection [19], photocatalysis [20, 21] and different forms of membrane filtration [22, 23, 24]to the use of nanoparticles [25, 26, 27, 28]to bring about disinfection and coagulation-flocculation [29, 30]. Conventional methods of water disinfection and decontamination are targeted towards large systems and hence, are energy intensive, requiring considerable capital infusion and engineering expertise. The infrastructure required for such elaborate systems is unavailable in most of the developing countries. Additionally, intensive chemical treatment creates unwanted contamination (like disinfection byproducts) of treated water. Additionally, the developing nations suffer from diverse socio-economic and political constraints which require a broader approach incorporating sustainable water resource management. Hence, recent research has focused on reducing chemical treatment through engineered natural systems for drinking water production and reduction of residual chemicals from distribution systems. An area of particular interest in this aspect is the exploration of the available nature-based solutions to the water purification problem. Unlike conventional approaches, bio-inspired solutions have the advantages of being naturally available at a fraction of a cost with the added convenience of being renewable, biodegradable and environment-friendly.

It is evident that the integration of science and technology can help to address the overarching aim of providing safe drinking water to every community across the world. Inspired by natural systems, engineering technology can produce a new generation of inexpensive devices suitable for the masses. In keeping with this spirit, the main objectives of this thesis are outlined in the next section.

1.2 Objectives

The main objectives of this thesis work are:

- Development of an efficient pre-concentrator for microfluidics based water quality monitoring devices.
- Establishment of the bactericidal effectiveness of the seed extract of naturally available *Moringa oleifera* plant.
- Development of a sustainable water treatment device targeting waterborne pathogens.

The major drawbacks for portable concentration devices mentioned earlier, can be alleviated to a certain extent through the use of hollow fiber filter modules which can be manually operated. Importantly, these can provide the required sample volumes necessary for the miniaturized detection devices without any external power requirement. They are easily portable, re-useable and available at a comparatively lower cost. The present study aims to increase the efficiency of the hollow fiber modules in concentrating microorganisms from water. A highly effective surfactant-based elution fluid is developed to aid this cause and provide the desired sample required by the microfluidics based detection devices.

Similarly for water treatment, the primary drawbacks of implementing traditional processes in limited resource settings can be encountered through the use

of widely available natural substances which are non-toxic in nature. A number of naturally occurring antimicrobial substances can help eliminate pathogen contamination of drinking water sources. In this study, we analyze the effectiveness of the *Moringa oleifera* seed extract on Gram-negative and Gram-positive bacteria in water to determine its potential as a rapid and cost effective water treatment solution. Furthermore, this natural substance is integrated with a paper substrate to create a highly efficient water treatment device, capable of removing bacterial pathogens from water. The device uses a combination of paper strips laced with chemoattractants to lure bacterial cells out of the bulk water and subsequently kills them using the *Moringa oleifera* antimicrobial protein adsorbed on the paper substrate. The entire device is completely made of bio-degradable substances which are environment-friendly and readily available at a very low cost.

1.3 Thesis organization

This thesis has been organized based on published and submitted papers. the guidelines from the Faculty of Graduate Studies (FGS) at York University have been followed to prepare this paper based thesis. The thesis consists of five chapters.

Chapter 1 (the present chapter) serves as the introduction of the thesis providing the motivation, background and objective of the thesis.

Chapter 2 presents the development of the rapid concentration process which plays a key role in point-of-use water quality monitoring systems.

Chapter 3 focuses on the selection and analysis of a naturally available antibacterial substance, the *Moringa oleifera* seed extract and its effectiveness in reducing bacterial loads in non-turbid potable water.

Chapter 4 illustrates the development of a cost effective and sustainable wa-

ter treatment device using a simple configuration of paper strips laced with environment-friendly chemoattractants and bactericidal agents.

Finally, Chapter 5 draws conclusion with significant observations and introduces potential directions of future work based on the research outcomes.

Bibliography

[1] Igor A Shiklomanov. Appraisal and assessment of world water resources. *Water International*, 25(1):11–32, 2000.

[2] United Nations World Water Assessment Programme. *The United Nations World Water Development Report 2016: Water and Jobs*. UNESCO, 2016.

[3] United Nations World Water Assessment Programme. *The United Nations World Water Development Report 2015: Water for a Sustainable World*. UNESCO, 2015.

[4] UN Water. *Investing In Water And Sanitation: Increasing Access, Reducing Inequalities*. World Health Organization, 2014.

[5] WHO/UNICEF Joint Water Supply and Sanitation Monitoring Programme and World Health Organization. *Progress on Drinking Water and Sanitation: 2014 Update*. World Health Organization, 2014.

[6] Pina M Fratamico, Solomon K Sackitey, Martin Wiedmann, and Ming Yi Deng. Detection of *Escherichia coli* O157: H7 by multiplex PCR. *Journal of Clinical Microbiology*, 33(8):2188–2191, 1995.

[7] Olivier Lazcka, F Javier Del Campo, and F Xavier Munoz. Pathogen detection: A perspective of traditional methods and biosensors. *Biosensors and Bioelectronics*, 22(7):1205–1217, 2007.

[8] Jeremy Olstadt, J Schauer, Jon Standridge, and Sharon Kluender. A comparison of ten usepa approved total coliform/e. coli tests. *Journal of Water and Health*, 5(2):267–282, 2007.

[9] Asim K Bej, Meena H Mahbubani, Richard Miller, Joseph L DiCesare, Lawrence Haff, and Ronald M Atlas. Multiplex PCR amplification and immobilized capture probes for detection of bacterial pathogens and indicators in water. *Molecular and Cellular Probes*, 4(5):353–365, 1990.

[10] Douglas A Boehm, Philip A Gottlieb, and Susan Z Hua. On-chip microfluidic biosensor for bacterial detection and identification. *Sensors and Actuators B: Chemical*, 126(2):508–514, 2007.

[11] Chuanmin Ruan, Liju Yang, and Yanbin Li. Immunobiosensor chips for detection of *Escherichia coli* O157: H7 using electrochemical impedance spectroscopy. *Analytical Chemistry*, 74(18):4814–4820, 2002.

[12] Ming Hua, Shujuan Zhang, Bingcai Pan, Weiming Zhang, Lu Lv, and Quanxing Zhang. Heavy metal removal from water/wastewater by nanosized metal oxides: a review. *Journal of Hazardous Materials*, 211:317–331, 2012.

[13] Ravi Chavali, Naga Siva Kumar Gunda, Selvaraj Naicker, and Sushanta K Mitra. Rapid detection of fluoride in potable water using a novel fluorogenic compound 7-o-tert-butyldiphenylsilyl-4-methylcoumarin. *Analytical Chemistry Research*, 6:26–31, 2015.

[14] Naga Siva Kumar Gunda, Selvaraj Naicker, Sujit Shinde, Sanjay Kimbahune, Sandhya Shrivastava, and Sushanta Mitra. Mobile water kit (MWK): A smartphone compatible low-cost water monitoring system for rapid detection of total coliform and *E. coli*. *Analytical Methods*, 6(16):6236–6246, 2014.

[15] Saumyadeb Dasgupta, Ravi Chavali, Naga Siva Kumar Gunda, and Sushanta K Mitra. Hollow fiber concentrator for water quality monitoring: role of surfactant based elution fluids. *RSC Advances*, 5(77):62439–62448, 2015.

[16] Ravi Chavali, Naga Siva Kumar Gunda, Selvaraj Naicker, and Sushanta K Mitra. Detection of escherichia coli in potable water using personal glucose meters. *Analytical Methods*, 6(16):6223–6227, 2014.

[17] Ian R Falconer, Heather F Chapman, Michael R Moore, and Geetha Ranmuthugala. Endocrine-disrupting compounds: a review of their challenge to sustainable and safe water supply and water reuse. *Environmental Toxicology*, 21(2):181–191, 2006.

[18] Jacek Nawrocki and Przemysław Andrzejewski. Nitrosamines and water. *Journal of Hazardous Materials*, 189(1):1–18, 2011.

[19] Stuart W Krasner, Howard S Weinberg, Susan D Richardson, Salvador J Pastor, Russell Chinn, Michael J Sclimenti, Gretchen D Onstad, and Alfred D Thruston. Occurrence of a new generation of disinfection byproducts. *Environmental Science & Technology*, 40(23):7175–7185, 2006.

[20] Sixto Malato, P Fernández-Ibáñez, MI Maldonado, J Blanco, and W Gernjak. Decontamination and disinfection of water by solar photocatalysis: recent overview and trends. *Catalysis Today*, 147(1):1–59, 2009.

[21] Je-Wen Liou and Hsin-Hou Chang. Bactericidal effects and mechanisms of visible light-responsive titanium dioxide photocatalysts on pathogenic bacteria. *Archivum Immunologiae et Therapiae Experimentalis*, 60(4):267–275, 2012.

[22] Enrique Samper, M Rodríguez, MA De la Rubia, and D Prats. Removal of metal ions at low concentration by micellar-enhanced ultrafiltration (meuf) using sodium dodecyl sulfate (sds) and linear alkylbenzene sulfonate (las). *Separation and Purification Technology*, 65(3):337–342, 2009.

[23] Mohamed Kheireddine Aroua, Fathiah Mohamed Zuki, and Nik Meriam Sulaiman. Removal of chromium ions from aqueous solutions by polymer-enhanced ultrafiltration. *Journal of Hazardous Materials*, 147(3):752–758, 2007.

[24] Hatijah Basri, Ahmad Fauzi Ismail, and Madzlan Aziz. Polyethersulfone (pes)–silver composite uf membrane: effect of silver loading and pvp molecular weight on membrane morphology and antibacterial activity. *Desalination*, 273(1):72–80, 2011.

[25] Yanhui Li, Fuqiang Liu, Bing Xia, Qiuju Du, Pan Zhang, Dechang Wang, Zonghua Wang, and Yanzhi Xia. Removal of copper from aqueous solution by carbon nanotube/calcium alginate composites. *Journal of Hazardous Materials*, 177(1):876–880, 2010.

[26] Theresa A Dankovich and Derek G Gray. Bactericidal paper impregnated with silver nanoparticles for point-of-use water treatment. *Environmental Science & Technology*, 45(5):1992–1998, 2011.

[27] T Pradeep et al. Noble metal nanoparticles for water purification: a critical review. *Thin Solid Films*, 517(24):6441–6478, 2009.

[28] Chanil Jung, Ahjeong Son, Namguk Her, Kyung-Duk Zoh, Jaeweon Cho, and Yeomin Yoon. Removal of endocrine disrupting compounds, pharmaceuticals, and personal care products in water using carbon nanotubes: A review. *Journal of Industrial and Engineering Chemistry*, 2015.

[29] AG El Samrani, BS Lartiges, and F Villiéras. Chemical coagulation of combined sewer overflow: Heavy metal removal and treatment optimization. *Water Research*, 42(4):951–960, 2008.

[30] Qing Chang and Gang Wang. Study on the macromolecular coagulant pex which traps heavy metals. *Chemical Engineering Science*, 62(17):4636–4643, 2007.

Chapter 2

Hollow fiber concentrator for water quality monitoring: role of surfactant based elution fluids[1]

2.1 Overview

Simple and efficient concentrators are indispensable to the development of portable biosensor based detection platforms for the determination of microbial contamination in water. This requires design of concentrator which could operate for large liquid volume yet maintain the desired microbial concentration in the resultant concentrate. In the present study, we have modified a commercially available hollow fiber filter modules to test for the concentration and recovery of *Escherichia coli (E.coli)* from water with the aid of a combination of surfactants and a chemical dispersant as the elution fluid. The process involves the tangential flow filtration of contaminated water through the hollow fiber filter to reduce the sample volume, followed by an elution step to facilitate the transfer

[1]A version of this chapter has been published. Saumyadeb Dasgupta, Ravi Chavali, Naga Siva Kumar Gunda and Sushanta K. Mitra. Hollow fiber concentrator for water quality monitoring: role of surfactant based elution fluids. *RSC Advances*, 5(77):62439-6244, 2015.

of the concentrated mixture onto a portable hand-held water monitoring device. The effect of using two non-ionic surfactants (Tween-20 and Tween-80) and a chemical dispersant, Sodium hexametaphosphate (SHMP), as elution fluids have been discussed in the present work. The lowest recovery efficiencies were associated with the case of concentration without elution. Recovery efficiencies using a mixture of SHMP and surfactants (1:1 ratio) were found to be greater than when the surfactants were used individually as the elution fluid. A mixture of Tween 20 and Tween 80 (1:1 ratio) was even more efficient with greater than 80% recovery being observed. The best results were obtained using the elution fluid made up of a combination of Tween 20, Tween 80 and SHMP (1:2:1 ratio) with greater than 90% efficiency of bacteria recovery associated with it. Recovery efficiencies increased by 25% using this combination as elution fluid as compared to the case where no elution fluid was used.

2.2 Introduction

Several concentration techniques like centrifugation[1, 2], immunomagnetic separation [3, 4], on-chip microfluidics based pre-concentration [5, 6] and membrane filtration [7, 8]are available in literature. Concentration of microorganisms using centrifugation is not recommended due to the possibility of cell structure damage [9] which would cause significant errors in their detection. Immunomagnetic separation is a very expensive as well as a complex process and hence, is not suitable for on-field applications. Among the other two concentration procedures, separation techniques using microfluidic channels like hydrodynamic filtration using pinched flow fractionation[10, 5], ratchet based filtration using funnel shaped microfluidic ratchets [11], lateral displacement array based separation [12, 13] and electro-kinetic separation using electrophoresis [14, 15, 6]and di-electrophoresis[16, 17] have been found to be continuous,

quick and efficient for the concentration of microorganisms. Nonetheless, such on-chip pre-concentrators and micro-mixers are still inadequate to handle large sample volumes to ensure proper estimation of microbial contamination.

The problem of large sample volume can be effectively addressed by the use of membrane filtration techniques. Membrane filters are polymer films with specific pore ratings. They retain particles and microorganisms larger than their pore sizes on the membrane surface by acting as a physical barrier. This filtration technique is efficient and finds its use in most conventional methods of pathogen detection from contaminated water[18, 19]. Many commercially available bacterial detection kits like Colilert® , Colibag® and others employ the membrane filtration technique for the concentration of microorganisms [20, 21, 22]. Syringe filters are capable of concentrating pathogens from large volumes of water onto the filter surface but the transfer of the microorganisms from the filter surface to the microfluidics based platform poses a considerable challenge. Concentration procedure using membrane filtration is limited by filter fouling problems. Additionally, the entrapment of microorganisms within the filter pores poses a further challenge in their recovery and consequent detection. However, using a different configuration of the membrane filter alleviates the difficulty of such a transfer. One such configuration of membrane filters is the hollow fiber filter which has a very good potential to be effective as a field-deployable concentrating device for monitoring water quality.

Hollow fiber microfiltration and ultrafiltration techniques have gained popularity since the early 1970s when polio-I virus samples were first concentrated from water using this technique [23]. Hollow fibers, as the name suggests, consist of a number of hollow cylindrical membranes, the walls of which are permeable to water but not to particles larger than the specified pore size. A hollow fiber filter module consists of a large number of these fibers bundled together, thereby increasing the effective surface area available for filtration. The modules

usually consist of two end-ports for the sample input and a side port to collect the permeate as the sample passes through the filter. Ultrafiltration techniques have been used for the concentration and recovery of a wide range of microorganisms [24, 25, 26, 27, 28, 29] from a variety of water sources [30, 31, 32]. These studies focus on the use of hollow fiber filters based on the tangential flow filtration process where the sample water is pumped tangentially across the hollow fibers through the end ports and recirculated under a set pressure until the desired volume of the concentrated mixture (retentate) is obtained. Size exclusion retains contaminants of sizes larger than the pore rating within the retentate which is consequently recovered. The hollow fibers can be used in a dead-end configuration as well, by closing one of the end-ports. The sample is forced to flow radially to the membrane surface resulting in the exit of the filtrate through the side port and the entrapment of the contaminants on the inner surface of the filter. Recovery of the contaminants in this case, is facilitated by a backwash step[33]. Despite dead-end filtration being a clearer choice regarding process simplicity, there is an associated disadvantage of membrane fouling and reduced flow rate. The choice of the tangential flow configuration becomes appropriate when a subsequent elution step with suitable elution fluid leads to a significant increase in recovery efficiencies[34, 35].

The effect of non-ionic surfactants on the adhesion of bacteria to solid surfaces is well documented [36]. Most of the linear ethoxylated surfactants have an anti-adhesive effect which helps to prevent bacterial attachment to surfaces. This property has been used to increase bacteria recovery efficiencies during hollow fiber concentration process [34]. Surfactants like Tween 20 and Tween 80 reduce the hydrophobic interactions between microbes and filter surfaces. The surfactant molecules orient themselves on the filter surfaces and help to desorb microorganisms adhered to the surfaces [35, 37].

In our present study, we investigated the effects of a combination of surfac-

tants (Tween-20 and Tween-80) and a chemical dispersant (SHMP) as elution fluid on the concentration and recovery efficiencies of bacteria using hollow fiber filter modules of different pore sizes. Initially, polystyrene latex particles were concentrated using this technique to obtain an estimate of the working conditions. Thereafter we investigated the effectiveness of two different hollow fibers under the same set of operating conditions for the recovery of *E.coli* from water using syringe operated tangential flow filtration. Finally we explored the effect of the different combination of surfactants on the recovery efficiencies using a wide range of bacteria test concentrations which will help to improve this concentrating technique. It is to be pointed out that one of our goals were to establish that one can directly use the commercial hollow fiber filters and an efficient recovery process can be governed solely by the introduction of elution fluids without pre-treating the surface of a commercially available hollow fiber filter, as carried out in the literature[35, 34].

2.3 Materials and methods

2.3.1 Materials

Ultrapure water was obtained from $Barnstead^{TM}$ $Nanopure^{TM}$ water purifier (Model No. D11971, Thermo Scientific, Waltham, Massachusetts). Blue polystyrene latex particles, 1 μm in diameter (Catalogue No. PSB3788-0813) were procured from Magsphere Inc., Pasadena, CA. *E.coli* K-12 strain was obtained from New England Biolabs, Ipswich, Massachusetts, USA. Lauryl Tryptose Broth (Catalogue No. DF0241-15-2) used as the medium for growing *E.coli* was obtained from Fischer Scientific, Canada. The concentration procedure was performed using two separate polyethersulfone membrane hollow fiber filter modules (MicroKros, C06-P20U-10-S) of 0.2 μm pore size and one modified polyethersulfone

membrane hollow fiber module (MicroKros, C02-E750-10-N) of 750 kD pore size obtained from Spectrum Laboratories Inc., Compton, CA. The total surface area of the hollow fiber filters are 0.0041 m^2 with the inner and outer diameters being 1 mm and 1.25 mm, respectively, as shown in Fig. 2.1. The concentration and recovery procedures were performed using four plastic, sterile 5 mL syringes with leur-lock tips (BD309695, BD Medicals, New Jersey, USA). Different combinations of non-ionic surfactants Tween 20 (Catalogue No. P1379) and Tween 80(Catalogue No. P1754) and a chemical dispersant Sodium hexametaphosphate (Catalogue no. 305553) were used as the elution fluid. All the chemicals were obtained from Sigma Aldrich, Canada. A 60 mL syringe (BD309653, BD Medicals, New Jersey, USA) was used for the filter cleaning purposes in between experiments.

2.3.2 Equipment

Absorbance values were measured using a spectrophotometer (Varian Cary 100 Bio, Agilent Technologies, Santa Clara, CA). Scanning Electron Microscopy (Quanta 3D FEG, FEI, Hillsboro, Oregon, USA) (SEM) was used to image the hollow fiber cross sections. Bacterial growth reagents were autoclaved (Primus Sterilizer Co., NE, USA) whenever required.

2.3.3 Preparation of latex solutions

One milliliter of 10% latex particle solution was mixed with 40 mL of water and the solution was serially diluted to prepare different concentrations (expressed in particles per mL units) used in this study. Corresponding absorbance values were obtained from spectrophotometric readings taken at 570 nm and used to prepare the calibration curve.

2.3.4 Preparation of bacteria concentrations

One hundred milliliters of Lauryl Tryptose Broth was prepared and autoclaved to be used as the growth medium. *E.coli* was grown in this media by incubating for 24 hours at 37°C. Thereafter, bacteria solutions were prepared using the serial dilution technique. The resulting concentrations were determined using the plate counting method and expressed in Colony Forming Units (CFU) per mL. Absorbance values recorded at 600 nm were used to prepare the corresponding calibration curve.

2.3.5 Experimental procedure

Prior to the start of the experiments the filter was rinsed with de-ionized water using the 60mL syringe. The Concentration and recovery procedures were performed by attaching three 5 mL syringes to the hollow fiber module and closing one of the side ports (permeate ports). The attachments and step by step procedure are shown in Fig. 2.2. Sample syringe 1 (SS 1), containing the sample was attached to one of the end ports. The sample was subjected to a couple of passes through the hollow fibers until the final volume of approximately 0.5 ml (retentate without elution) was obtained in Sample Syringe 2 (SS 2) attached to the other end port of the module. The permeate was collected in a Permeate syringe (PS) attached to the open permeate port. Four different combinations of the surfactants (0.01% Tween 20, vol/vol and 0.1% Tween 80, vol/vol) and chemical dispersant (0.01% SHMP, by weight) were used as the elution fluid. 0.5 mL of elution fluid was administered through the fibers using an elution fluid syringe attached to the end port by replacing SS 1. The elution fluid, after passing through the fiber lumen, was collected in SS 2, increasing the final volume to approximately 1 mL (retentate with elution). Similar protocols were followed for experiments with both bacteria and latex particles.

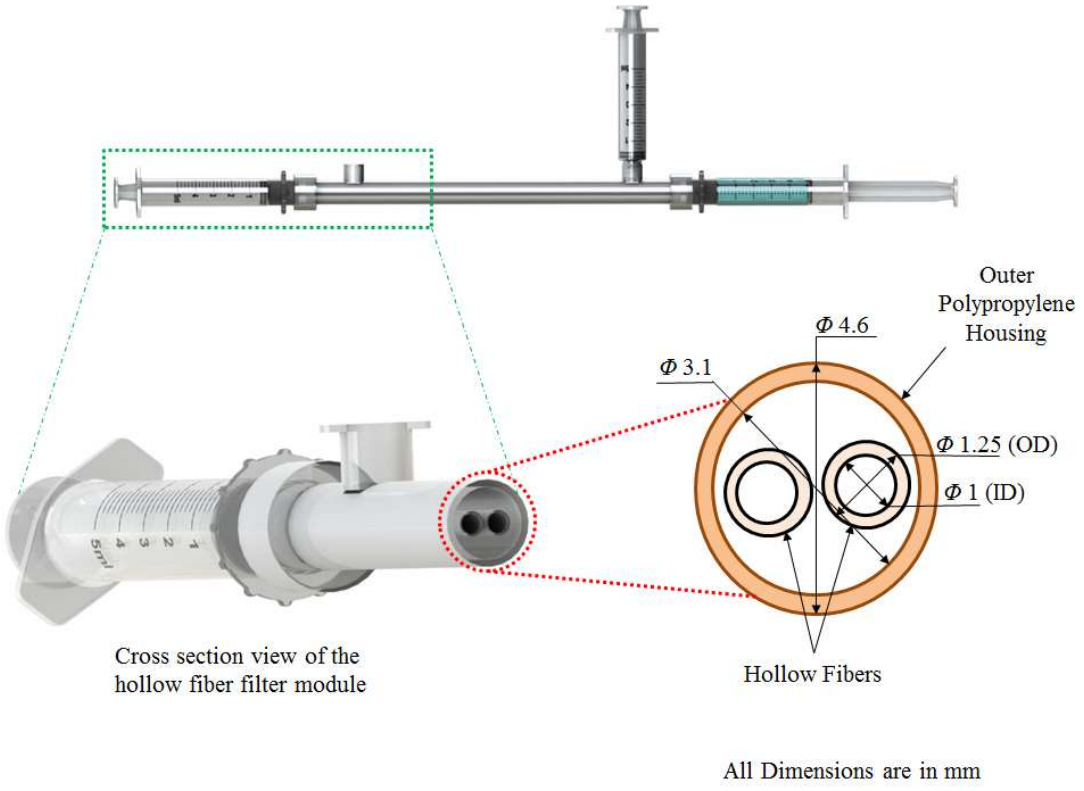
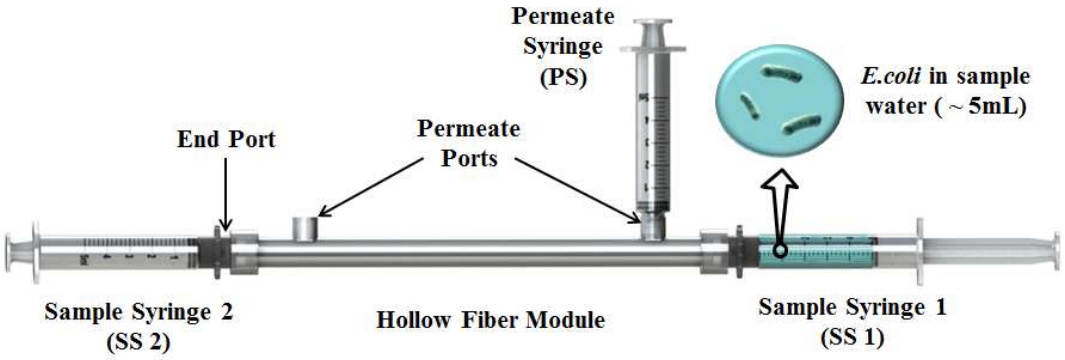
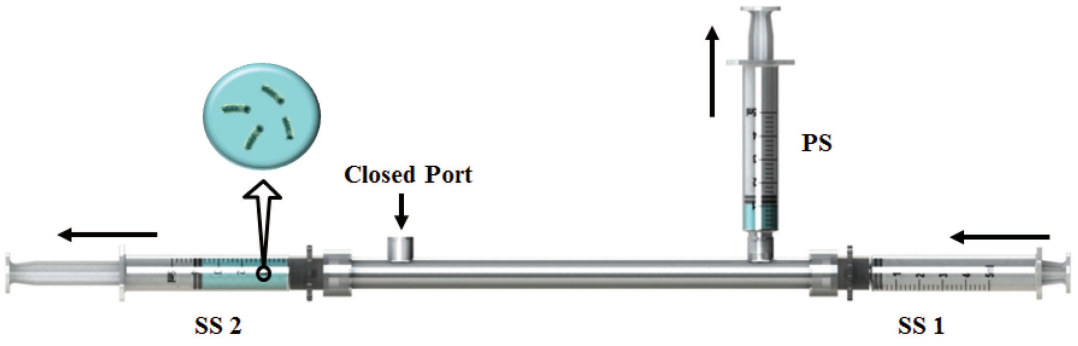


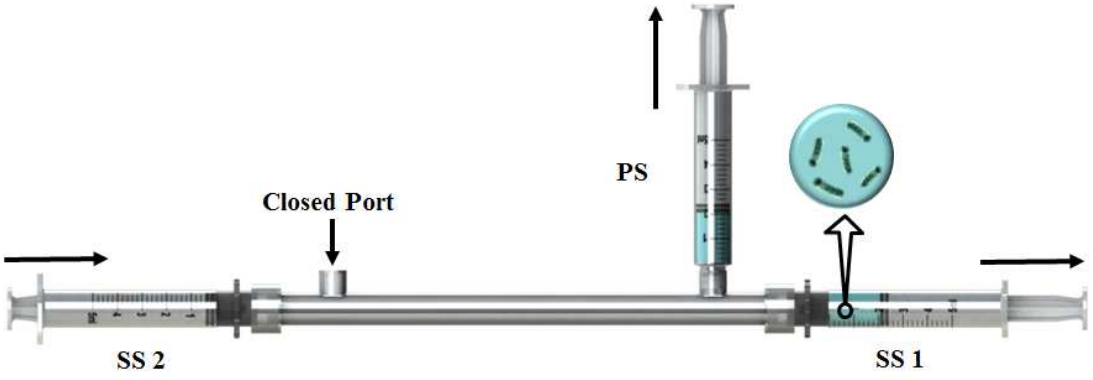
Figure 2.1: Schematic of the hollow fiber filtration module cross-section for the concentration of *E.coli* and polystyrene latex particles from sample water. The relevant dimensions in mm are indicated in the figure.



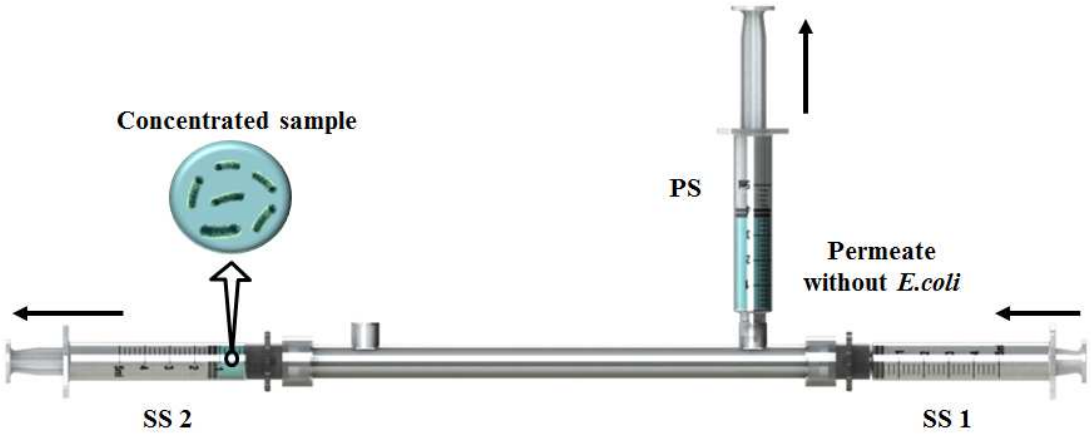
(a) Step I



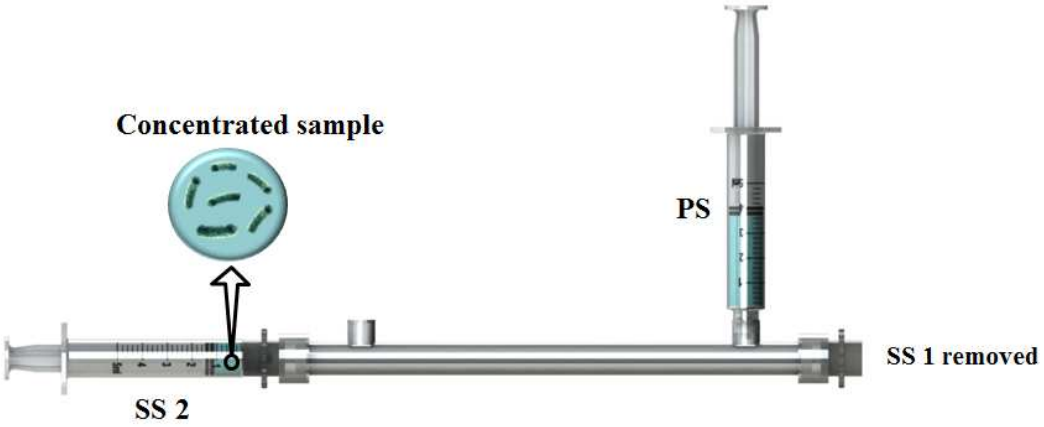
(b) Step II



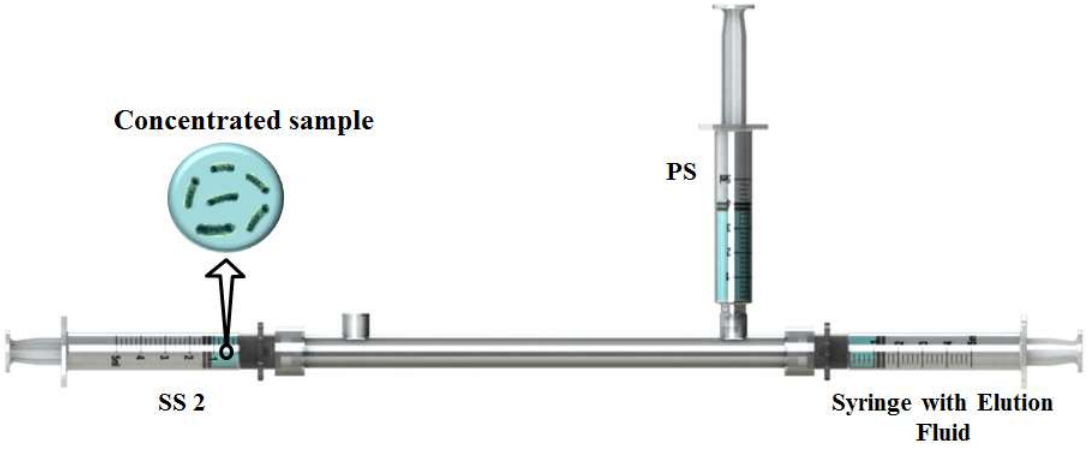
(c) Step III



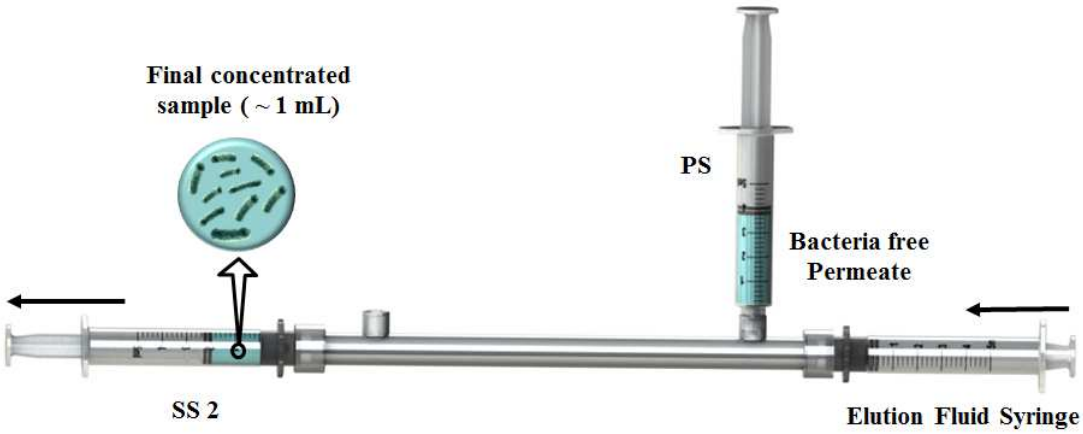
(d) Step IV



(e) Step V



(f) Step VI



(g) Step VII

Figure 2.2: Step by step procedure: a. (Step I) Sample syringe 1 with water sample is attached to the hollow fiber module; b. (Step II) Plunger of sample syringe 1 is pushed to pass the sample through the fiber and into the sample syringe 2 while some permeate is collected in the permeate syringe; c. (Step III) Plunger of sample syringe 2 is pushed and a more concentrated sample is collected in sample syringe 1 with the increase in permeate volume; d. (Step IV) Steps II and III are repeated until final sample volume is 1 mL in sample syringe 2; e. (Step V) Sample syringe 1 is removed from the module f. (Step VI) Sample syringe 1 is replaced with a syringe containing 1 mL of elution fluid; g. (Step VII) The elution fluid is forced through the filter and the final concentrated mixture is collected in Sample syringe 2

For each of the test concentrations, several replicates were performed. Moreover, to maintain uniformity in measurements, the final concentrated volumes were increased to 5 mL by adding water and the corresponding absorbance values were recorded. A cleaning protocol, involving the flushing of the filter first with water and then by air, using a 60 mL syringe, was undertaken at the end of each experiment. Recovery efficiencies were calculated by dividing the final concentration by the initial concentration and multiplying the result by 100 to report the recovery in percentage.

$$\text{Recovery efficiency (\%)} = \frac{\text{Final concentration}}{\text{Initial concentration}} * 100$$

To investigate filter surface characteristics and particle entrapment within the surface pore structures, a concentration procedure was undertaken without elution, using a latex particle solution. Once the final concentrate was obtained, the polypropylene housing was broken to remove the hollow fibers from the module. Small sections of the used hollow fibers were used to conduct Scanning Electron Microscopy (SEM) imaging studies. The samples were sputter-coated with gold and placed inside the base of the scanning electron microscope. Images were taken at high vacuum and high voltage (20 kV) with the beam current set at 7.5 pA and dwell time of 1 μs.

2.4 Results and discussions

2.4.1 Concentration and recovery of polystyrene latex particles in solution

Polystyrene latex microspheres were used as test particles to estimate the experimental conditions for the subsequent experiments with *E.coli*. The calibration

curve, shown in Fig.2.3, was used to calculate the final concentrations after the recovery experiments.

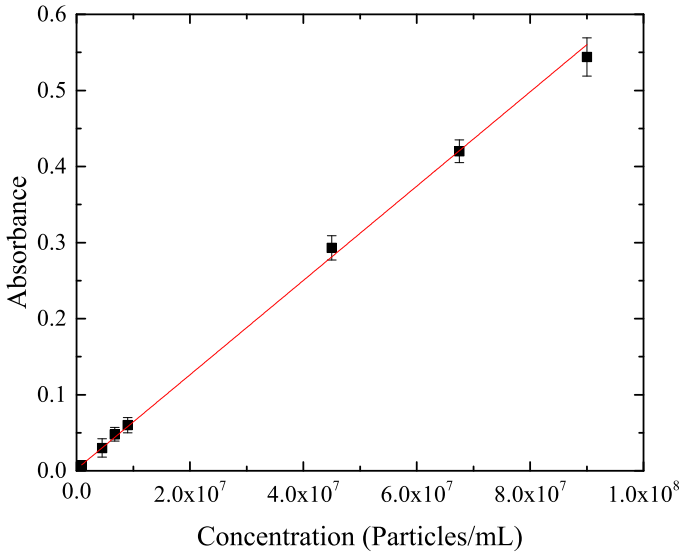


Figure 2.3: Calibration curve for 1 μm polystyrene latex particles in solution. Correlation coefficient is 0.99945 indicating a good positive linear relationship between the variables.Ten replicate experiments were performed with the error bars referring to the corresponding standard errors.

The recovery efficiencies for latex particles with and without elution are shown in Fig.2.4. The results indicate a distinct trend of increasing efficiency with the aid of elution using Tween 20 (0.01%, vol/vol) for all of the test concentrations. However, a steep drop in recovery efficiency is observed for the solution with a concentration of 4.5×10^6 particles per mL. Overall, the data is not very promising with only a single case portraying greater than 50% recovery with the aid of the elution step. On the other hand, these experiments provided an estimate of the working conditions like the number of passes to reach desired volume, time taken for each experiment and the optimum filter cleaning procedure, thereby supplying the necessary framework for the bacteria recovery experiments.

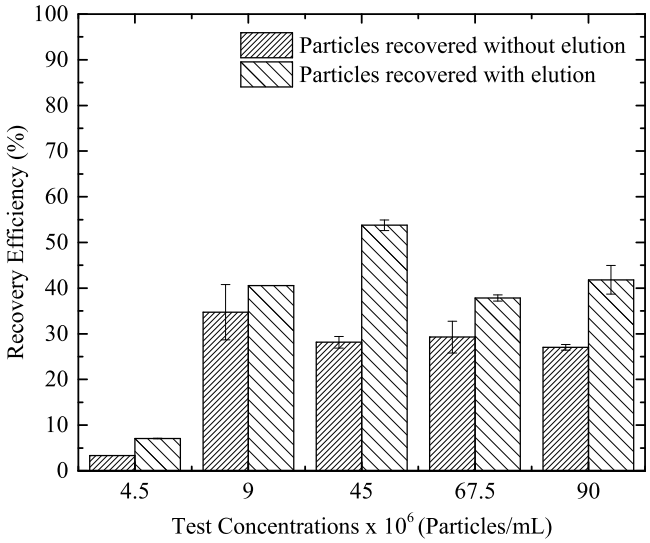


Figure 2.4: Comparison of recovery efficiencies with and without the elution step during the concentration of 1 μm diameter polystyrene latex particles from water. Elution fluid is Tween 20 (0.01%, vol/vol). Error bars indicate the standard errors from three replicate experiments.

2.4.2 Concentration and recovery of *E.coli* (K-12 strain)

Experiments dealing with concentration and recovery of *E.coli* from water were conducted using filters of two different pore sizes of 0.2 μm and 750 kD. The calibration curve used for all relevant calculations regarding bacteria concentrations is shown in Fig. 2.5. The performances of the two filters under the same set of operating conditions were evaluated, as shown in Fig. 2.6. High initial sample concentrations, ranging between 10^7 and 10^8 CFU per mL, were used. Tween 20 (0.01%, vol/vol) was used as the elution fluid for these set of experiments.

The filter with the lower pore size (750 kD) was found to be much more efficient as compared to the other (0.2 μm pore size) one. The results shown in Fig. 2.6 indicate an increase in recovery of nearly 20% for the 750 kD pore sized filter when no elution fluid was used. The introduction of the elution step resulted in significant improvements in recovery efficiencies for both the filters.

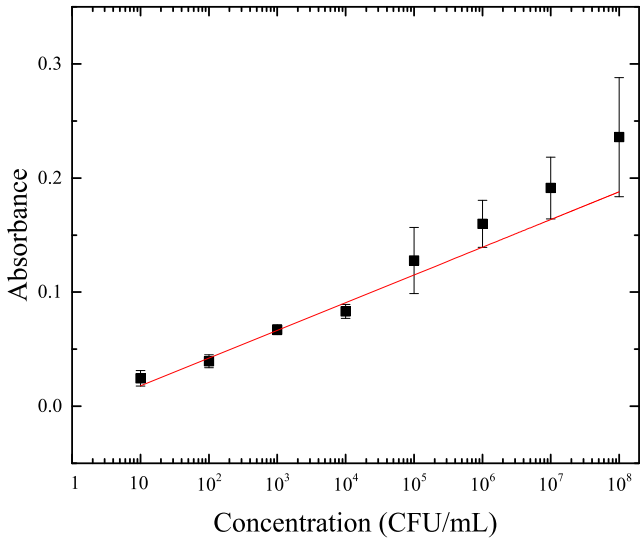


Figure 2.5: Calibration curve for bacteria (*E.coli*) concentrations in water. Correlation coefficient is 0.94365. Error bars indicate the standard errors obtained from ten replicate experiments.

In this case the 0.2 μm filter was associated with efficiencies greater than 80% while the 750 kD filter was more efficient and showed more than 90% recovery for every experiment performed in our study. This increased recovery of the 750 kD pore size filter can be attributed to the lower anisotropy (asymmetry) and pore size of the modified polyethersulfone membrane. Hence, along with the type of elution fluid, these parameters play a prominent role in the concentration and recovery of bacteria (*E.coli*) using hollow fiber filtration technique.

The effectiveness of different elution fluids on the recovery efficiency was investigated using the 750 kD pore sized filter. Sample concentrations ranging from 10^2 to 10^5 CFU per mL were used in this assessment. The calibration curve shown in Fig. 2.5 was used for all relevant calculations regarding bacteria concentrations. The corresponding experimental results are shown in Fig. 2.7. Among the individual surfactants, Tween 80 (0.1%, vol/vol) was found to be a better alternative to Tween 20 (0.01%, vol/vol) as an elution fluid. The recovery levels increased when each of the two surfactants were combined with SHMP

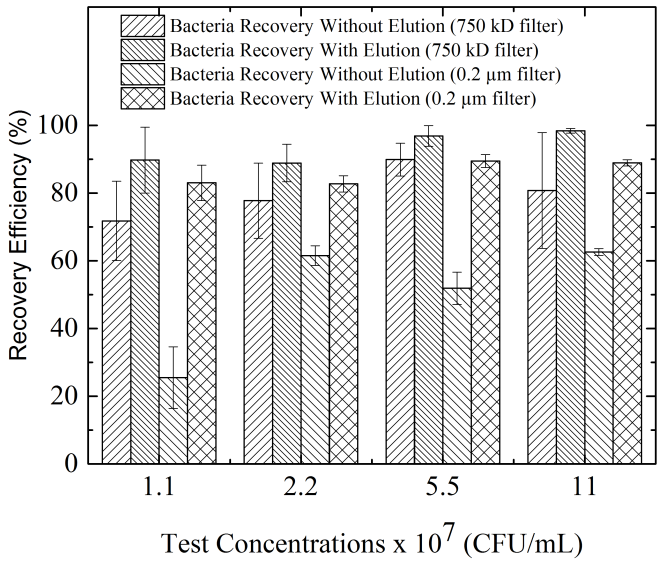


Figure 2.6: Comparison of bacteria (*E.coli*) recovery efficiencies with two different hollow fiber filters using high concentration samples of the order of 10^7 CFU/mL. Elution fluid is Tween 20 (0.01%, vol/vol). Three replicates of each of the experiments were performed. Error bars indicate the standard errors obtained thereof.

(0.01%, by weight) in a 1:1 ratio to constitute the elution fluid. Even higher recovery efficiencies were obtained with a mixture of Tween 20 and Tween 80 (in a 1:1 ratio) as the elution fluid, exhibiting greater than 85% recovery for all the test concentrations. However, the best elution fluid for concentration and recovery purposes was found to be a mixture of Tween 20, Tween 80 and SHMP (in a 1:2:1 ratio), yielding more than 90% recovery of *E.coli* from water. The recovery efficiency corresponding to 10^5 CFU per mL was found to be 98% using this mixture as the elution fluid. Recovery efficiencies were greater by 25% using this combination when compared to the case without the elution step.

2.4.3 Hollow fiber SEM image analysis

The low recovery values observed for the latex particles in the concentration procedure indicate a distinct anisotropic nature of the 0.2 μm pore size hollow

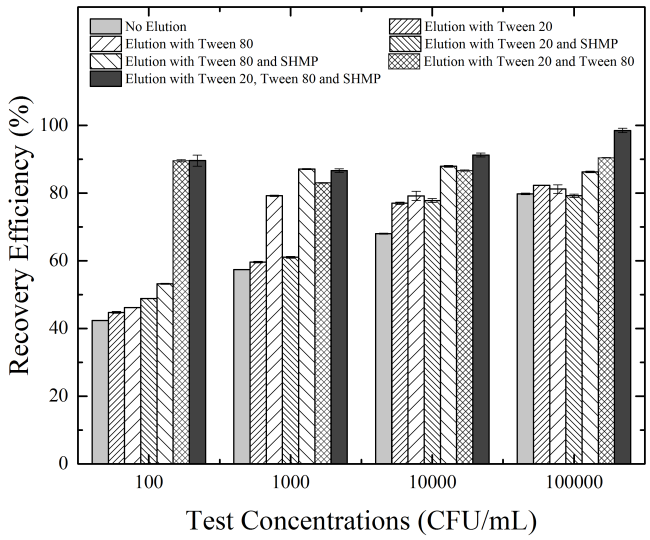


Figure 2.7: Comparison of recovery efficiencies using a combination of surfactants (Tween 20 and Tween 80) and chemical dispersant (SHMP) as elution fluids. Pore size of the hollow fiber filter is 750 kD. Experiments were replicated three times with the error bars indicating the corresponding standard errors.

fiber membrane with most of the particles being captured on the inner surface pores. SEM images presented in Fig. 2.8 show the different cross sections of the hollow fiber after a concentration procedure using latex particles. Figure 2.8A shows that particles were not only entrapped at the surface pores but rather a considerable number of these were embedded within the inner pore structures. There is a gradual increase in pore size from the outer to the inner wall as can be observed in Fig. 2.8C. This points out to the fact that inner surface pore sizes are larger than the specified 0.2 μm. Some surface pore sizes even exceed 1 μm resulting in the embedding of latex particles within the inner pore structures. The increase in recovery efficiency due to elution using Tween 20 (0.01%, vol/vol) can be attributed to the scouring effect of the surfactant which resulted in the recovery of the particles stuck on the filter surface. However, the elution fluid was unable to recover the particles embedded within the inner pore structure of the hollow fiber membrane. The embedded particles can be dislodged with the

introduction of a subsequent back-flushing step. Figure 2.8B shows the absence of any latex particle on the outer wall of the hollow fiber. Hence, we conclude that the low recovery of latex particles using this procedure is the result of the inherent asymmetry of the (0.2 μm pore size) polyethersulfone membrane.

2.4.4 Analysis of bacteria recovery: role of surfactants

A comparison of the recovery rate obtained in this study is presented in Table 2.1 with some of the relevant studies in this field. The high recovery rates were obtained without any prior filter treatment or sample amendment techniques, thereby reducing the complexity and duration of the procedure.

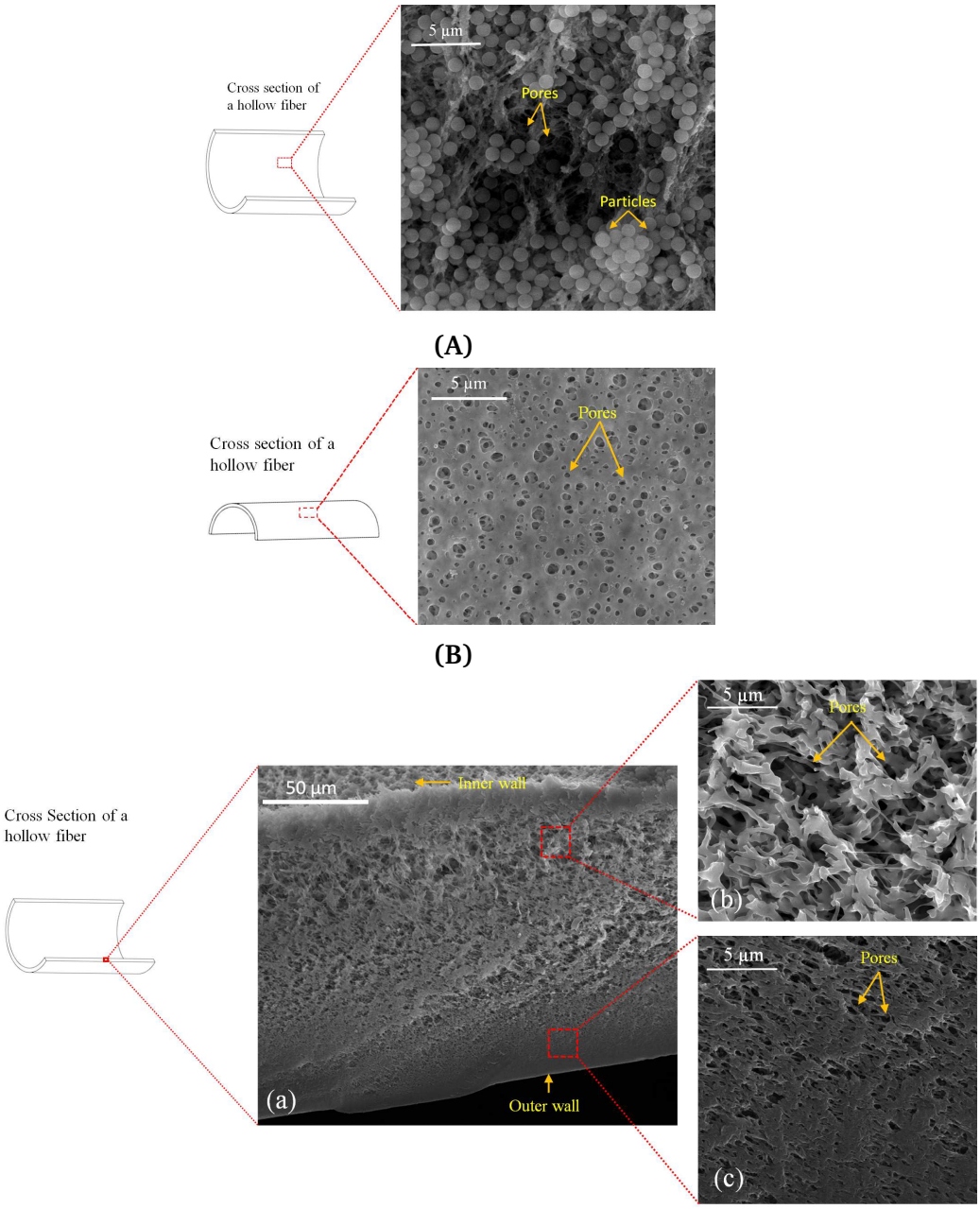


Figure 2.8: SEM images of hollow fiber cross sections; A. Inner surface showing particle entrapment; B. Pore distribution on the outer surface; C.(a) Pore size distribution across the cross section, (b) Pore structures near the inner wall, (c) Pore structures near the outer wall. Images were taken after an experiment with latex particle solution.

Table 2.1: Comparison between the operating conditions and highest recovery efficiencies of the present work with those obtained in previous studies

Paper	Operating Conditions				Tested Microbes	Highest Recorded Recovery Efficiency (%)		
	Filter Pretreatment	Sample Amendment	Backflushing/ Elution	Backflushing/ Elution agent		Bacteria	Virus	Protozoa
V.R.Hill et al., 2005[35]	5% FBS(Fetal Bovine Serum) and NaPP (0.1% and 0.01%)	NaPP (0.1% and 0.01%) and Tween 80 (0.01% and 0.002%)	Backflushing	Tween 80, Tween 20, NaPP	*MS2, Echovirus, Salmonella E. faecalis, B.globigii, C.parvum, E.coli*	98 ± 8 (*E.coli*)	97 ± 58 (*Echovirus*)	98 ± 17 (*C.parvum*)
H.A.Morales-Morales et al., 2003[34]	5% FBS 1X PBS, 0.1% Tween 80	-	-	-	*E.coli, C.parvum, T1 phage, PP7 phage*	95 ± 7.8 (*E.coli*)	62.8 ± 5.8 (*PP7 phage*)	31.6 ± 11.9 (*C.parvum*)
Smith and Hill, 2009[33]	-	-	Backflushing (Dead End Filtration)	Tween 80, NaPP, Y-30 antifoam	*E.faecalis, C.perfringens, MS2, C.parvum*	94 ± 22 (*C.perfringens*)	82 ± 14 (*MS2*)	87 ± 18 (*C.parvum*)
Kuhn and Oshima, 2002[29]	5% FBS	5% FBS	-	-	*C.parvum* oocysts	-	-	74.1 ± 2.8
J. Olszewski et al., 2005[27]	5% FBS	Glycine-NaOH	-	-	*PP7* and *T1* phage, *Poliovirus 2*	-	87.1 ± 24 (*PP7*)	-
L.J. Winona et al., 2001[26]	5% Beef extract, 1% and 5% FBS	10X PBS	Elution	0.05M Glycine, 10% FBS	*PP7* and *T1* phage, *Poliovirus 2*	-	98 ± 7 (*Poliovirus*)	-
V.R. Hill et al., 2007[25]	5% FBS	NaPP (0.01% w/v)	Elution	0.01% Tween 80, 0.01% NaPP, 0.001% Y-30	*PhiX174, MS2, E.faecalis, C.perfringens, C.parvum, G.intestinalis*	120 (*E.faecalis, C.perfringens*)	120 (*MS2*)	88 (*C.parvum*)
Present work	-	-	Elution	0.01% Tween 20, 0.1% Tween 80, 0.01% SHMP (1:2:1 ratio)	*E.coli*	98.5 ± 0.63	-	-

E.coli recovery for all the cases discussed in this study are substantially high due to the deformable nature of bacterial cells and the hydrophilic nature of the polyethersulfone (0.2 μm) and modified polyethersulfone membranes (750 kD). Experiments with different combinations of surfactants and dispersant were conducted to evaluate concentration and recovery characteristics. Use of Tween 20 and Tween 80 as elution fluids led to better recoveries of *E.coli* as compared to the case where concentration was done without the additional elution step. The surfactants used in the study are characterized by a polyoxyethylene hydrophilic group and a long chain fatty acid hydrophobic group. Non-ionic surfactants aid in lowering hydrophobic interactions between bacteria and the filter surface by making the filter surfaces more hydrophilic [38, 34]. The hydrophobic and hydrophilic parts of the surfactant molecules congregate and orient themselves at the solid liquid interfaces preventing bacterial adhesion to the filter surface and helps keep the bacteria in suspension[39]. The addition of 0.01% SHMP to the surfactant solution resulted in recovery efficiencies higher than that of the experiments with individual Tween 20 or Tween 80 as the elution fluid. SHMP falls in the category of sodium polyphosphates which are highly negatively charged chemical compounds. SHMP is a six unit compound which prevents bacterial adhesion to the filter surface by steric effects and surface charge alterations. The polyphosphate molecules are adsorbed onto the surfaces of *E.coli*. This imparts a high negative charge to the bacterial surface which, in turn, favours repulsion. The attractive forces between the bacteria are thereby reduced which facilitates the dispersion of *E.coli* in solution. The recovery levels corresponding to the low test concentration (100 CFU/mL) were surprisingly low for all other conditions except those two which resulted in 90% recovery. However, these levels gradually increased with the increasing bacterial concentration in the sample. Here we postulate that the increase in repulsion due to higher concentration of bacterial cells in water, facilitates the dispersion of bacteria rather than deposition onto

the filter surface. The high recoveries obtained using a combination of Tween 20 and Tween 80 (1:1 proportion) can be attributed to the augmentation of the hydrophilic characteristics of the filter surface by the surfactant molecules. Interestingly, the effect of SHMP (0.01% by weight) on bacteria dispersion is not masked by the presence of the two surfactants already in the solution as can be observed from the highest recoveries recorded using a combination of the surfactants and SHMP. The recovery efficiencies obtained using a combination of Tween 20 and Tween 80 and that using a combination of the surfactants and SHMP, do not exhibit any significant variance with respect to bacterial concentration in water sample. The reason can be attributed to the significant increase in membrane hydrophilicity and bacterial surface charge augmentation which prevents the attachment of the bacterial cells with he filter walls even at low cell concentrations.

Tween 80 has potential membrane fouling characteristics as reported in previous hollow fiber ultrafiltration study[35]. The surfactant, when used as a water amendment, resulted in lower flow rates on continuous recirculation. However, in the present work, the surfactants used as the elution fluids are only subjected to a single pass through the filter. Moreover, the device being hand operated with the aid of syringes, the change in flow rate could not be quantified to indicate the effects of filter fouling. Further studies based on the fouling effects and disadvantages of using the elution fluid would constitute a part of our future work in this area.

2.5 Summary

In the present study, we developed a combination of surfactants and chemical dispersant as elution fluid for very high bacteria recovery efficiencies. Using a hand-held hollow fiber filtration device, *E.coli* was efficiently concentrated and

recovered from 5 mL water samples. This study indicates that along with the type of elution fluid, filter anisotropy and pore size are the principle parameters dictating bacteria recovery efficiencies using hollow fiber filter concentration technique. There is a great potential for the integration of the procedure discussed here with portable microfluidics based biosensor devices for pathogen detection in contaminated water. The high recovery rates would ensure accurate estimation of pathological contaminants and with further modifications, the process can be integrated with the portable biosensor platforms for effective water quality monitoring at the point of use.

Bibliography

[1] I Fliss, E Emond, RE Simard, and S Pandian. A rapid and efficient method of lysis of listeria and other gram-positive bacteria using mutanolysin. *Biotechniques*, 11(4):453–456, 1991.

[2] JH Tjhie, FJ Van Kuppeveld, Robert Roosendaal, WJ Melchers, Roel Gordijn, David M MacLaren, JM Walboomers, CJ Meijer, and AJ Van Den Brule. Direct PCR enables detection of *mycoplasma pneumoniae* in patients with respiratory tract infections. *Journal of Clinical Microbiology*, 32(1):11–16, 1994.

[3] Yun Ju Sung, Ho-Jun Suk, Hwa Young Sung, Taihua Li, Haryoung Poo, and Min-Gon Kim. Novel antibody/gold nanoparticle/magnetic nanoparticle nanocomposites for immunomagnetic separation and rapid colorimetric detection of *Staphylococcus aureus* in milk. *Biosensors and Bioelectronics*, 43:432–439, 2013.

[4] Xiao-Li Su and Yanbin Li. Quantum Dot Biolabeling Coupled with Immunomagnetic Separation for Detection of *Escherichia coli* O157: H7. *Analytical Chemistry*, 76(16):4806–4810, 2004.

[5] Emil Chmela, Robert Tijssen, Marko T Blom, Han JGE Gardeniers, and Albert van den Berg. A chip system for size separation of macromolecules and particles by hydrodynamic chromatography. *Analytical Chemistry*, 74(14):3470–3475, 2002.

[6] Daniel E Raymond, Andreas Manz, and H Michael Widmer. Continuous sample pretreatment using a free-flow electrophoresis device integrated onto a silicon chip. *Analytical Chemistry*, 66(18):2858–2865, 1994.

[7] WAM Hijnen, DA Van Veenendaal, WMH Van der Speld, Ate Visser, W Hoogenboezem, and D Van der Kooij. Enumeration of faecal indicator bacteria in large water volumes using on site membrane filtration to assess water treatment efficiency. *Water Research*, 34(5):1659–1665, 2000.

[8] Graham L Pettipher, Roderick Mansell, Charles H McKinnon, and Christina M Cousins. Rapid membrane filtration-epifluorescent microscopy technique for direct enumeration of bacteria in raw milk. *Applied and Environmental Microbiology*, 39(2):423–429, 1980.

[9] Brandon W Peterson, Prashant K Sharma, Henny C van der Mei, and Henk J Busscher. Bacterial cell surface damage due to centrifugal compaction. *Applied and Environmental Microbiology*, 78(1):120–125, 2012.

[10] Masumi Yamada, Megumi Nakashima, and Minoru Seki. Pinched flow fractionation: Continuous size separation of particles utilizing a laminar flow profile in a pinched microchannel. *Analytical Chemistry*, 76(18):5465–5471, 2004.

[11] Sarah M McFaul, Bill K Lin, and Hongshen Ma. Cell separation based on size and deformability using microfluidic funnel ratchets. *Lab on a Chip*, 12(13):2369–2376, 2012.

[12] Kevin Loutherback, Kevin S Chou, Jonathan Newman, Jason Puchalla, Robert H Austin, and James C Sturm. Improved performance of deterministic lateral displacement arrays with triangular posts. *Microfluidics and Nanofluidics*, 9(6):1143–1149, 2010.

[13] Lotien Richard Huang, Edward C Cox, Robert H Austin, and James C Sturm. Continuous particle separation through deterministic lateral displacement. *Science*, 304(5673):987–990, 2004.

[14] Vladislav Dolnik and Shaorong Liu. Applications of capillary electrophoresis on microchip. *Journal of Separation Science*, 28(15):1994–2009, 2005.

[15] Agnes T Aranas, Armando M Guidote Jr, and Joselito P Quirino. Sweeping and new on-line sample preconcentration techniques in capillary electrophoresis. *Analytical and Bioanalytical Chemistry*, 394(1):175–185, 2009.

[16] Conrad D James, Murat Okandan, Seethambal S Mani, Paul C Galambos, and Randy Shul. Monolithic surface micromachined fluidic devices for dielectrophoretic preconcentration and routing of particles. *Journal of Micromechanics and Microengineering*, 16(10):1909, 2006.

[17] Naga Siva Kumar Gunda, Subir Bhattacharjee, and Sushanta K Mitra. Study on the use of dielectrophoresis and electrothermal forces to produce on-chip micromixers and microconcentrators. *Biomicrofluidics*, 6(3):034118, 2012.

[18] Naga Siva Kumar Gunda, Selvaraj Naicker, Sujit Shinde, Sanjay Kimbahune, Sandhya Shrivastava, and Sushanta Mitra. Mobile water kit (MWK): A smartphone compatible low-cost water monitoring system for rapid detection of total coliform and *E. coli*. *Analytical Methods*, 6(16):6236–6246, 2014.

[19] J De Jong, RGH Lammertink, and M Wessling. Membranes and microfluidics: a review. *Lab on a Chip*, 6(9):1125–1139, 2006.

[20] William P Hamilton, Moonil Kim, and Edward L Thackston. Comparison of commercially available *Escherichia coli* enumeration tests: Implications for attaining water quality standards. *Water Research*, 39(20):4869–4878, 2005.

[21] M Manafi. New developments in chromogenic and fluorogenic culture media. *International Journal of Food Microbiology*, 60(2):205–218, 2000.

[22] Jeremy Olstadt, J Schauer, Jon Standridge, and Sharon Kluender. A comparison of ten usepa approved total coliform/e. coli tests. *Journal of Water and Health*, 5(2):267–282, 2007.

[23] G Belfort, Y Rotem, and E Katzenelson. Virus concentration using hollow fiber membranes. *Water Research*, 9(1):79–85, 1975.

[24] Pengbo Liu, Vincent R Hill, Donghyun Hahn, Trisha B Johnson, Yi Pan, Narayanan Jothikumar, and Christine L Moe. Hollow-fiber ultrafiltration for simultaneous recovery of viruses, bacteria and parasites from reclaimed water. *Journal of Microbiological Methods*, 88(1):155–161, 2012.

[25] Vincent R Hill, Amy M Kahler, Narayanan Jothikumar, Trisha B Johnson, Donghyun Hahn, and Theresa L Cromeans. Multistate evaluation of an ultrafiltration-based procedure for simultaneous recovery of enteric microbes in 100-liter tap water samples. *Applied and Environmental Microbiology*, 73(13):4218–4225, 2007.

[26] LJ Winona, AW Ommani, J Olszewski, JB Nuzzo, and KH Oshima. Efficient and predictable recovery of viruses from water by small scale ultrafiltration systems. *Canadian Journal of Microbiology*, 47(11):1033–1041, 2001.

[27] John Olszewski, Linda Winona, and Kevin H Oshima. Comparison of 2 ultrafiltration systems for the concentration of seeded viruses from environmental waters. *Canadian Journal of Microbiology*, 51(4):295–303, 2005.

[28] Stephaney D Leskinen and Daniel V Lim. Rapid ultrafiltration concentration and biosensor detection of *enterococci* from large volumes of Florida recreational water. *Applied and Environmental Microbiology*, 74(15):4792–4798, 2008.

[29] Ryan C Kuhn and Kevin H Oshima. Hollow-fiber ultrafiltration of Cryptosporidium parvum oocysts from a wide variety of 10-l surface water samples. *Canadian Journal of Microbiology*, 48(6):542–549, 2002.

[30] Peter SK Knappett, Alice Layton, Larry D McKay, Daniel Williams, Brian J Mailloux, Md R Huq, Md J Alam, Kazi Matin Ahmed, Yasuyuki Akita, Marc L Serre, et al. Efficacy of hollow-fiber ultrafiltration for microbial sampling in groundwater. *Groundwater*, 49(1):53–65, 2011.

[31] Karen Shapiro, Jonna AK Mazet, Alexander Schriewer, Stefan Wuertz, Heather Fritz, Woutrina A Miller, John Largier, and Patricia A Conrad. Detection of Toxoplasma gondii oocysts and surrogate microspheres in water using ultrafiltration and capsule filtration. *Water Research*, 44(3):893–903, 2010.

[32] HD Alan Lindquist, Stephanie Harris, Sasha Lucas, Margaret Hartzel, Diana Riner, Paul Rochele, and Ricardo DeLeon. Using ultrafiltration to concentrate and detect Bacillus anthracis, Bacillus atrophaeus subspecies globigii, and Cryptosporidium parvum in 100-liter water samples. *Journal of Microbiological Methods*, 70(3):484–492, 2007.

[33] Carmela M Smith and Vincent R Hill. Dead-end hollow-fiber ultrafiltration for recovery of diverse microbes from water. *Applied and Environmental Microbiology*, 75(16):5284–5289, 2009.

[34] Hugo A Morales-Morales, Guadalupe Vidal, John Olszewski, Channah M Rock, Debanjana Dasgupta, Kevin H Oshima, and Geoffrey B Smith. Optimization of a reusable hollow-fiber ultrafilter for simultaneous concentration of enteric bacteria, protozoa, and viruses from water. *Applied and Environmental Microbiology*, 69(7):4098–4102, 2003.

[35] Vincent R Hill, Amy L Polaczyk, Donghyun Hahn, Jothikumar Narayanan, Theresa L Cromeans, Jacquelin M Roberts, and James E Amburgey. Development of a rapid method for simultaneous recovery of diverse microbes in drinking water by ultrafiltration with sodium polyphosphate and surfactants. *Applied and Environmental Microbiology*, 71(11):6878–6884, 2005.

[36] Martyn Humphries, Joseph F Jaworzyn, John B Cantwell, and Allan Eakin. The use of non-ionic ethoxylated and propoxylated surfactants to prevent the adhesion of bacteria to solid surfaces. *FEMS Microbiology Letters*, 42(1):91–101, 1987.

[37] Javier Méndez, Ana Audicana, Ana Isern, Julián Llaneza, Belén Moreno, Maria Luisa Tarancón, Juan Jofre, and Francisco Lucena. Standardised evaluation of the performance of a simple membrane filtration-elution method to concentrate bacteriophages from drinking water. *Journal of Virological Methods*, 117(1):19–25, 2004.

[38] John H Paul and Wade H Jeffrey. The effect of surfactants on the attachment of estuarine and marine bacteria to surfaces. *Canadian Journal of Microbiology*, 31(3):224–228, 1985.

[39] M Lepesteur, S Blasdall, and NJ Ashbolt. Particle dispersion for further Cryptosporidium and Giardia detection by flow cytometry. *Letters in Applied Microbiology*, 37(3):218–229, 2003.

Chapter 3

Evaluation of the antimicrobial activity of *Moringa oleifera* seed extract as a sustainable solution for potable water[1]

3.1 Overview

Pathogen contamination in drinking water sources is of great concern, particularly for limited resource communities across the globe. At the same time, there is a pressing need to develop water treatment solutions which are sustainable and are 'green'. To address this issue we have provided a detailed antimicrobial study of the seed extract of *Moringa oleifera* which is a common medicinal plant found all over south east Asia and Africa. In this study we report the efficacy of the extract in inhibiting bacterial growth on agar and in nutrient medium (Lauryl Tryptose Broth) and the role of different parameters of bacteria concen-

[1]A version of this chapter has been published. Saumyadeb Dasgupta, Naga Siva Kumar Gunda, and Sushanta K. Mitra. Evaluation of the antimicrobial activity of *Moringa oleifera* seed extract as a sustainable solution for potable water. *RSC Advances*, 6(31):2591825926, 2016.

tration and extract concentration on its antimicrobial activity. This study further involves the determination of the decay rates of a gram-negative (*Escherichia coli*) and a gram-positive (*Bacillus subtilis*) bacteria in 100 mL of non-turbid water in the presence of 2, 3, 5 and 10 mL of the seed extract.The seed extract volume of 10 mL affected a maximum bacterial decay of 93.2% for *E. coli* and 96.2% for *B. subtilis*. The bacterial decay data were fitted to exponential curves and three different regimes of decay were observed over a period of 6 h. *B. subtilis* showed a 35% re-growth during the third regime as compared to the initial test concentration, which can be attributed to the resistance created by the modification of the peptidoglycan backbone of the cell wall.

3.2 Introduction

Natural substances can help purify water through the mechanisms of adsorption and coagulation-flocculation of contaminants along with inhibiting the growth of pathogenic organisms. Different forms of naturally available inexpensive material like potato peels [1], sawdust [2], jatropha husks [3], eggshells [4], orange and banana peels [5], coffee husks [6], flowers [7], treated bark [8] etc. have been the target of considerable research for their effectiveness in heavy metal and industrial dye adsorption. Other biosorbents like algal [9] and microbial biomass [10] (which include bacteria, fungi and yeast) are abundant in nature offering efficient removal of metal ions from wastewater at optimum pH levels. The use of natural [11] and surface modified zeolites [12, 13] provide treatment possibilities for a wide range of industrial effluents as well as heavy metals in water. There also exists different options like pads of *Cactus opuntia* and *Cactus latifaria*, tannin (an extract from *Acasta mearnsii*) [14] and chitosan (obtained from crustacean shells) [15] to choose from when using coagulation-flocculation based water treatment options which helps in reducing the total

contaminant load in water. Moving into the realm of elimination and inhibition of pathogenic growth in water, only a few options are readily available [16, 17, 18, 19]. The current work focuses on the plant known as *Moringa oleifera* which has many, well documented medicinal properties [20]. Our principal area of interest is the properties of *Moringa oleifera* seeds which significantly help improving the quality of contaminated water, especially in removing the water-borne pathogen *E. coli*. Various studies have revealed and quantified the effectiveness of the shelled seeds in powder form in removing heavy metals like cadmium [21], chromium [22], nickel, copper and zinc [23] from water and the effect of pH [24] on such biosorption studies. The seeds, in their shelled and powdered form, are also capable of removing organic pollutants like benzene, toluene, ethylbenzene and cumene from water through adsorption [25]. The seeds can act as a powerful natural coagulant and has been found to be effective in removing dyes [26] and surfactants [27] from wastewater. Furthermore, *Moringa oleifera* seed extract has been reported to reduce the contaminant load in highly turbid natural waters [28, 29, 30]. In addition to the coagulation/flocculation and adsorption potential of the seeds (shelled and in powder form), the seed extract has also been found to exhibit antimicrobial properties [31, 32, 33] owing to the presence of a cationic flocculating polypeptide known as *Moringa oleifera* cationic protein (MOCP) [34, 28].

Although extensive research has been devoted to its effectiveness in highly turbid natural water samples, there is a dearth of studies regarding quantification of its efficacy in the context of bacterial load reduction in low turbidity water, which is often the case for potable water sources. In the present work, we focused on the determination of the rate of decay of bacteria in turbidity-free water due to the bactericidal effect of *Moringa oleifera* seed extract. Our organism of interest was the gram-negative bacteria *Eshcherichia coli*, a major species in the faecal coliform group and a common indicator of water quality. We also

extend the investigation to include the gram-positive bacteria, *Bacillus subtilis* (*B. subtilis*) to establish the efficacy of the seed extract over a wide range of pathogens. Different volumes of the seed extract was used to quantify the decay rates in order to obtain an estimate of its practical usability in cases of bacterial contamination of drinking water sources.

3.3 Materials and methods

3.3.1 Materials

Ultrapure water used in all experiments was obtained from $Barnstead^{TM}$ $Nanopure^{TM}$ water purifier (Model No. D11971, Thermo Scientific, Waltham, Massachusetts). *E. coli* (K-12 strain) was procured from New England Biolabs, Ipswich, Massachusetts, USA and *B. subtilis* (MI112 strain) from Cedarlane, Burlington, Ontario, Canada. Lauryl Tryptose Broth (Catalogue No. CA90001-768) was used as the medium of growth for *E. coli*. *B. subtilis* was grown in a medium consisting of Veal Infusion Broth (Catalogue No. CA90002-674) and Yeast extract (Catalogue No. 90000-726) in a 5:1 ratio. For enumeration purposes, all bacteria were grown on Luria Bertani (LB) Agar (Catalogue No. CA90003-344). All bacterial growth media were obtained from VWR, Canada. Dried, unshelled *Moringa oleifera* seeds were obtained from Purelife Herbs, USA. A common household kitchen blender was used to convert the seeds into powder form. Stericup® filters (Catalogue No. SCHVU01RE) fitted with a vacuum pump (Catalogue No. EZSTREAM1) and a membrane filtration setup (Catalogue No.EZFITMIC01 and MIHAWG100) from EMD Millipore, Massachusetts, USA, were used for the preparation of the seed extract and the collection of bacteria from water for further enumeration. Bacterial growth reagents were autoclaved (Primus Sterilizer Co., NE, USA) as and when required during the experiments. 100 mL

glass beakers (Catalogue No. 89000-200) from VWR, Canada, were used in all the experiments. The turbidity measurements were performed using a portable turbidity meter (2100Q, Hach Company, CO, USA). A UV-Vis spectrophotometer (Genesys® 10S) from Thermo Scientific, USA was used to obtain optical density for the growth experiments.

3.3.2 Preparation of *Moringa oleifera* seed extract

Moringa oleifera seeds were obtained in a dry, unshelled form. The chemical composition of the seeds in different forms has been studied in details by Ndabigengesere et al.[28]. Shelled seeds contain, by mass, 36.7% proteins, 34.6% lipids and 5% carbohydrates while the non-shelled ones have 27.1% proteins, 21.1% lipids and 5.5% carbohydrates by mass, in powdered form. Shelled seeds are composed of 6.1% nitrogen, 54.8% carbon and 8.5% hydrogen by mass as the principal constituents with the remaining 30% being composed of oxygen and trace elements. The non-shelled seeds contain all these elements in marginally smaller quantities. The total mass of each non-shelled seed is constituted by the seed cotyledon (70%) and the outer shell (30%). The husk enveloping the inner kernel was removed and the shelled seeds were stored in dry conditions at a room temperature of 22°C. Before starting the extract preparation process, the shelled seeds were washed twice with deionized water to remove any contaminants from the surface and further dried at 37°C for 5 h to remove any moisture. Thereafter the seeds were pulverized using a common kitchen blender and five different amounts (1, 2, 3, 4 and 5 g) of the powder was suspended in 100 mL of deionized water to obtain 1%, 2%, 3%, 4% and 5% concentrations (w/v). The mixture was stirred at 300 RPM for 2 h using a magnetic stirrer. Immediately after, the mixture was filtered using a 0.45 μm, polyvinylidene fluoride (PVDF) *Stericup*^{R} filter aided by a vacuum pump. The

resulting filtrate was used as the seed extract in all subsequent experiments. The chemical information of this crude extract is well documented in literature [28, 30, 34]. The quantity of extractable matter from the seeds using water is 25% by mass [28]. The lipid content of the extract from shelled seeds is 0.8% and protein content is around 1% by mass [28, 34]. Henceforth, simple calculations show that the amount of extractable matters in the five different concentrations (1%, 2%, 3%, 4% and 5%) were 0.25, 0.5, 0.75, 1 and 1.25 g, respectively while the total protein content in the extracts were 0.01, 0.02, 0.03, 0.04 and 0.05 g, respectively. When not in use, the seed extract was kept refrigerated in tightly sealed containers.

3.3.3 Preparation of bacteria concentrations

E. coli was grown in 100 mL of Lauryl Tryptose Broth (LTB) by incubating for 24 h at 37°C. Growth media for *B. subtilis* consisted of a 5:1 mixture of Veal Infusion Broth and Yeast Extract ($ATCC^R$ Medium 1179:VY medium). *B. subtilis* was grown in this media for 24 h at 30°C. Subsequently the bacteria were enumerated using serial dilution followed by plate counting. For the experiments dealing with bacterial decay, the concentrations for *E. coli* and *B. subtilis* were chosen to be 250 and 160 Colony Forming Units (CFU), respectively, in 100 mL of deionized water.

3.3.4 Bacteria growth experiments

Approximately, 10^5 cells of *E. coli* were inoculated into 100 mL of LTB along with 5 mL or 10 mL of the seed extract and allowed to grow at 37°C. During incubation the flasks were kept under constant shaking at 130 RPM. Five different concentrations of the extract was used to get ten different sets of data. For each set of data, a different calibration curve was used in order to minimize the effects

of variation of optical density due to the addition of different concentrations of extract. This way the uniformity in bacterial count was ensured. For each data set, *E. coli* grown in LTB was serially diluted using the proper ratio of LTB and *Moringa* extract to get 8 different concentrations from 100 CFU per mL to 10^9 CFU per mL. The OD600 readings of these concentrations were plotted to obtain the respective calibration curves. To obtain the growth curves, bacterial concentration was determined every h, for 11 h, by measuring absorbance values at 600 nm using a spectrophotometer.

3.3.5 Growth inhibition experiments

To examine the bactericidal activity of the extract, bacterial growth inhibition tests were performed with *E. coli*. LB agar plates were supplemented with different concentrations of the extract. This procedure involved spreading 100 μL of the seed extract on the agar surface followed by drying for 30 min. These plates were subsequently used to grow *E. coli* for 24 h at 37°C. Two separate sets of experiments were performed to determine the effects of extract concentration and bacterial concentration on the inhibition of growth caused by the extract. To determine the effects of extract concentration, all five concentrations were used on agar plates and approximately 500 CFU of *E. coli* was allowed to grow in them. For the effects of different bacterial concentrations, 85, 170, 850, 1000 and 1700 CFU of *E. coli* were grown on agar plates supplemented with 5% extract concentration.

3.3.6 Bacteria decay experiments

Simple jar tests were performed to evaluate the decay rates. The process was carried out as follows. The glass beakers were filled with deionized water and seeded with the bacteria. Separate set of experiments were performed for *E. coli*

and *B. subtilis*. Initial concentrations for both the bacteria were maintained as mentioned in the previous section. Four sets of data were obtained by adding four different volumes of extract (2mL, 3mL, 5mL and 10mL) of 5% concentration to the samples. Bacteria count in the samples were noted in 20 intervals (0, 3, 6, 9, 12, 15, 30, 45, 60, 90, 105, 120, 135, 150, 165, 180, 240, 300 and 360 min) over a period of 6 h. After every interval, the entire content of the beaker was filtered out on a gridded 0.45 μm cellulose ester membrane filter. Following this, the filters were transferred aseptically to agar plates with their gridded side facing upward. Care was taken to ensure that there were no air gaps between the filter and agar surface and that the entire filter was in contact with the agar surface. The agar plates were then inverted and incubated for 24 h at 37°C. After each filtration process the surfaces of the membrane filtration apparatus which came in contact with bacteria were cleaned using 70% Ethanol and subsequently air dried to prevent inaccuracies in bacterial count. After 24 h, the agar plates were taken out and the number of colonies grown on the filters were counted manually and noted. The same procedures were followed for both *E. coli* and *B. subtilis*. Control experiments were performed by measuring bacterial decay in water in absence of any extract. Experiments were conducted so as to absolutely minimize the chances of cross contamination. For each time interval, several replicates were performed for every set of data obtained by using different extract volumes. All the experiments were carried out inside a bio-safety cabinet.

The water samples used in the decay experiments were measured for their turbidity after the addition of bacteria and seed extract. 25 mL vials were filled with the sample and turbidity values were obtained in NTU (Nephelometric Turbidity Units).

3.3.7 Decay curve modeling method

The Curve Fitting application of MATLAB© was used to find the best fit curve for the set of multiple data points. The axes of the graph was normalized - the time axis by 360, the total experimental time in min and the vertical axis (bacteria count in water) by 250, the initial bacteria count in the samples. This course of representation was undertaken in order to conveniently establish a relation between the two non-dimensional parameters viz., bacteria count (N) and time (τ). The data points were found to fit best the exponential decay curves of the form

$$N = ae^{b\tau} \tag{3.1}$$

In this context, the parameters of curve fitting must be mentioned as follows. The same parameters were applied when dealing with both *E. coli* and *B. subtilis* data plotted in Figures 3.4 and 3.5. The non-linear least squares method employing the Levenberg-Marquardt algorithm was applied to find out the best fitting curve. The maximum function evaluations and iterations were restricted to 600 and 400, respectively as no further changes were observed by increasing these numbers. The minimum change in a and b for finite difference gradients was 10^{-8} and the corresponding maximum allowed change was 0.1. Iterations were stopped when the error for evaluation of the coefficients dropped lower than 10^{-6}.

3.4 Results and discussions

3.4.1 *E. coli* growth curves

The trends of bacterial growth in the presence of seed extract in the nutrient medium is shown in Fig. 3.1. The results indicate a tendency of diminished growth with increasing extract concentration. For the same extract concentration, an increased volume resulted in a significant reduction in bacterial growth. The quickest growth was observed for 5 mL of 1% concentrated extract when *E. coli* showed increased growth just after 3 h. The slowest growth was caused by 10 mL of 5% concentrated extract which did not allow growth for nearly 9 h. In fact a close observation reveals a dip in the growth curve indicating that the bacterial count in the medium went well below 10^5 CFU. Only the two volumes of 5% concentrated extract were able to initiate bacteria reduction in the medium. The inset picture in Fig. 3.1 shows the control experiment where growth occurred in absence of any extract. Compared to control, bacteria growth was lower by two orders of magnitude when extracts were used in the growth media over the same period of time. The inset curve is magnified and shown in Fig. A1 in the Appendix section.

3.4.2 Inhibition tests

Figure 3.2 exhibits the inhibition characteristics of different extract concentrations. Following the trends of the growth curves, the highest inhibition to bacterial growth was affected by agar plates supplemented with 5% concentrated extract. Only 10% of the plated bacteria were able to grow back in this case. The worst results were exhibited by the lowest concentration of seed extract which could inhibit the growth of only 14% of the plated bacteria. The trends suggest an increase in bactericidal effect with increased concentration.

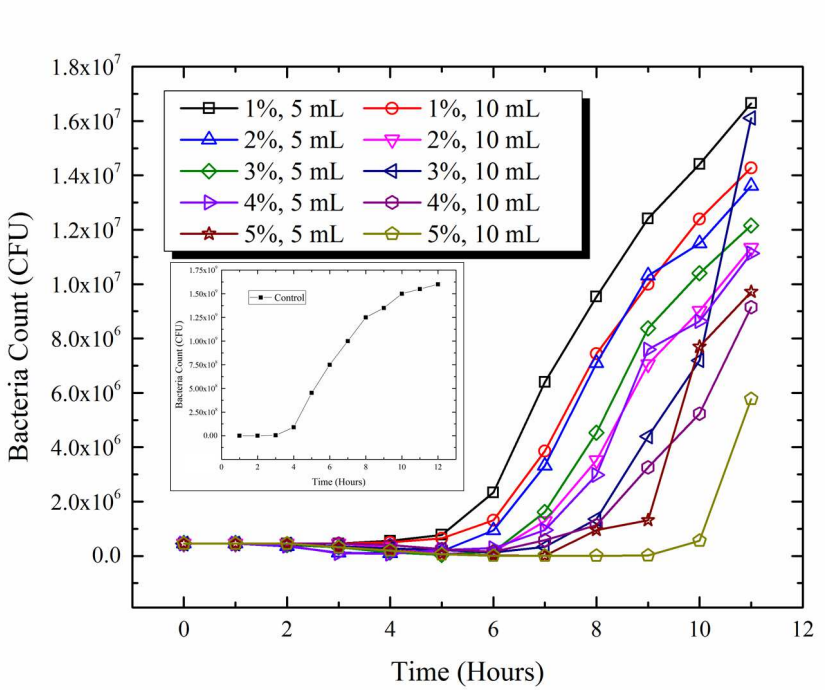


Figure 3.1: Growth curves of *E. coli* in Lauryl Tryptose Broth in the presence of different concentrations (expressed in percentages) and quantity (expressed in the units of mL) *Moringa oleifera* seed extract. The inset picture shows the control experiment where *E. coli* was grown in LTB only. Experiments were performed in triplicates for each data point.

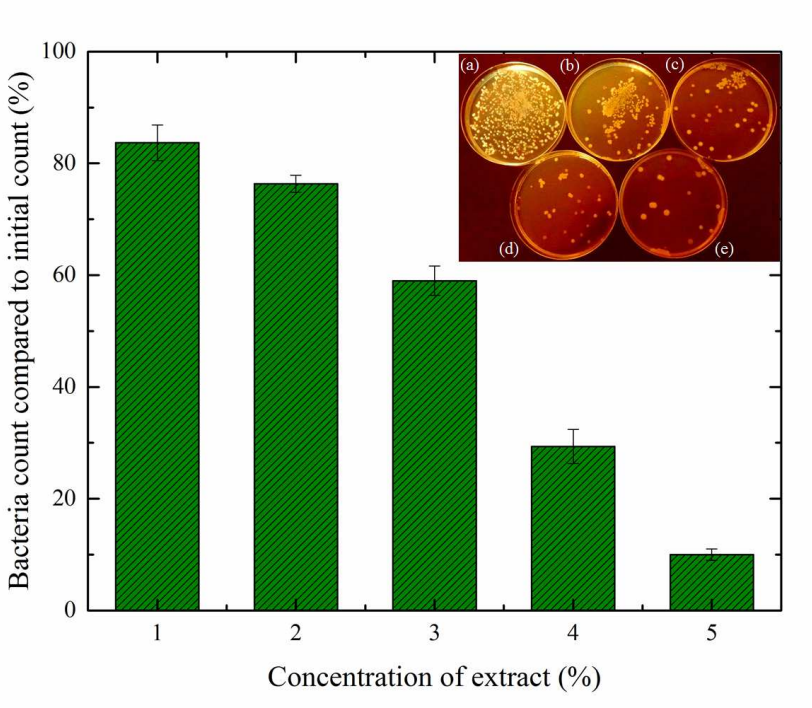


Figure 3.2: Number of *E. coli* colonies as a function of the concentration of *Moringa oleifera* seed extract in LB agar plates expressed as a percentage of the number of colonies grown on extract-free control plates. The inset picture shows LB plates containing different concentrations of 100 μL of the seed extract: (a) 0%, (b) 2%, (c) 3%, (d) 4%, (e) 5%. Error bars indicate the standard errors obtained from three replicate experiments.

Figure 3.3 displays the effectiveness of the seed extract with increasing bacterial load. For low bacteria counts, the extract prohibited the growth of more than 90% of the bacteria. This effectiveness, however, deteriorates with the introduction of higher number of *E. coli* cells. When 1700 CFU were plated, nearly 40% of the cells were able to grow back.

3.4.3 Decay characteristics of *E. coli*

Four different volumes of *Moringa oleifera* seed extract was used to obtain the data points indicating the decay rates of *E. coli* in deionized water as shown in Fig.3.4. Based on the time interval, three distinctly different decay regimes were observed from the curve fitting analysis (Fig.3.4) with different values of

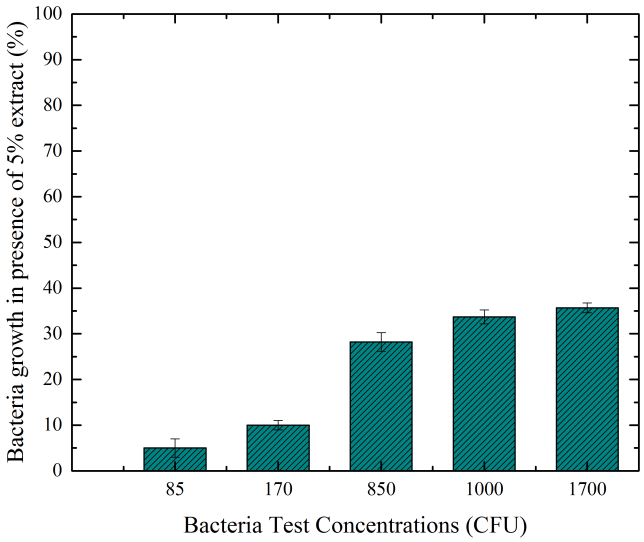


Figure 3.3: Number of *E. coli* colonies grown up on LB agar plates containing 100 μL of 5% *Moringa oleifera* seed extract as a function of the number of colony forming units (CFU) applied in the experiments expressed as a percentage of the original counts. Error bars indicate the standard errors obtained from three replicate experiments.

the coefficient a and exponent b (Equation 3.1), as shown in Table 3.1. Regime I, comprising of the initial 15 min of the experiment, represents a zone involving a very high rate of bacterial decay which is reflected by the best fitting curve F1.

Similarly, the best fit curves were obtained for regimes II and III, as indicated by the curves F2 and F3 in Fig.3.4. Regime II was observed over the time interval spanning from 15 min to 165 min where the decay rate reduced substantially as indicated by the much lower value of the exponent b. Regime III, observed between 165 and 360 min of experimental time, is the last stage of bacterial decay observed in our experiments. It is not starkly different from regime II but is differentiated by lower values of both a and b. We observed the minimum bacteria count of 17 CFU using 10 mL of the seed extract at the end of 6 h. For all other volumes of extract the final bacteria count was less than 50 CFU.

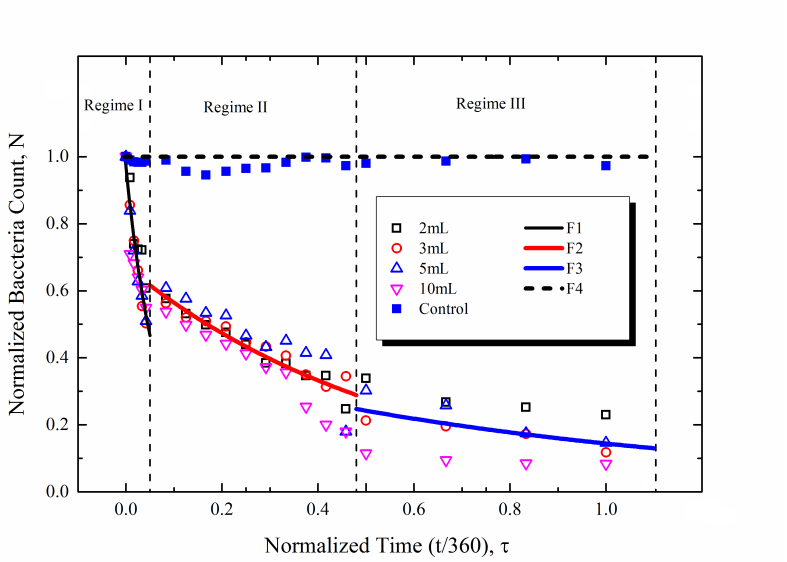


Figure 3.4: Decay curve for *E. coli* in the presence of 2, 3, 5 and 10 mL of 5% concentrated *Moringa oleifera* seed extract. Experiments were performed in triplicates for each data point. The error bars are not shown. F1, F2 and F3 indicate the best fitted curves for regimes I, II and III, respectively. The adjusted R-square values of F1, F2 and F3 were 0.83, 0.74, 0.26, respectively. F4 drawn through $N = 1$ provides a reference for the control test data.

Table 3.1: Coefficients and exponents of the best-fit curves for the different regimes of decay of *E. coli*

Regime	*a*	95% Confidence Interval	*b*	95% Confidence Interval
I	0.97	(0.92, 1.02)	-14.61	(-17.01, -12.19)
II	0.57	(0.53, 0.61)	-1.76	(-2.07, -1.46)
III	0.34	(0.07, 0.62)	-1.04	(-2.17, 0.10)

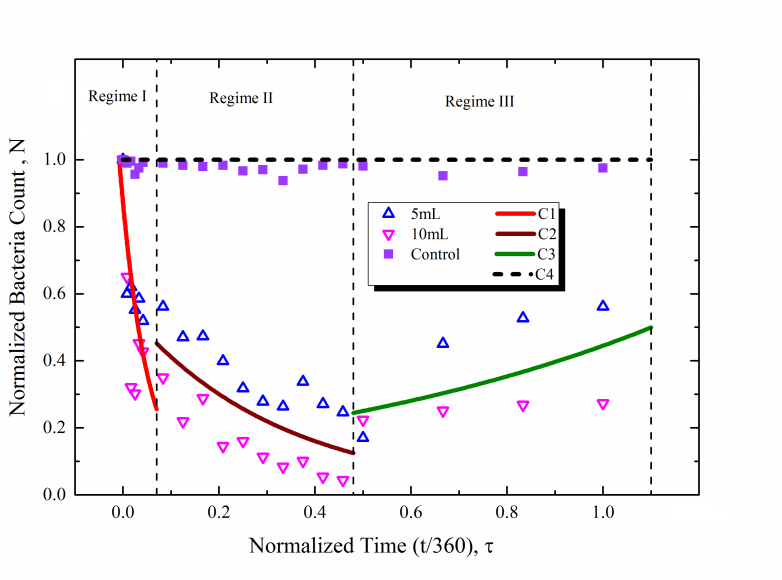


Figure 3.5: Decay curve for *B. subtilis* in the presence of 5 and 10 mL of 5% concentrated *Moringa oleifera* seed extract. Experiments were performed in triplicates for each data point. The error bars are not shown. C1, C2 and C3 indicate the best fitted curves for regimes I, II and III, respectively. The adjusted R-square values of C1, C2 and C3 were 0.74, 0.56, 0.17, respectively. C4 drawn through $N = 1$ provides a reference for the control test data.

3.4.4 Decay characteristics of *B. subtilis*

The encouraging results obtained with the gram-negative strain necessitated experiments with a gram-positive strain and as such we chose *B. subtilis* for this purpose. The decay rates obtained with *B. subtilis* is promising, as shown in Fig. 3.5 and the duration of the experiment can once again be divided into three different regimes. The initial bacteria count was 160 CFU in 100 mL of deionized water and hence, the vertical axis of the plot in Fig. 3.5 has been normalized by 160. Following similar curve fitting methods, the data were found to best fit exponential models as described by equation 3.1. The best fit curves are denoted as C1, C2 and C3 in Fig. 3.5 and the values of the coefficient a and exponent b are shown in Table 3.2. Also in this plot, for the sake of clarity, we have excluded the datasets for extract volumes of 2 mL and 3 mL.

Regime I (0 to 15 min),once again, exhibits a very high rate of bacterial decay.

The curve C1 is steeper than F1 with a higher value of the exponent b. Regime II indicates a region of decreased decay characteristics spanning for a duration of 2.5 h after the initial decay regime. Interesting characteristics were obtained in the last stretch of these set of data, where, instead of decay, we observed bacterial re-growth after 165 min of continuous decay. Regime III indicates bacterial growth reflected by the positive value of the exponent b in Table 3.2.

The minimum bacteria count of 6 CFU in 100 mL of the sample was observed after 165 min using 10 mL of *Moringa oleifera* seed extract. The maximum bacteria count at the end of 6 h was observed to be 76 CFU for the 5 mL extract volume data as can be observed in Fig. 3.5. The bacterial re-growth resulted in a final count of 37 CFU at the end of the experiments for the 10 mL extract volume data. The data obtained using 2 mL and 3 mL extract volume in the samples are shown in Table 3.3. These data were excluded from the plots because they did not show significant decay characteristics. For both sets of experiment with 2 mL and 3 mL extract volumes the bacterial decay ceased after 45 min and remained around the value 80 CFU with some degree of variability over the rest of the time period. The control experiments revealed that there was no significant decay without the extract in water over the period of 6 h.

Moringa oleifera seed extract helped reduce a maximum 93.2% of *E. coli* and 96.2% of *B. subtilis* from non-turbid deionized water. The volume of extract found to be most effective in reducing bacterial load from water was 10 mL.

Figure 3.6 shows the turbidity levels of the samples after the addition of different volumes of extract. Data were taken after seeding the samples with bacteria. 2 mL extract volume increased the turbidity of water by 5 NTU while 10 mL increased the same by 70 NTU. The addition of bacteria alone did not appreciably raise the turbidity of the water samples.

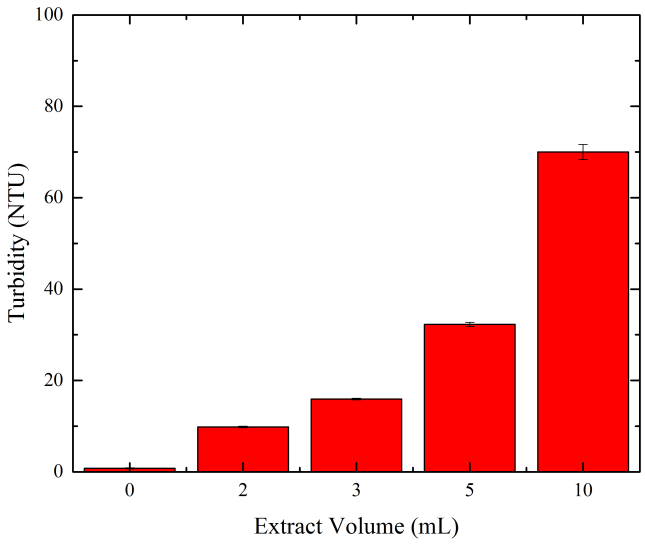


Figure 3.6: Increase in turbidity of sample water seeded with bacteria due to addition of different volumes of *Moringa oleifera* seed extract. Error bars indicate the standard errors from six replicate readings.

Table 3.2: Coefficients and exponents of the best-fit curves for the different regimes of decay of *B. subtilis*

Regime	*a*	95% Confidence Interval	*b*	95% Confidence Interval
I	0.87	(0.66, 1.07)	-21.22	(-34.11, -8.32)
II	0.48	(0.28, 0.67)	-3.14	(-5.05, -1.24)
III	0.12	(-0.07, 0.31)	1.15	(-0.76, 3.06)

Table 3.3: Temporal decay behavior of *B. subtilis*

Time (Min)	Extract volume, 2mL		Extract Volume, 3 mL	
	Mean bacteria count (CFU)	Standard Deviation (CFU)	Mean bacteria count (CFU)	Standard Deviation (CFU)
0	160	0	160	0
3	113	8	109	5
6	91	21	96	1
9	101	25	88	16
12	92	7	92	10
15	95	6	84	5
30	93	3	81	6
45	82	7	78	7
60	83	3	79	7
75	85	7	82	5
90	82	3	88	20
105	85	6	75	14
120	85	3	80	5
135	78	11	80	6
150	81	2	74	2
165	81	2	71	4
180	85	3	61	7
240	81	7	76	3
300	78	7	81	4
360	80	2	88	2

The bacteria decay curves obtained in the course of this work reinforces the effectiveness of *Moringa oleifera* as a natural, green alternative for water treatment. The antimicrobial activity of *Moringa oleifera* seed extract is due to the presence of a short, cationic protein within the seed. This protein, commonly known as the *Moringa oleifera* cationic protein (MOCP), has been shown to cause bacterial cell damage through rapid flocculation and fusion of their inner and outer membranes [34]. Our experiments reveal that this protein inhibits the bacterial growth and stronger concentrations facilitate higher bactericidal properties. However, this activity is dependent on the bacterial load and increased bacterial concentration would require a larger dose or a stronger concentration of the seed extract. From Fig. 3.1 we observed that 5 mL and 10 mL of the 5% concentrated extract was capable of causing bacterial cell death at a rate faster than their growth in presence of a nutrient rich medium. This necessitated the experiments to estimate the bacterial decay rates to understand the range of its effectiveness. Moreover, the initial inhibition experiments facilitated the determination of the proper bacterial load in the samples that the 5% concentrated extract would be effective in reducing.

The mechanism of coagulation-flocculation of the active flocculating protein is also well documented [28, 31]. The inactivation of bacteria in water occurs through a combination of aggregation due to flocculation and cell death due to membrane fusion. The three different regimes obtained in our case for the gram-negative bacteria, *E. coli*, can be explained by the variation of aggregation rates with time [35]. It is reported that in the presence of lysozyme, which is similar to MOCP in many aspects [36], the rates of aggregation were very high for another gram-positive bacteria, *Streptococcus sanguinis*, in the initial stages and slowed down after some time. We propose that the bactericidal property of the MOCP is linked to this coagulation-flocculation process. Dynamic light scattering experiments on model cell membranes show a rapid increase in ag-

gregation and fusion in presence of MOCP [34]. Shebek et al. reported a rapid increase in size starting from as early as 175 s which is in agreement with our results for both *E. coli* and *B. subtilis* where we observed a decrease in bacterial count as early as 3 min from the beginning of the decay experiments. The initial 15 min of the experiment can be linked to the initial regime of coagulation when the negative charge on the bacterial cell wall is neutralized allowing them to aggregate into microflocs. As the floc sizes increase, we observe over the next two and a half h, a reduction in the bacteria decay rate as evidenced by the reduced value of the exponent b between the regime I and II for *E. coli* (refer Table 3.1) and *B. subtilis* (refer Table 3.2). The data points towards a correlation between floc formation and the antimicrobial activity of MOCP. Regime I for both bacteria was characterized by rapid membrane fusion leading to cell death. Both the processes of membrane fusion and coagulation takes place simultaneously and we can consider them as competing forces while looking into bacterial decay. In the beginning, cell damage due to membrane fusion occurs unrestricted when the coagulating effects of *Moringa* seed extract augments the effectiveness of the process. However, as Shebek et al. points out [34] the need of multiple MOCP peptides for causing membrane fusion, the availability of protein decreases with time leading to the diminished decay rate in regime II. Moreover, during this period the flocculation activity decreases substantially [35] which adds to the sluggish rate of decay. In regime III we observed different characteristics for *E. coli* and *B. subtilis*. The *E. coli* decay rate subsided further, as evident from reduced value of exponent b (Table 3.1) which can be attributed to the progressive decrease in the amount of MOCP in the sample. By this time, the large aggregation of the bacterial cells owing to flocculation also hampers the interaction of the viable cells with the MOCP. We also predict that the intracellular substances along with the dead bacterial cells now present in the medium cause a major hindrance for MOCP to bind to the favorable sites of the viable cells.

In contrast, *B. subtilis* showed increased cell growth in regime III, completely negating the effect of the MOCP. The rate of growth follows an exponential curve steadily for the last 3 h of the experiment as given by the positive value of b in Table 3.2. The mechanism of this re-growth is not fully clear from existing studies in the literature [34, 28, 30, 32]. We try to explain this phenomenon by drawing a similarity of the MO cationic protein with lysozyme which is an antimicrobial cationic protein as well, usually found on mucosal surfaces. Both of these proteins are characterized by comparable molecular weights [36] (13 kDa for the dimer of MOCP and 14.3 kDa for lysozyme). Studies depicting the mechanism of antimicrobial activity of lysozyme have revealed that gram-positive bacteria can develop a resistance to the damaging effects of the protein through the modification of the peptidoglycan backbone of the cell wall [37]. Due to the similarities between these two proteins and the mechanisms in which they affect the bacterial cells, we infer that the re-growth observed in our case is a result of the modification of the peptidoglycan layer of *B. subtilis* which made it immune to the impairing effects of MOCP. Interestingly, after developing resistance, the ability of *B. subtilis* to proliferate was not hindered by the coagulating effects of the flocculating protein.

Our study suggests that the multi-layered, thick peptidoglycan layer in the gram-positive bacteria plays an important role in regulating bacterial decay using MOCP. The gram-negative *E. coli*, with its much thinner and single layer of peptidoglycan, is more susceptible to the detrimental effects of the protein. This is reflected in Table 3.3 where we observed a cessation of bacterial decay in the presence of 2 mL and 3 mL of extract for *B. subtilis*. These volumes of extract are insufficient to cause the decay rates observed in Fig. 3.5 which indicates to the potential existence of a threshold quantity which is vital for maintaining a continuous decay for gram-positive bacteria. In all of these cases it is apparent that the antimicrobial activity is dependent on the number of bacterial cells as

well as the quantity of extract used.

Previous studies in this field have always dealt with bacterial load reduction in model turbid water or naturally available river water of varying turbidities. This is understandable considering *Moringa oleifera* being very effective (92%-99%) as a natural coagulant for highly turbid water [38, 30, 28]. Olayemi and Alabi [39] reported a 65% decrease in total coliform after treating Asa river water for four days. Madsen et al. [40] reported a 99.99% *E. coli* reduction in Blue Nile river water with initial turbidity of 3200 FTU within one h. Less turbid waters from the White Nile river and other sources when treated with *Moringa oleifera*, affected only 90% reduction of *E. coli* in the first one h. In a recent study, Nkurunziza et al. [29] developed a linear correlation between the initial turbidity of sample water and *E. coli* removal by using water from rivers Kadahokwa and Rwamamba. At 450 NTU the maximum efficacy of 96% was observed by the researchers. The major mechanism for the reduction of bacterial load in turbid water is due to the physical removal of bacterial cells from the supernatant together with the majority of the turbidity causing sediments owing to coagulation. Using the correlation equation of Nkurunziza et al., the predicted value of *E. coli* removal is 90.06% for 0 NTU which is close to the maximum of 93.2% obtained in our study. Moreover, the predominant mechanism of bacteria inactivation in non-turbid water is membrane fusion caused by the MOCP. Interestingly, in all the cases of turbid water [39, 40, 29] bacterial re-growth occurred owing to mainly bacterial release from the sediments. Although our study also reports a re-growth of *B. subtilis*, the reasons are rather different because of fundamental differences in the processes as we have conjectured before.

3.5 Summary

In the current work we present a detailed study of the antimicrobial properties of *Moringa oleifera* seed extract. The bactericidal efficacy of the cationic protein has been evaluated for non-turbid water at low bacteria concentrations by establishing bacteria decay curves. Although the seed extract was not able to reduce the bacteria count in water below the limits set by the World Health Organization (0 CFU per 100 mL of potable water), it can still find use in conjunction with another treatment process or in cases of lesser contamination of potable water sources. This mode of water treatment, being entirely constituted of naturally available means, finds considerable interest in the field of sustainable and environment friendly materials to solve drinking water problems in the developing world. Further research into the mechanism of bacterial decay in presence of the *Moringa oleifera* cationic protein would lead to a better insight into the specific areas of application of this bio-inspired solution to the water treatment problem.

Bibliography

[1] Tehseen Aman, Asrar Ahmad Kazi, Muhammad Usman Sabri, and Qudsia Bano. Potato peels as solid waste for the removal of heavy metal copper (ii) from waste water/industrial effluent. *Colloids and Surfaces B: Biointerfaces*, 63(1):116–121, 2008.

[2] Alka Shukla, Yu-Hui Zhang, P Dubey, JL Margrave, and Shyam S Shukla. The role of sawdust in the removal of unwanted materials from water. *Journal of Hazardous Materials*, 95(1):137–152, 2002.

[3] C Namasivayam, D Sangeetha, and R Gunasekaran. Removal of anions, heavy metals, organics and dyes from water by adsorption onto a new activated carbon from jatropha husk, an agro-industrial solid waste. *Process Safety and Environmental Protection*, 85(2):181–184, 2007.

[4] Kyozo Suyama, Yoshitaka Fukazawa, and Yoshiyaki Umetsu. A new biomaterial, hen egg shell membrane, to eliminate heavy metal ion from their dilute waste solution. *Applied Biochemistry and Biotechnology*, 45(1):871–879, 1994.

[5] G Annadurai, RS Juang, and DJ Lee. Adsorption of heavy metals from water using banana and orange peels. *Water Science & Technology*, 47(1):185–190, 2003.

[6] Leandro S Oliveira, Adriana S Franca, Thiago M Alves, and Sonia DF Rocha. Evaluation of untreated coffee husks as potential biosorbents for treatment of dye contaminated waters. *Journal of Hazardous Materials*, 155(3):507–512, 2008.

[7] TO Jimoh et al. Sorption study of co (ii), cu (ii) and pb (ii) ions removal from aqueous solution by adsorption on flamboyant flower (delonix regia). *American Journal of Chemistry*, 2(3):165–170, 2012.

[8] Murielle Haussard, Ibrahim Gaballah, Philipe de Donato, Odile Barrès, and André Mourey. Removal of hydrocarbons from wastewater using treated bark. *Journal of the Air & Waste Management Association*, 51(9):1351–1358, 2001.

[9] Jinsong He and J Paul Chen. A comprehensive review on biosorption of heavy metals by algal biomass: materials, performances, chemistry, and modeling simulation tools. *Bioresource Technology*, 160:67–78, 2014.

[10] Nisha Goyal, SC Jain, and UC Banerjee. Comparative studies on the microbial adsorption of heavy metals. *Advances in Environmental Research*, 7(2):311–319, 2003.

[11] Shaobin Wang and Yuelian Peng. Natural zeolites as effective adsorbents in water and wastewater treatment. *Chemical Engineering Journal*, 156(1):11–24, 2010.

[12] Robert S Bowman. Applications of surfactant-modified zeolites to environmental remediation. *Microporous and Mesoporous Materials*, 61(1):43–56, 2003.

[13] Youbao Sun, Qinghua Fang, Junping Dong, Xiaowei Cheng, and Jiaqiang Xu. Removal of fluoride from drinking water by natural stilbite zeolite modified with fe (iii). *Desalination*, 277(1):121–127, 2011.

[14] Mahmut Ozacar and I Ayhan Sengil. The use of tannins from turkish acorns(valonia) in water treatment as a coagulant and coagulant aid. *Turkish Journal of Engineering and Environmental Sciences*, 26(3):255–263, 2002.

[15] Defang Zeng, Juanjuan Wu, and John F Kennedy. Application of a chitosan flocculant to water treatment. *Carbohydrate Polymers*, 71(1):135–139, 2008.

[16] Jorge Gutierrez, Catherine Barry-Ryan, and Paula Bourke. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology*, 124(1):91–97, 2008.

[17] RS Farag, ZY Daw, FM Hewedi, and GSA El-Baroty. Antimicrobial activity of some egyptian spice essential oils. *Journal of Food Protection®*, 52(9):665–667, 1989.

[18] Serge Ankri and David Mirelman. Antimicrobial properties of allicin from garlic. *Microbes and Infection*, 1(2):125–129, 1999.

[19] Rupesh Kumar Basniwal, Harpreet Singh Buttar, VK Jain, and Nidhi Jain. Curcumin nanoparticles: preparation, characterization, and antimicrobial study. *Journal of Agricultural and Food Chemistry*, 59(5):2056–2061, 2011.

[20] Fozia Farooq, Meenu Rai, Avinash Tiwari, Abdul Arif Khan, and Shaila Farooq. Medicinal properties of moringa oleifera: An overview of promising healer. *Journal of Medicinal Plants Research*, 6(27):4368–4374, 2012.

[21] Parul Sharma, Pushpa Kumari, MM Srivastava, and Shalini Srivastava. Removal of cadmium from aqueous system by shelled moringa oleifera lam. seed powder. *Bioresource Technology*, 97(2):299–305, 2006.

[22] Parul Sharma, Pushpa Kumari, MM Srivastava, and Shalini Srivastava. Ternary biosorption studies of cd (ii), cr (iii) and ni (ii) on shelled moringa oleifera seeds. *Bioresource Technology*, 98(2):474–477, 2007.

[23] M Helen Kalavathy and Lima Rose Miranda. Moringa oleiferaa solid phase extractant for the removal of copper, nickel and zinc from aqueous solutions. *Chemical Engineering Journal*, 158(2):188–199, 2010.

[24] SMI Sajidu, EMT Henry, I Persson, WRL Masamba, and D Kayambazinthu. ph dependence of sorption of cd 2+, zn 2+, cu 2+ and cr 3+ on crude water and sodium chloride extracts of moringa stenopetala and moringa oleifera,/i¿. *African Journal of Biotechnology*, 5(23), 2006.

[25] Mubeena Akhtar, S Moosa Hasany, MI Bhanger, and Shahid Iqbal. Sorption potential of moringa oleifera pods for the removal of organic pollutants from aqueous solutions. *Journal of Hazardous Materials*, 141(3):546–556, 2007.

[26] R Krishna Prasad. Color removal from distillery spent wash through coagulation using moringa oleifera seeds: Use of optimum response surface methodology. *Journal of hazardous materials*, 165(1):804–811, 2009.

[27] J Beltrán-Heredia and J Sánchez-Martín. Removal of sodium lauryl sulphate by coagulation/flocculation with moringa oleifera seed extract. *Journal of Hazardous Materials*, 164(2):713–719, 2009.

[28] Anselme Ndabigengesere, K Subba Narasiah, and Brian G Talbot. Active agents and mechanism of coagulation of turbid waters using moringa oleifera. *Water Research*, 29(2):703–710, 1995.

[29] T Nkurunziza, J Nduwayezu, E Banadda, and I Nhapi. The effect of turbidity levels and moringa oleifera concentration on the effectiveness of coagulation in water treatment. 2009.

[30] Anselme Ndabigengesere and K Subba Narasiah. Quality of water treated by coagulation using moringa oleifera seeds. *Water Research*, 32(3):781–791, 1998.

[31] Kebreab A Ghebremichael, KR Gunaratna, Hongbin Henriksson, Harry Brumer, and Gunnel Dalhammar. A simple purification and activity assay of the coagulant protein from moringa oleifera seed. *Water Research*, 39(11):2338–2344, 2005.

[32] Miquel Lürling and Wendy Beekman. Anti-cyanobacterial activity of moringa oleifera seeds. *Journal of Applied Phycology*, 22(4):503–510, 2010.

[33] Ping-Hsien Chuang, Chi-Wei Lee, Jia-Ying Chou, M Murugan, Bor-Jinn Shieh, and Hueih-Min Chen. Anti-fungal activity of crude extracts and essential oil of moringa oleifera lam. *Bioresource Technology*, 98(1):232–236, 2007.

[34] Kevin Shebek, Allen B Schantz, Ian Sines, Kathleen Lauser, Stephanie Velegol, and Manish Kumar. The flocculating cationic polypetide from moringa oleifera seeds damages bacterial cell membranes by causing membrane fusion. *Langmuir*, 31(15):4496–4502, 2015.

[35] ELLIS E Golub, JOSEPH Cheruka, B Boosz, C Davis, and D Malamud. A comparison of bacterial aggregation induced by saliva, lysozyme, and zinc. *Infection and Immunity*, 48(1):204–210, 1985.

[36] Uma V Katre, CG Suresh, M Islam Khan, and Sushama M Gaikwad. Structure–activity relationship of a hemagglutinin from moringa oleifera seeds. *International Journal of Biological Macromolecules*, 42(2):203–207, 2008.

[37] Kimberly M Davis and Jeffrey N Weiser. Modifications to the peptidoglycan backbone help bacteria to establish infection. *Infection and Immunity*, 79(2):562–570, 2011.

[38] Farooq Anwar, Sajid Latif, Muhammad Ashraf, and Anwarul Hassan Gilani. Moringa oleifera: a food plant with multiple medicinal uses. *Phytotherapy Research*, 21(1):17–25, 2007.

[39] AB Olayemi and RO Alabi. Studies on traditional water purification using moringa oleifera seeds. *African Study Monographs*, 15(3):135–142, 1994.

[40] M Madsen, J Schlundt, and EF Omer. Effect of water coagulation by seeds of moringa oleifera on bacterial concentrations. *The Journal of Tropical Medicine and Hygiene*, 90(3):101–109, 1987.

Chapter 4

Development of a sustainable water treatment device[1]

4.1 Overview

An innovative process of effective 'fishing, trapping and killing' of *Escherichia coli (E. coli)* in contaminated water samples is proposed here. A naturally occurring sugar, D-glucose, has been adsorbed onto blotting paper strips to facilitate the 'fishing' of *E. coli* cells by inducing their chemotactic response towards the chemoattractant (glucose) concentration gradient. By dipping paper strips laced with 0.1M glucose in sample contaminated water, we report a maximum bacterial removal efficiency of 99.5% after a 90 minute duration. The porous nature of the paper strip facilitates the 'trapping' of the bacterial cells within it's inherent porous network. Antimicrobial paper strips produced through the adsorption of bactericidal *Moringa oleifera* cationic protein (MOCP) were used to 'kill' the 'trapped' *E. coli* through prolonged contact. Finally, we have combined

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these individual processes into an integrated 'fishing, trapping, and killing' water treatment system, whereby the top and the bottom paper strips containing chemoattractant glucose are glued with the middle one containing the antimicrobial MOCP. Based on the results of the study, we anticipate that this approach can lead to the development of a new generation of inexpensive and portable water treatment devices which can passively remove and neutralize deleterious pathogens from water.

4.2 Introduction

Contaminated water sources continue to pose a major challenge in the twenty-first century towards providing clean and safe drinking water to the local communities[1, 2, 3, 4, 5, 6]. Freshwater resources all over the world are plagued by contaminants like traditional chemical wastes from agricultural and industrial sites, heavy metals, fecal coliforms, viruses as well as new-age pollutants such as endocrine disruptors and nitrosoamines [7, 8, 9, 10, 11]. Notably, potable water sources contaminated by deleterious pathogens pose significant public health concerns impacting nearly 1.8 billion people globally[12]. This is compounded by the limitations in implementing reliable water treatment solutions [13, 14, 15, 16] in limited resource communities where chlorine based disinfection procedures are still in vogue despite their considerable drawbacks and long-term health effects[17, 18, 19, 20, 21]. Emerging water treatment techniques using nanoparticles[22, 23] and photocatalysts[24, 25] show considerable promise for point-of-use purposes but their long term health impacts remain under continued scrutiny[26, 27]. Hence, there is a pressing need to remove harmful pathogens without creating toxic by products in compromised water sources. Effective point-of-use devices and techniques which are cheap, easily available, portable and non-toxic with minimal energy requirements can serve

this purpose towards providing clean and safe drinking water to the limited resource and marginalized communities.

Our approach towards removing bacteria from water uses the phenomenon of directed movement of bacterial cells (chemotaxis) towards a favorable chemical agent. The chemotactic behavior of bacterial cell dispersions has been widely studied[28, 29, 30, 31, 32]. In the presence of favorable chemical agents, called chemoattractants, the bacterial cells have been found to migrate towards regions of higher concentration along the chemoattracting gradient. Certain amino acids[33] (like aspartate and serine) and sugars[34] (like glucose, fructose and lactose) function as chemoattractants with varying degree of effectiveness depending upon it's concentration and the type of bacterial species[35, 29]. Such controlled behavior has been used in a number of applications ranging from increasing the efficiency of contact-killing surfaces[36] to regulated mixing in microfluidic systems[37] and payload delivery in micro-robotics[38]. Our strategy involved incorporating a suitable chemoattractant on blotting paper to lure *Escherichia coli (E. coli)*, the organism of interest in the study, out of water. Based on previous research[39, 40], D-glucose or dextrose which is a naturally occurring sugar, was chosen as the model chemoattractant. This approach is akin to the mechanism of 'fishing' using glucose as 'bait' to capture *E. coli* from water. We note that the use of a porous substrate like paper, instead of an impervious one, facilitates the 'trapping' of the 'fished' bacterial cells within the internal porous network. In the present study, we report a maximum bacterial removal of 99.5% from water using this 'fishing' procedure and subsequently 'trapping' them within the blotting paper.

It is important to eventually 'kill' the trapped bacteria for a complete water treatment solution. There are a number of natural materials, such as plant essential oils [41, 42], allicin[43] and curcumin[44] which have significant bactericidal properties. In our previous work focusing on nature inspired solutions

for water treatment, [45] we established the efficiency of *Moringa oleifera* seed extract in reducing bacterial loads in non-turbid water. *Moringa oleifera* is a multipurpose medicinal plant found in regions of tropical climate, especially in southeast Asia and Africa[46]. Besides having a high nutritional value, the crude *Moringa* seed extract has been shown to contain a water soluble flocculating cationic protein which has been characterized[47, 48, 49] and used in the treatment of highly turbid water sources[50, 51, 52]. Moreover, this *Moringa oleifera* cationic protein (MOCP), present in the seed extract, exhibits antimicrobial activity by causing bacterial cell death through the mechanism of membrane fusion [53, 54]. Naturally, owing to it's effectiveness and non-toxic nature, we proceeded with incorporating the antimicrobial MOCP on blotting paper to 'kill' the trapped bacteria through prolonged contact.

By using a simple combination of these mechanisms of 'fishing, trapping and killing', we present, in this study, a novel and sustainable water treatment technique, which is inherently made up of naturally available, biodegradable substances. The engineered natural system consists of three key components: highly porous, bio-degradable top and bottom blotting paper strips (Grade GB003, Whatman) laced with D-glucose and a similar central paper strip laced with *Moringa oleifera* seed extract. The principal ingredients, glucose and *Moringa oleifera* seeds, are harmless natural products which are conveniently available worldwide. The glucose works as a chemoattractant to lure the bacteria into the paper strips which are further killed after coming in contact with the MOCP present in the *Moringa oleifera* seed extract. We note that the use of blotting papers ensured a uniform capillary action facilitating the passive transport of fluid within our proposed system without the need of any additional pumps thereby reducing the overall cost of such a system. Our organism of interest was *E. coli*, a common Gram-negative bacteria of the fecal coliform group, present in abundance in compromised potable water sources. Herein, we demonstrate the

proof-of-concept study of this environment-friendly water treatment approach by successfully removing 99.5% of *E. coli* (strain K12) from water.

4.3 Materials and methods

4.3.1 Materials

Sample water used in all experiments was obtained from Barnstead™ Nanopure™ water purifier (Model No. D11971, Thermo Scientific, Waltham, Massachusetts). The model microorganism, *Escherichia coli* (K-12 strain), used in this study was obtained from New England Biolabs, Ipswich Massachusetts, USA. The blotting paper (Catalogue no. WHA10427804) used to prepare the paper strips was procured from Sigma Aldrich, Canada. D-glucose or dextrose (Catalogue no. 3260-1-70) was obtained from Caledon Laboratories Ltd, Ontario, Canada. Lauryl Tryptose Broth (LTB) (Catalogue no. CA90001-768) was used as the growth medium for *E. coli*. Luria Bertani (LB) agar (Catalogue no. CA90002-674) was used to plate the bacteria for the purposes of enumeration. All bacterial growth media were purchased from VWR, Canada. Sodium chloride (NaCl) obtained from Sigma Aldrich, Canada, was used to prepare a 0.85% concentrated solution which was used as a medium to suspend bacteria prior to fluorescent staining. Dry, unshelled *Moringa oleifera* seeds were procured from Purelife Herbs, USA. Standard issue cardboard and adhesives were used to prepare the device. 100 mL glass beakers (Catalogue no. 89000-200) from VWR, Canada were used to conduct the experiments. A two-pronged clamp (Catalogue no. 80063-610) from VWR, Canada was used to hold the paper strip combinations in place during all experiments. *E. coli* were stained for fluorescence using Live/Dead® BacLight™ bacterial viability kit (Catalogue no. L7012) from ThermoFisher Scientific, USA. Yellow-green fluorescent nanoparticles, 200 nm in diameter, (Cat-

alogue no. F8848, ThermoFisher Scientific, USA) were used to determine the protein adsorption on paper fibers. All bacterial growth media were autoclaved (Primus Sterilizer) according to experimental requirements.

4.3.2 Experimental setup

Figure 4.1 shows the experimental setup used to test the water treatment device. A two-prong extension clamp obtained from VWR Canada, was attached to a clamp stand to hold the cardboard scaffold containing the three paper strips in position. The height of the clamp was adjusted to keep only the lower edge of the bottom glucose strip (BGS) dipped inside sample water. Similar clamp arrangements were used in tracking the liquid/air interface in control experiments using single strips of NS and GS and in determining the efficiency of different GS. Glass beakers containing 60 mL of sample water were used in all the experiments.

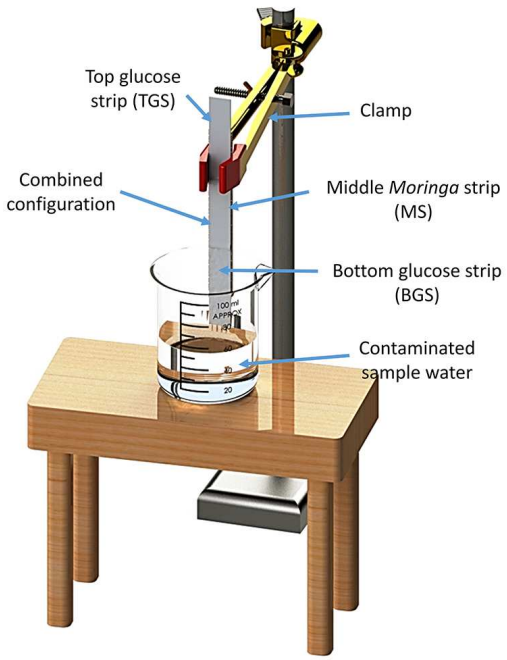

Figure 4.1: The experimental setup used to demonstrate the 'fishing, trapping and killing' of *E. coli*.

4.3.3 Imaging and characterization system

A DSLR camera (Nikon D5200) was used to capture all the images used in this study. To capture images of the fluorescence distribution on paper strips, a dark enclosure was created to prevent interference from ambient light. The light source and DSLR camera were affixed to the top of the enclosure while the paper strips were placed on the bottom wooden support (see Fig. 4.2). The mounted LED light source (Catalogue no. M490L3) used to observe the fluorescence from stained bacterial cells was attached to an adjustable collimator lens (Catalogue no. SM2P50-A) and connected to a LED driver (Catalogue no. LEDD1B) which controlled the current input. A 575 nm bandpass filter was used in conjunction with the camera to capture the fluorescence from stained *E. coli* cells. All lighting and filter accessories were obtained from Thorlabs, USA. The LED light source was attached with a collimator lens to narrow the light rays to our region of interest. A scanning electron microscope (Quanta 3D FEG, FEI) was used to characterize bacterial distributions within the paper strips. For the purpose of scanning electron microscopy (SEM) imaging, small portions of the paper strips were cut from the regions of interest and attached to SEM stubs using double sided carbon tape. The samples were then sputter coated with gold and imaged under high vacuum (10^{-5} Torr) and high voltage (20 kV) with the electron microscope. A confocal laser scanning microscope (Zeiss LSM 700) was used for the purposes described in this study. An optical upright microscope (Omax Epi-fluorescent Trinocular Compound Microscope) was used to capture bacterial distribution in the chemotaxis assay. The images of the bacterial dispersions in the chemotaxis assay were captured with an Omax digital camera mounted to an optical microscope using a ToupView software (ToupTek Photonics). A 0.5X reduction lens was connected to the camera using an appropriate adapter for reducing the image size to match the sensor of the digital camera.

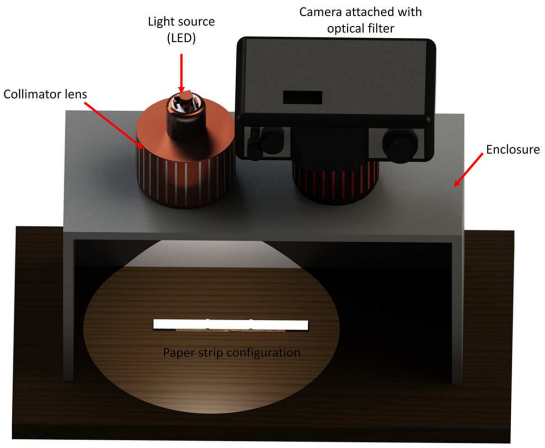

Figure 4.2: The fluorescence and imaging arrangement used to capture the liquid/air interface and bacteria cells within the paper strips. A 575 nm bandpass filter is attached to the camera to capture the fluorescent emissions from the stained bacteria on paper.

4.3.4 Bacterial growth characterization and fluorescent staining

E. coli was grown overnight (24 hours) in 100 mL of autoclaved LTB at 37°C inside an incubator under a constant shaking speed of 150 rpm. The resulting culture was enumerated using the plate counting technique following a serial dilution protocol. For the plate counting procedure, LB agar plates were used and the plated bacteria were incubated overnight (24 hours) at 37°C before enumeration. The concentration of bacterial culture prepared before each set of experiments was maintained at 10^9 CFU/mL. For staining purposes, the BacLight^{TM} bacterial viability kit was used. Prior to staining, *E. coli* cells were doubly washed in deionized (DI) water according to manufacturer guidelines and re-suspended in 0.85% sodium chloride solution (NaCl). The fluorescent dyes Syto 9 and propidium iodide (PI) were mixed in a 1:1 ratio and 3 μL of the mixture was added per mL of bacterial culture and mixed thoroughly using a vortex shaker. Before using it in the experiments, the stained culture was kept

in dark for 15 minutes at room temperature. Only freshly stained cultures were used in the experiments to ensure uniformity in results.

4.3.5 Preparation of glucose and *Moringa* paper strips

The blotting paper obtained from Sigma Aldrich, Canada, was cut into 55 mm by 14 mm strips. Solutions of D-glucose of five different concentrations (0.0001M, 0.001M, 0.01M, 0.1M and 1M) were prepared by adding requisite amounts of glucose in DI water. The paper strips were completely immersed in these solutions and kept on a shaker for 2 hours at room temperature. Thereafter, the wet strips were removed from the solutions and dried overnight at room temperature and used as glucose paper strips (GS) in subsequent experiments.

Moringa oleifera seed extract (5% concentration, w/v) was prepared using previously reported procedures [45]. As with glucose, the paper strips of the same dimensions were kept immersed in the seed extract for 2 hours under constant shaking. The strips were dried overnight prior to use in the experiments and are here onward referred to as *Moringa* paper strips (MS). The 2 hour immersion time was found to be sufficient for the adsorption of glucose and *Moringa oleifera* cationic protein (MOCP) onto the paper surfaces.

4.3.6 'Fishing' experiments with glucose paper strips (GS)

The chemoattracting effect of the modified paper strips (GS) was qualitatively analyzed. We modified the conventional capillary chemotaxis assay [39] by replacing the capillary containing chemoattractants with a GS on the glass slide, as shown in Fig. 3.1. The rest of the chemotaxis setup, as reported by Adler [39], was kept unchanged. It consists of a U-shaped spacer covered by a coverslip to create the bacterial chamber. At the start of the experiment, 20 μL of bacterial culture of concentration 10^9 CFU/mL was placed carefully inside the chamber.

Thereafter a small GS was inserted carefully inside the bacterial chamber. After 5 minutes, images of bacterial dispersion near the edge of the paper strip were obtained using the camera attached to an optical microscope. As a control experiment, a similar piece of paper without glucose (NS) was inserted in the assay and images of the region around it's edge were taken after 5 minutes.

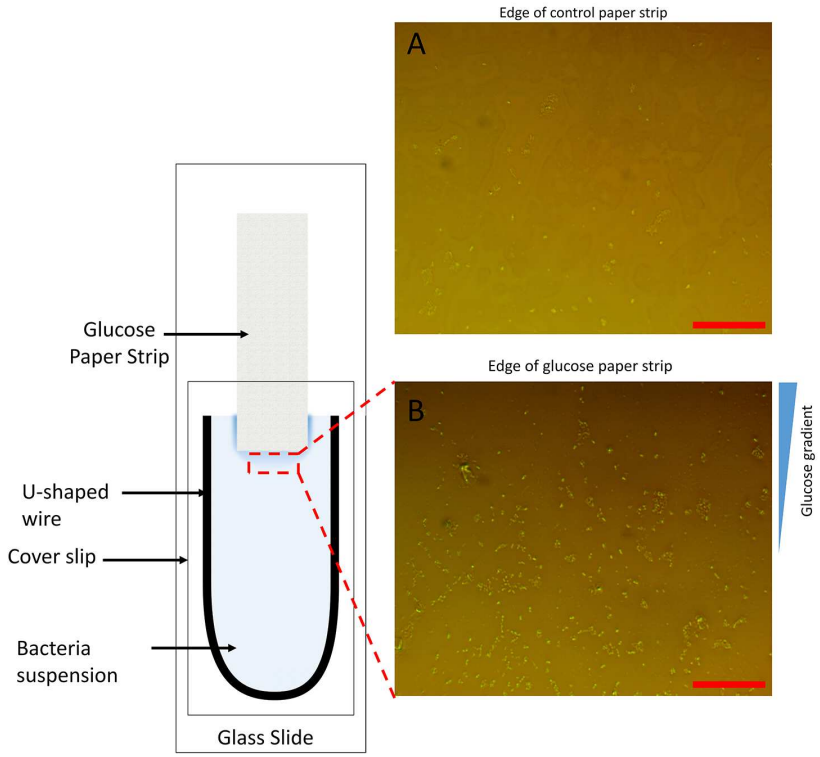

Figure 4.3: Schematic of the modified chemotaxis assay using paper strip treated with glucose solution. A) Microscope image showing bacterial distribution near the control paper (NS, without any glucose additive) edge after 5 minutes. B) *E. coli* cells near the edge of the glucose paper strip (GS) after 5 minutes. The glucose concentration gradient is shown. Images taken using a 40X brightfield objective. Scale bar is 50 μm.

An initial set of screening experiments were also performed to investigate the most effective glucose concentration for bacterial chemotaxis. Different GS were created using five different glucose concentrations (1M, 10^{-1}M, 10^{-2}M, 10^{-3}M, 10^{-4}M) and tested for effectiveness in water samples containing an initial bacterial concentration of 1.5 X 10^6 CFU/mL. The experimental procedure involved dipping the end of a vertical GS in 60 mL of model contaminated water sample

with a known initial bacterial concentration. A clamp was used to hold the paper strips in a stable position (similar to the arrangement shown in Fig. 4.1). The GS was removed from water after 90 minutes followed by the enumeration of bacterial count in the residual water sample by serial dilution and plate counting techniques. LB agar plates were incubated at 37°C for 24 hours before counting the number of colonies. Control experiments were conducted using a NS in lieu of the GS.

4.3.7 Characterization of *Moringa* paper strips (MS)

The presence of MOCP in the dried MS was verified using yellow-green fluorescent particles (200 nm in diameter, sulphate modified) carrying a negative surface charge. 10 mL solution of the fluorescing particles with a concentration of 2.65 X 10^{10} particles per mL was prepared using DI water. MS were completely immersed in solutions containing above mentioned fluorescent particles and kept overnight under constant shaking. This ensured sufficient time for the electrostatic adsorption of the particles to the surface of the MS due to the underlying positive charge of the MOCP coating. Thereafter, the samples were removed from the solution, dried and imaged using a confocal laser scanning microscope. Untreated paper strips (NS) were subjected to the same experimental procedure and used as control.

4.3.8 Experiments with the water treatment device

The water treatment device was constructed by a combination of three paper strips. Using a cardboard scaffold and appropriate adhesives, two GS and a MS of similar dimensions (55 mm by 14 mm), obtained through the processes described earlier, were fixed end to end with an approximately 5 mm overlap between them so that the entire water treatment system is composed of a bottom

glucose strip (BGS), a middle *Moringa* strip (MS) and a top glucose strip (TGS), as shown in Fig. 4.4. During experiments the cardboard scaffold was held with a clamp to ensure stability. Only the lower edge of the BGS was kept in contact with contaminated water. The detailed experimental setup is shown in Fig. 4.1. The contact between the individual strips at the overlap regions was properly monitored to ensure the smooth transition of water and bacteria along the strips. Fluorescent stained bacteria, obtained through the procedure described before, were used in these set of experiments by adding requisite amounts to the sample water. For all the experiments with the device, 60 mL water samples were used with an initial *E. coli* concentration of 1.5 X 10^6 CFU/mL. The experimental time was varied to track the liquid/air interface at different positions on the paper strips with the maximum experimental duration being 90 minutes. After the conclusion of the experiments, the configuration was removed from water and kept inside a dark enclosure for imaging, as shown in Fig 4.2. In order to confirm the presence of stained bacteria in the paper, we used an excitation wavelength of 490 nm and captured images using a 575 nm bandpass filter attached to the camera.

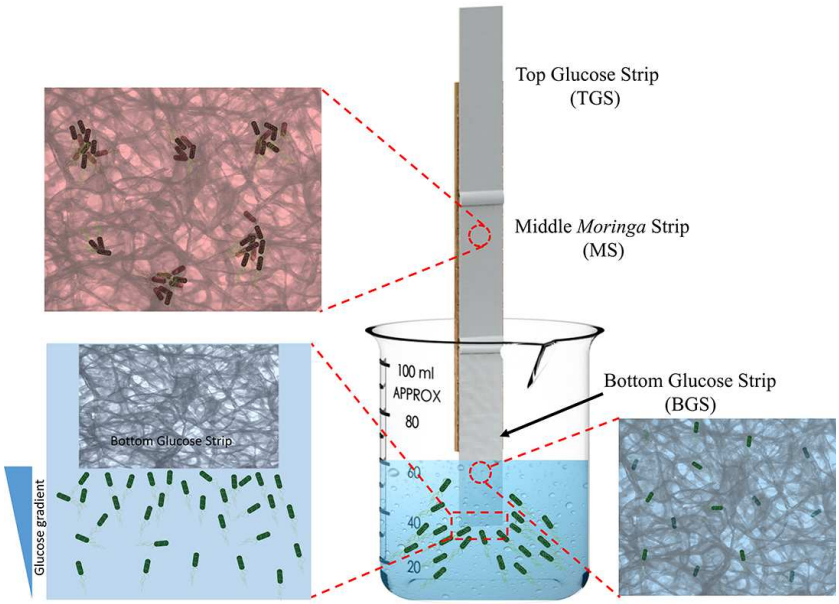

Figure 4.4: Schematic of the water treatment system showing three paper strips attached end to end with the lower edge of the paper strip dipped in sample water. The magnified images show *E. coli* moving through the pores of the blotting paper strips. Green color indicates viable, motile bacteria getting attracted and moving through the bottom glucose strip. The red color denotes the non-viable cells due to contact with the middle *Moringa* strip. The glucose concentration gradient is shown on the left.

4.4 Results and discussions

4.4.1 'Fishing' efficiency of Glucose Strips

We hypothesized that a chemoattractant concentration gradient in the bulk media surrounding the GS would cause the directed migration of bacterial cells towards and into the GS. A two-fold approach was undertaken to verify this chemoattracting efficiency of GS. Our findings with a modified chemotaxis assay (see Fig. 4.3) confirmed that a GS was more efficient in luring bacteria towards it than a NS. On being introduced into the bacterial suspension, the GS, unlike the NS, induces a chemoattractant concentration gradient (shown in Fig. 4.3B) through diffusion to promote the active movement of bacteria along the glucose

concentration gradient. This was confirmed by the presence of a higher density of *E. coli* cells in the area near the edge of the GS after 5 minutes as compared to the NS (Figure 4.3A).

Figure 4.5 shows that the paper strips dipped in 0.1M glucose solutions were the most efficient in removing *E. coli* from water among the different glucose concentrations (0.0001M, 0.001M, 0.01M, 0.1M and 1M) that were tested. The GS laced with 0.1M glucose removed 94.5% of the bacterial load, which was nearly three times more than the bacteria removal facilitated by the NS (Indicated by the 0 glucose concentration in Fig. 4.5). The 33.3% bacterial removal caused by the NS can be attributed to the inherent capillary transport of *E. coli* suspension inside the porous paper. 1M and 0.0001M glucose concentrations were least effective among the GS with 64% and 67% bacteria removal, respectively, while both the 0.01M and 0.001M GS facilitated greater than 80% bacteria removal. We note that existing literature [36, 34] suggest that the optimum glucose concentration to promote *E. coli* chemotaxis is in the vicinity of 10^{-3}M. Hence, we conclude that the strips dipped in 0.1M glucose solution are able to create a chemoattracting gradient similar to that caused by 10^{-3}M glucose solution in capillary chemotaxis assays.

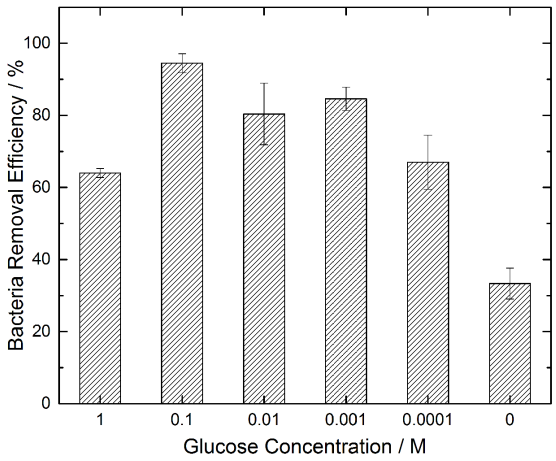

Figure 4.5: Bacteria removal efficiency of different glucose trips (GS) with varied glucose concentration. A zero glucose concentration refers to the control experiment with an untreated paper strip (NS). For all the cases, initial bacteria concentration was 1.5 X 10^6 CFU/mL. Error bars indicate the standard errors from three replicate experiments.

Figure 4.6 shows the efficiency of the 0.1M GS over a wide range of bacterial concentrations (10^2, 10^3, 10^4, 10^5 and 10^6 CFU/mL). The maximum bacteria removal efficiency of 99.5% was obtained for samples with an *E. coli* concentration of 10^4 CFU/mL. The results indicate the consistency of bacteria removal by GS, registering greater than 90% efficiency for all the considered cases. Even for the worst cases of 10^2 and 10^5 CFU/mL, only 7% of the initial contamination remained in the residual water. Hence, all subsequent experiments were conducted utilizing the most effective 0.1M GS.

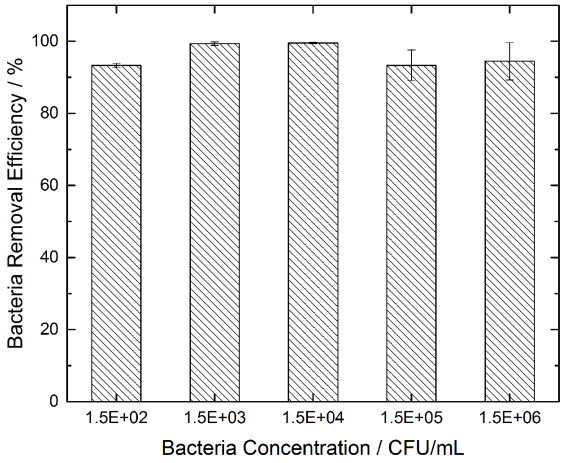

Figure 4.6: Efficiency of the 0.1M glucose strip (GS) against varying bacterial concentration. Error bars indicate the standard errors from three replicate experiments.

4.4.2 The 'trapping' process

The use of the porous paper substrate was found to be highly beneficial for the entire process. Besides facilitating the continuous migration of bacterial cells inside the paper strips through capillary action and hence removing them from the bulk contaminated water, it's permeable nature helps in 'trapping' the same bacterial cells within it's porous network. The pores being much larger than individual cells, do not hinder the movement of the cells away from the bulk water source. The preliminary experiments showed that the bacterial cells 'fished' from water migrated till the very end of the of the paper strips along with the liquid/air front (results not shown here for brevity). Hence, the presence of a porous network is fundamental to the 'trapping' process, which could not have been achieved otherwise by using a substrate of impermeable nature.

4.4.3 MOCP adsorption on paper

Previously, the antimicrobial properties of MOCP were achieved on the surfaces of silica microparticles[55] by simply suspending them in a solution of the *Moringa oleifera* seed extract. We hypothesized that the blotting paper strip would similarly be able to adsorb the water soluble MOCP from the extract and the protein would retain it's antimicrobial properties in a dried form. Figure 4.7 shows the images taken of a MS and NS after overnight incubation in a solution of yellow-green fluorescent particles. The confocal microscopy images show a very high particle density and hence, a higher fluorescence intensity in the MS compared to the NS. This served as a definitive proof of the presence of the MOCP on paper because the negatively charged fluorescent particles are greatly adsorbed due to the cationic charge of the underlying protein in the MS. The images clearly depict the distribution of the fluorescing particles along the paper fibers. The NS, devoid of any cationic surface charge, showed minimal nanoparticle retention owing to the interaction of surface forces.

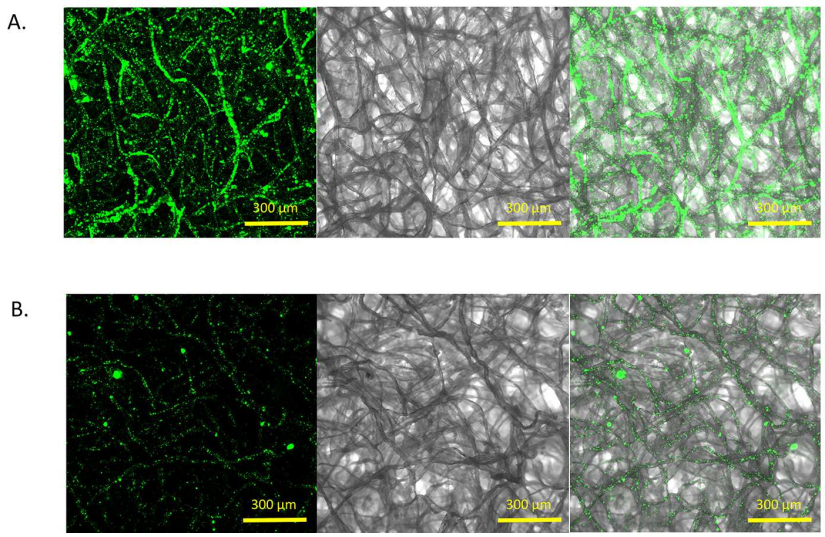

Figure 4.7: A) Intense fluorescence observed in the *Moringa* paper strip (MS). Negatively charged fluorescent nanoparticles (200 nm) are adsorbed onto the paper fiber surface owing to the presence of underlying MOCP coating. B) Very low fluorescence observed in case of the control paper strip (NS), indicating significantly lower adsorption of nanoparticles. The confocal (the images at the left) and the differential interference contrast (DIC) images (at the middle) were taken using a 5X objective and merged (the images on the right) to create a perspective of the position of the nanoparticles with respect to the paper fibers.

4.4.4 'Killing' of *E. coli*

Following the successful 'fishing' of bacteria from water, we proceeded towards 'killing' the 'trapped' *E. coli* on paper using the MOCP. Earlier studies with MOCP [45, 53] has established that the flocculating and antimicrobial characteristics of the protein takes effect approximately 3 minutes after initiation of contact. This feature necessitated a certain minimum length of the *Moringa* paper strip (MS) for the motile bacteria to be in contact with the cationic protein for more than 3 minutes. By tracking the progress of the liquid/air interface, we determined that a 55 mm long MS would provide a sufficient contact time for this purpose. Hence, the length of GS was also maintained at 55 mm to maintain uniformity. We anticipated that the *E. coli* cells after being 'fished' out of water by the BGS, would smoothly progress into the MS, where they would be neutralized owing

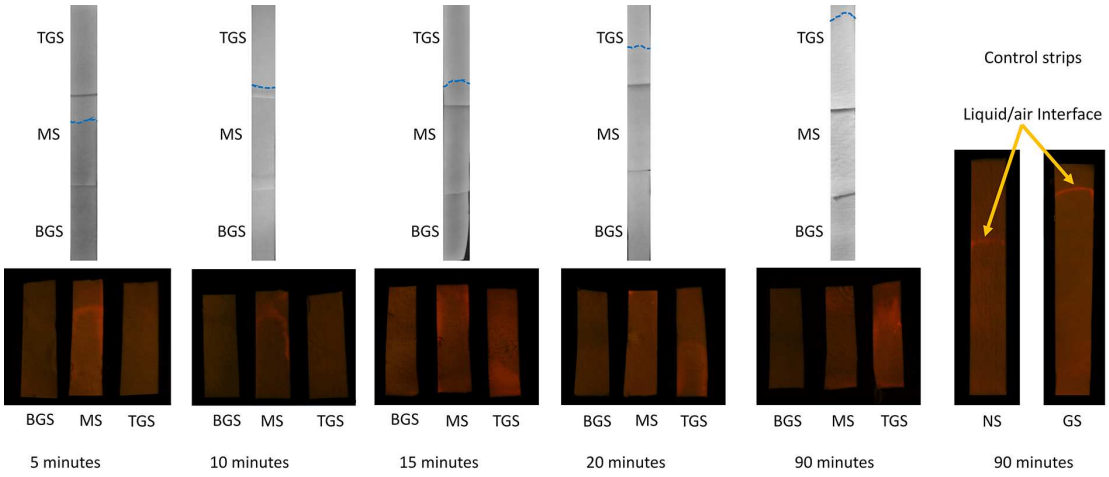

Figure 4.8: Position of the liquid/air interface (shown by dotted blue line) and the corresponding bacterial distribution at different experimental times are shown in the top and the bottom panel, respectively. Yellowish-orange fluorescence on the paper strips, in the bottom panel, indicates the distribution of bacteria at the interface and away from it during different stages of the experiment. Fluorescence on the control strips (GS and NS) is shown on the right after 90 minutes from the start of the experiment.

to prolonged (greater than 3 minutes) contact with MOCP adsorbed onto the paper fibers. The principal function of the TGS is in facilitating the transfer of any viable *E. coli* cells, which didn't get killed in contact with MOCP located in the MS, to the topmost paper strip and essentially trapping them further. The position of the liquid/air interface and the corresponding bacterial distributions was tracked at different experimental times. The images obtained from this set of experiments are shown in Fig. 4.8.

We observed that the *E. coli* cells move with the interface, rising steadily owing to capillary action through the porous paper strips, as indicated by the concentrated fluorescence intensity (emitted by the bacterial cells stained with fluorescing dyes Syto 9 and propidium iodide) tracing the liquid/air front in Fig. 4.8. We also performed control studies using two separate strips - NS and GS, each 140 mm long. During the entire 90 minute span of the front tracking experiments using control strips (images not shown), bacterial cells always followed the liquid/air interface. This is a classic example of motile bacterial cell behav-

ior, which can also be observed in droplets of bacterial suspensions[56] where the cells seek towards the droplet/air interface. The combined configuration of BGS-MS-TGS, on the other hand, indicated an interesting scenario. During the first 5 minutes, we observed a smooth front movement with the bacterial cells faithfully following the liquid/air interface, similar to that in the control experiments (see Fig. 4.8). However, as soon as the duration of the front within MS extended beyond three minutes, we observe a faint fluorescent trail behind the interface. After 10, 15 and 20 minutes of dipping time, we observed distinct regions with increasing fluorescence away from the interface and along the edges of the MS and TGS indicating bacterial cell deposits in these areas. Close to the end of the experiment i.e., at the 60 and 90 minutes mark, we witnessed a nearly complete separation of the bacterial cells from the interface, as is evident from the widespread prevalence of highly fluorescing zones away from the interface in the TGS. At the end of the experiment, the fluorescence intensity at the interface in the TGS is found to be very low as compared to areas away from it, indicating a much lower concentration of viable *E. coli* cells at the liquid/air interface area. This unusual behavior can be explained from the detailed scanning electron microscopy (SEM) images of the paper strips, which is discussed later. All the images shown in Fig. 4.8 are representative images as the exact location and the shape of the interface would vary due to the inherent nature of randomness in the porous paper strips.

We further confirm here that the fluorescence from paper strip is indeed due to 'trapped' bacterial cells. For this purpose, direct evidence was gathered from scanning electron microscopy (SEM) images of the used paper strips. The fluorescing zones were identified and viewed under high vacuum settings of the microscope. The SEM images were first captured for the control strip (NS), as shown in Fig. 4.9 to establish a reference data set. Naturally, under such high vacuum setting of the SEM, the *E. coli* cells were not able to retain their char-

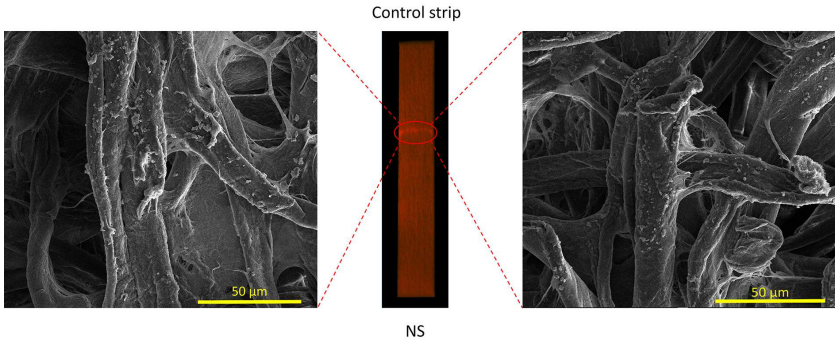

Figure 4.9: SEM images of the control strip (NS) showing bacterial deposition. The bacterial cells appear fragmented due to the damage caused by the high vacuum inside the SEM.

acteristic rod like shape and appear fragmented in the images. Images taken all along the fluorescing interface of the control strip reveals an uniform distribution of bacterial cells on the individual paper fibers. Except for the fluorescing liquid/air interface (see right panel in Fig. 4.8), we did not observe significant bacterial presence elsewhere on the NS.

Subsequently, the three paper strips of the combined configuration were subjected to a similar inspection after the 'fishing, trapping and killing' maneuver. Figure 4.10 displays the bacterial distributions observed in the TGS, MS and BGS, respectively. The BGS did not show any fluorescence because all the bacterial cells had moved onto the MS and TGS as is evident from the SEM images at the bottom panel of Fig. 4.10. In the ensuing investigation of the fluorescing regions for the middle MS we were able to observe large deposits of aggregated bacteria, as shown in Fig. 4.10. These agglomerates are typical consequences of the flocculating action of MOCP. As reported earlier [45, 53], the mechanisms of bacterial neutralization by the MOCP is due to a combination of flocculation and membrane fusion processes leading to non-viable aggregates of bacterial cells. As the liquid/air interface crosses over to the MS from the BGS, the MOCP, being water soluble, begins to interact with the bacterial cells at the interface. After the three minutes mark, both the flocculating and antimicrobial characteristics

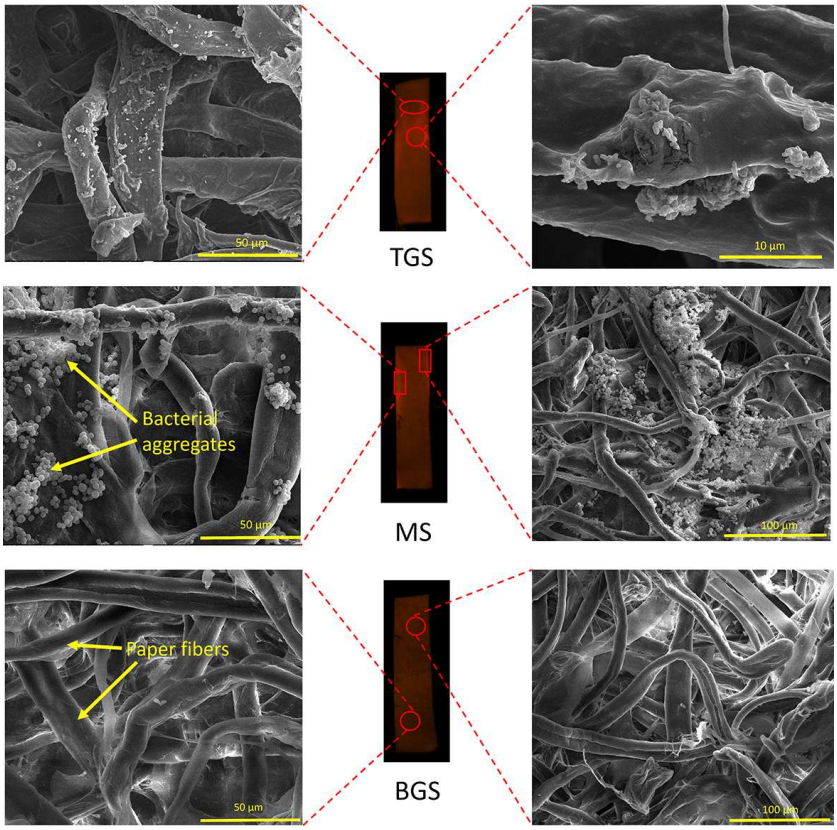

Figure 4.10: SEM images of the TGS, MS and BGS taken under high vacuum. The left and right panels have different magnifications.

start taking into effect and the bacterial surface charges are neutralized causing them to form clusters. Shortly after, the neutralized and clustered bacteria are unable to follow the liquid/air interface and begin to trail behind. The large clusters which become comparable to the pore sizes of the paper, are deposited towards the edges and away from the interface owing to hindrance caused by the paper fibers. The remaining viable *E. coli* cells continue to follow the interface up to the TGS where we observe a reduced fluorescence intensity. The smaller clusters are also pulled along up to the TGS where they lag behind the interface leaving a trail of non-viable bacteria cells spread across the TGS, as is evident from the images in the top panel of Fig. 4.10.

4.4.5 Analysis of fluorescence distribution on paper strips

One of the critical challenges in performing fluorescence experiments with paper strips containing both the bacteria and the *Moringa* extract is that the MOCP present in the extract also fluoresce along with the tagged bacteria within an overlapping wavelength. The inherent fluorescence of most proteins arises due to the presence of the amino acids tryptophan and tyrosine[57]. When excited by a light source at 295 nm, the emission spectra of purified MOCP is found to be dominated by tryptophan emissions[58] with a maxima at the wavelength of 343 ± 2 nm. Moreover, existing literature suggests that the emission spectrum of MOCP and Syto 9 (used for staining *Escherichia coli* cells) overlap around the 440 nm - 450 nm region [59, 58]. Therefore, if the paper strips are excited with a light of wavelength 490 nm (outside the emission spectrum of MOCP), we would only observe fluorescent emissions from Syto 9 and propidium iodide, devoid of any contribution from tryptophan and tyrosine, which would then confirm the presence and distribution of bacteria at the fluorescing regions. Based on the studies of Stocks [59] and Kwaambwa and Maikokera [58], we

also hypothesized that a 302 nm excitation wavelength would reveal a fluorescent distribution on the paper strips with contributions from both MOCP and the stained bacteria. This was confirmed in our observations displayed in Fig. 4.11. The paper strips illuminated by a 302 nm ultraviolet light source (UV Lamp, Cole Parmer, Canada) showed increased fluorescence when compared with those imaged using a 490 nm light source, the former indicating the additional fluorescent emissions from tryptophan residues within the protein. The protein, being water soluble, is transported from the MS to the TGS along with the bacterial aggregates leading to larger fluorescing zones within TGS (see images after 15 minutes dipping time in Fig. 4.11). The control strip (GS) did not exhibit any additional fluorescing zones under 302 nm excitation thereby confirming the absence of any protein. The fluorescence in the control strips is solely due to the fluorescent stained bacteria.

We note a couple of key points in analyzing the fluorescent distributions caused by the protein and fluorescent tagged bacterial cells. First and foremost, quantification of the live and dead cells from these images based on the fluorescence intensity distribution were not carried out because of the porous nature and the considerable thickness of the blotting paper strips. Also Syto 9 has been known to show strong bleaching effect [60] which considerably decreases the fluorescing intensity with time giving unrealistic results. Secondly, in our study we have used crude *Moringa oleifera* seed extract on paper. The *Moringa* seeds contain nearly 27% proteins by mass [50] of which only 1.2% is constituted by MOCP[53]. This introduces a lot of water soluble proteins unaccounted for within the seed extract, which do not possess any flocculating or antimicrobial properties but contribute towards the fluorescence owing to the ubiquitous presence of tryptophan and tyrosine. Although using a 302 nm excitation wavelength minimizes the fluorescence emissions of tyrosine, the overlapping tryptophan emissions from the other water soluble proteins, make it

almost impossible to distinguish the exact locations of MOCP in the used paper strips.

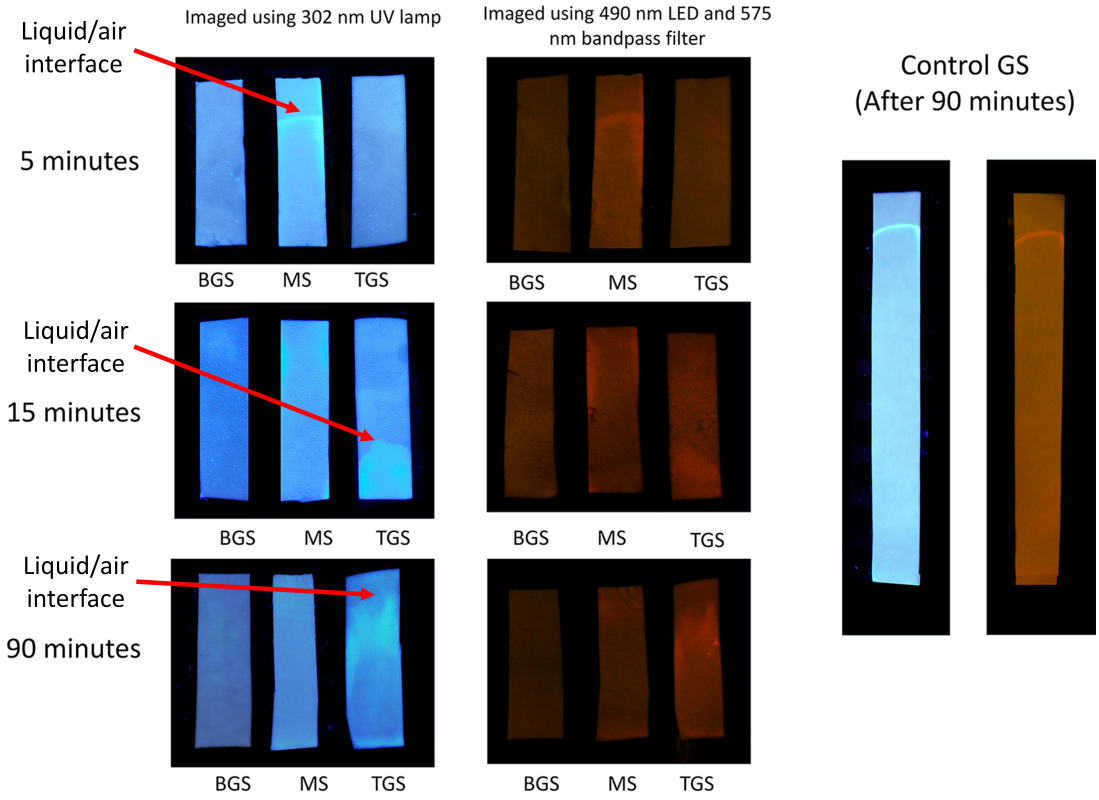

Figure 4.11: Fluorescence observed on paper under different set of excitation wavelengths for different time instants during the experiment. The intact configuration was dismantled and laid out as three separate strips - bottom glucose strip (BGS), middle Moringa strip (MS), and top glucose strip (TGS) for visualization purpose. In the first 5 minutes, the liquid/air interface has moved into MS, as indicated here with appreciable fluorescence for both the light sources. After 15 minutes, the front has moved in the TGS, as indicated by the position of the liquid/air interface. At the end of experiment (90 minutes), a broader spread of fluorescence indicates the presence of bacterial clusters along with water soluble MOCP that has transported from MS to TGS. Comparison between the the left and the right panels for the dismantled strips shows greater intensity of fluorescence under UV light indicating the contribution of MOCP. The control strip (GS) at the far right panel did not show any extra fluorescing regions under UV light confirming that no additional fluorescence arises in the absence of *Moringa* seed extract.

4.5 Summary

In the current work we present a novel technique of 'fishing, trapping and killing' bacterial cells which has a significant potential in point-of-use water treatment. This proof-of-concept study indicates that the process can be integrated into an innovative water treatment device constituted of easily available blotting paper strips laced with natural materials like glucose and *Moringa oleifera* seed extract. Using the chemotactic response of *E. coli* towards glucose, the device is capable of luring 99.5% of bacterial load from sample water onto the paper strip. The innovative configuration of top and bottom glucose strips help in trapping the bacteria within the paper substrate where the *E. coli* cells are killed by the centrally placed *Moringa* strips due to the bactericidal action of the MOCP, the presence of which has been confirmed using negatively charged fluorescent nanoparticles. The distribution of the fluorescent tagged *E. coli* cells on paper strips have been characterized through detailed SEM imaging. Overall, the findings demonstrate a sustainable water treatment technique using the antimicrobial activity of MOCP and the chemoattracting properties of glucose. Our paper device was effective in 'fishing, trapping and killing' *E. coli* in a very simple and efficient manner. One of the possibilities to deploy this kind of new water treatment device is in the form of a dip pen, where one can package the three strips as a cartridge of a pen and then dip such pen to treat a glass of water. The pen can be immersed in the water for a specified time to remove the bacterial contaminant. Figure **??** showcases one such possible design and we named it as a DipTreat device. DipTreat device would be a powerful hand-held tool for rapid treatment of water in remote communities and even for military applications, where one can dip this DipTreat device inside water collected in a glass (typically 250 mL size) for more than 3 min to get the water cleaned. The use of a chemoattractant to lure bacteria from water towards an antimicrobial protein in a paper based scaffold has

significant potential to be a 'clean and green' technology as well as an economic solution towards removing pathogens from potable water. The efficiency of the system can be enhanced by preparing the MS with higher extract concentrations thereby incorporating more protein molecules which would augment bacteria neutralization [45]. We anticipate that this technique can be generalized for a large number of deleterious waterborne pathogens by using a combination of effective chemoattractants and contact killing antimicrobials which can be easily integrated with paper scaffolds. Moreover, this technique can be expanded to remove pathogens from other contaminated beverages like milk and fruit juices. This strategy can also find use towards targeted pathogen removal from water or any liquid media through the use of species specific chemoattractants. The use of glucose and *Moringa oleifera* in our case, ensures that no harmful chemicals are leached into the sample water, which could otherwise have an adverse impact to public health. We envision that this technique can be converted into a compact point-of-use product for water treatment in limited resource communities across the globe.

Bibliography

[1] Mark A Shannon, Paul W Bohn, Menachem Elimelech, John G Georgiadis, Benito J Marinas, and Anne M Mayes. Science and technology for water purification in the coming decades. *Nature*, 452(7185):301–310, 2008.

[2] René P Schwarzenbach, Beate I Escher, Kathrin Fenner, Thomas B Hofstetter, C Annette Johnson, Urs Von Gunten, and Bernhard Wehrli. The challenge of micropollutants in aquatic systems. *Science*, 313(5790):1072–1077, 2006.

[3] Naga Siva Kumar Gunda, Ravi Chavali, and Sushanta K Mitra. A hydrogel based rapid test method for detection of escherichia coli (e. coli) in contaminated water samples. *Analyst*, 141(10):2920–2929, 2016.

[4] Naga Siva Kumar Gunda, Selvaraj Naicker, Sujit Shinde, Sanjay Kimbahune, Sandhya Shrivastava, and Sushanta Mitra. Mobile water kit (MWK): A smartphone compatible low-cost water monitoring system for rapid detection of total coliform and *E. coli*. *Analytical Methods*, 6(16):6236–6246, 2014.

[5] Ravi Chavali, Naga Siva Kumar Gunda, Selvaraj Naicker, and Sushanta K Mitra. Detection of escherichia coli in potable water using personal glucose meters. *Analytical Methods*, 6(16):6223–6227, 2014.

[6] Naga Siva Kumar Gunda, Selvaraj Naicker, Maryam S Ghoraishi, Subir Bhattacharjee, Thomas G Thundat, and Sushanta K Mitra. Microspot with integrated wells (msiw) for the detection of e. coli. *ASME 2013 11th International Conference on Nanochannels, Microchannels, and Minichannels*, pages V001T10A001–V001T10A001, 2013.

[7] Ian R Falconer, Heather F Chapman, Michael R Moore, and Geetha Ranmuthugala. Endocrine-disrupting compounds: a review of their challenge to sustainable and safe water supply and water reuse. *Environmental Toxicology*, 21(2):181–191, 2006.

[8] Jacek Nawrocki and Przemysław Andrzejewski. Nitrosamines and water. *Journal of Hazardous Materials*, 189(1):1–18, 2011.

[9] Andreas Musolff, Sebastian Leschik, Frido Reinstorf, Gerhard Strauch, and Mario Schirmer. Micropollutant loads in the urban water cycle. *Environmental Science & Technology*, 44(13):4877–4883, 2010.

[10] Mark J Benotti, Rebecca A Trenholm, Brett J Vanderford, Janie C Holady, Benjamin D Stanford, and Shane A Snyder. Pharmaceuticals and endocrine disrupting compounds in us drinking water. *Environmental Science & Technology*, 43(3):597–603, 2008.

[11] Ravi Chavali, Naga Siva Kumar Gunda, Selvaraj Naicker, and Sushanta K Mitra. Rapid detection of fluoride in potable water using a novel fluorogenic compound 7-o-tert-butyldiphenylsilyl-4-methylcoumarin. *Analytical Chemistry Research*, 6:26–31, 2015.

[12] WHO/UNICEF Joint Water Supply and Sanitation Monitoring Programme and World Health Organization. *Progress on Drinking Water and Sanitation: 2014 Update*. World Health Organization, 2014.

[13] Manfred Clara, Birgit Strenn, Oliver Gans, Elena Martinez, Norbert Kreuzinger, and Helmut Kroiss. Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. *Water Research*, 39(19):4797–4807, 2005.

[14] Vinka A Oyanedel-Craver and James A Smith. Sustainable colloidal-silver-impregnated ceramic filter for point-of-use water treatment. *Environmental Science & Technology*, 42(3):927–933, 2007.

[15] John Bratby. *Coagulation and Flocculation in Water and Wastewater Treatment*. IWA publishing, 2006.

[16] John J Macauley, Zhimin Qiang, Craig D Adams, Rao Surampalli, and Melanie R Mormile. Disinfection of swine wastewater using chlorine, ultraviolet light and ozone. *Water Research*, 40(10):2017–2026, 2006.

[17] David L Sedlak and Urs von Gunten. The chlorine dilemma. *Science*, 331(6013):42–43, 2011.

[18] Mark J Nieuwenhuijsen, Mireille B Toledano, Naomi E Eaton, John Fawell, and Paul Elliott. Chlorination disinfection byproducts in water and their association with adverse reproductive outcomes: a review. *Occupational and Environmental Medicine*, 57(2):73–85, 2000.

[19] Stuart W Krasner, Howard S Weinberg, Susan D Richardson, Salvador J Pastor, Russell Chinn, Michael J Sclimenti, Gretchen D Onstad, and Alfred D Thruston. Occurrence of a new generation of disinfection byproducts. *Environmental Science & Technology*, 40(23):7175–7185, 2006.

[20] Michael J Plewa, Elizabeth D Wagner, Deborah H Metz, Ramesh Kashinkunti, Katherine J Jamriska, and Maria Meyer. Differential toxic-

ity of drinking water disinfected with combinations of ultraviolet radiation and chlorine. *Environmental Science & Technology*, 46(14):7811–7817, 2012.

[21] H Jiang, C Adams, N Graziano, A Roberson, M McGuire, and D Khiari. Occurrence and removal of chloro-s-triazines in water treatment plants. *Environmental Science & Technology*, 40(11):3609–3616, 2006.

[22] T Pradeep et al. Noble metal nanoparticles for water purification: a critical review. *Thin Solid Films*, 517(24):6441–6478, 2009.

[23] Theresa A Dankovich and Derek G Gray. Bactericidal paper impregnated with silver nanoparticles for point-of-use water treatment. *Environmental Science & Technology*, 45(5):1992–1998, 2011.

[24] Meng Nan Chong, Bo Jin, Christopher WK Chow, and Chris Saint. Recent developments in photocatalytic water treatment technology: a review. *Water Research*, 44(10):2997–3027, 2010.

[25] XZ Li, H Liu, LF Cheng, and HJ Tong. Photocatalytic oxidation using a new catalyst tio2 microsphere for water and wastewater treatment. *Environmental Science & Technology*, 37(17):3989–3994, 2003.

[26] Peter HM Hoet, Irene Brüske-Hohlfeld, and Oleg V Salata. Nanoparticles–known and unknown health risks. *Journal of Nanobiotechnology*, 2(1):12, 2004.

[27] Susan D Richardson, Alfred D Thruston, Timothy W Collette, Kathleen S Patterson, Benjamin W Lykins, and John C Ireland. Identification of tio2/uv disinfection byproducts in drinking water. *Environmental Science & Technology*, 30(11):3327–3334, 1996.

[28] Wenyuan Shi, Thilo Köhler, and David R Zusman. Chemotaxis plays a role in the social behaviour of myxococcus xanthus. *Molecular Microbiology*, 9(3):601–611, 1993.

[29] Rina Barak, I Nur, and Y Okon. Detection of chemotaxis in azospirillum brasilense. *Journal of Applied Bacteriology*, 54(3):399–403, 1983.

[30] DANIEL Zicha, Graham A Dunn, and ALASTAIR F Brown. A new direct-viewing chemotaxis chamber. *Journal of Cell Science*, 99(4):769–775, 1991.

[31] Vagn Leick and Jannie Helle. A quantitative assay for ciliate chemotaxis. *Analytical Biochemistry*, 135(2):466–469, 1983.

[32] Tanvir Ahmed, Thomas S Shimizu, and Roman Stocker. Microfluidics for bacterial chemotaxis. *Integrative Biology*, 2(11-12):604–629, 2010.

[33] Robert Mesibov and Julius Adler. Chemotaxis toward amino acids in escherichia coli. *Journal of Bacteriology*, 112(1):315–326, 1972.

[34] Julius Adler, Gerald L Hazelbauer, and MM Dahl. Chemotaxis toward sugars in escherichia coli. *Journal of Bacteriology*, 115(3):824–847, 1973.

[35] Thoyd Melton, Philip E Hartman, John P Stratis, Timothy L Lee, and Alan T Davis. Chemotaxis of salmonella typhimurium to amino acids and some sugars. *Journal of Bacteriology*, 133(2):708–716, 1978.

[36] Rishabh Jain, Nancy G. Faith, Andrew Milkowski, Kevin Nelson, David Busche, David M. Lynn, Charles J. Czuprynski, and Nicholas L. Abbott. *Angewandte Chemie*, 128:5792–5796, 2016.

[37] Min Jun Kim and Kenneth S Breuer. Controlled mixing in microfluidic systems using bacterial chemotaxis. *Analytical Chemistry*, 79(3):955–959, 2007.

[38] Dongwook Kim, Albert Liu, Eric Diller, and Metin Sitti. Chemotactic steering of bacteria propelled microbeads. *Biomedical Microdevices*, 14(6):1009–1017, 2012.

[39] J Adler. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by escherichia coli. *Microbiology*, 74(1):77–91, 1973.

[40] Howard C Berg and Douglas A Brown. Chemotaxis in escherichia coli analysed by three-dimensional tracking. *Nature*, 239(5374):500–504, 1972.

[41] Jorge Gutierrez, Catherine Barry-Ryan, and Paula Bourke. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology*, 124(1):91–97, 2008.

[42] RS Farag, ZY Daw, FM Hewedi, and GSA El-Baroty. Antimicrobial activity of some egyptian spice essential oils. *Journal of Food Protection*, 52(9):665–667, 1989.

[43] Serge Ankri and David Mirelman. Antimicrobial properties of allicin from garlic. *Microbes and Infection*, 1(2):125–129, 1999.

[44] Rupesh Kumar Basniwal, Harpreet Singh Buttar, VK Jain, and Nidhi Jain. Curcumin nanoparticles: preparation, characterization, and antimicrobial study. *Journal of Agricultural and Food Chemistry*, 59(5):2056–2061, 2011.

[45] Saumyadeb Dasgupta, Naga Siva Kumar Gunda, and Sushanta K Mitra. Evaluation of the antimicrobial activity of moringa oleifera seed extract as a sustainable solution for potable water. *RSC Advances*, 6(31):25918–25926, 2016.

[46] Farooq Anwar, Sajid Latif, Muhammad Ashraf, and Anwarul Hassan Gilani. Moringa oleifera: a food plant with multiple medicinal uses. *Phytotherapy Research*, 21(1):17–25, 2007.

[47] Kebreab A Ghebremichael, KR Gunaratna, Hongbin Henriksson, Harry Brumer, and Gunnel Dalhammar. A simple purification and activity assay of the coagulant protein from moringa oleifera seed. *Water Research*, 39(11):2338–2344, 2005.

[48] Ursula Gassenschmidt, Klaus D Jany, Tauscher Bernhard, and Heinz Niebergall. Isolation and characterization of a flocculating protein from moringa oleifera lam. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1243(3):477–481, 1995.

[49] M Broin, C Santaella, S Cuine, K Kokou, G Peltier, and T Joet. Flocculent activity of a recombinant protein from moringa oleifera lam. seeds. *Applied Microbiology and Biotechnology*, 60(1-2):114–119, 2002.

[50] Anselme Ndabigengesere, K Subba Narasiah, and Brian G Talbot. Active agents and mechanism of coagulation of turbid waters using moringa oleifera. *Water Research*, 29(2):703–710, 1995.

[51] Anselme Ndabigengesere and K Subba Narasiah. Quality of water treated by coagulation using moringa oleifera seeds. *Water Research*, 32(3):781–791, 1998.

[52] T Nkurunziza, J Nduwayezu, E Banadda, and I Nhapi. The effect of turbidity levels and moringa oleifera concentration on the effectiveness of coagulation in water treatment. 2009.

[53] Kevin Shebek, Allen B Schantz, Ian Sines, Kathleen Lauser, Stephanie Velegol, and Manish Kumar. The flocculating cationic polypetide from moringa

oleifera seeds damages bacterial cell membranes by causing membrane fusion. *Langmuir*, 31(15):4496–4502, 2015.

[54] Miquel Lürling and Wendy Beekman. Anti-cyanobacterial activity of moringa oleifera seeds. *Journal of Applied Phycology*, 22(4):503–510, 2010.

[55] Huda A Jerri, Kristin J Adolfsen, Lauren R McCullough, Darrell Velegol, and Stephanie B Velegol. Antimicrobial sand via adsorption of cationic moringa oleifera protein. *Langmuir*, 28(4):2262–2268, 2011.

[56] TV Kasyap, Donald L Koch, and Mingming Wu. Bacterial collective motion near the contact line of an evaporating sessile drop. *Physics of Fluids (1994-present)*, 26(11):111703, 2014.

[57] EA Burstein, NS Vedenkina, and MN Ivkova. Fluorescence and the location of tryptophan residues in protein molecules. *Photochemistry and Photobiology*, 18(4):263–279, 1973.

[58] HM Kwaambwa and R Maikokera. A fluorescence spectroscopic study of a coagulating protein extracted from moringa oleifera seeds. *Colloids and Surfaces B: Biointerfaces*, 60(2):213–220, 2007.

[59] SM Stocks. Mechanism and use of the commercially available viability stain, baclight. *Cytometry Part A*, 61(2):189–195, 2004.

[60] Philipp Stiefel, Sabrina Schmidt-Emrich, Katharina Maniura-Weber, and Qun Ren. Critical aspects of using bacterial cell viability assays with the fluorophores syto9 and propidium iodide. *BMC Microbiology*, 15(1):1, 2015.

Chapter 5

Conclusion

Different aspects of improving existing water quality monitoring and development of novel water treatment techniques have been explored in this study.

The first objective of this research was to develop a portable concentrating device to improve point-of-use microfluidics based pathogen detection systems.

In pursuance of this objective, commercially available hollow fiber filter modules which can be operated manually, were selected as the basic device to perform the concentration procedure based on their re-usable and portable nature. Employing the procedure of tangential filtration through manually operated syringes, an initial investigation was carried out using latex microspheres as model organisms. This examination provided an estimate of the optimum working conditions to be employed for actual bacteria recovery tests and also revealed the importance of an elution step in the process. Model indicator bacteria, *E. coli* (K-12 strain), was then used to perform a set of preliminary experiments under different operating conditions. Better recovery efficiencies were observed for the hollow fiber membranes with lower anisotropy (asymmetry) and pore sizes. These observations indicated that to achieve an efficient concentrating device, a suitable elution fluid was needed, which would reduce bacterial adhesion to the membrane walls and keep the cells in suspension. Due to their unique struc-

ture and properties, surfactants like Tween 20, Tween 80 and dispersants like SHMP were tested as elution fluids. Among the different combination of surfactants and dispersant tested in this study, a 1:2:1 mixture of Tween 20 (0.01%, vol/vol), Tween 80 (0.1%, vol/vol) and SHMP (0.01%, by weight) was found to be the most efficient elution fluid providing a high bacterial recovery efficiency of 98%. In addition to developing the optimized protocol for the use of the elution fluid, the concentration process was able to reduce the sample volume to a few hundred microliters and retain a viable concentration of target microorganism to accommodate the miniature detection devices.

Possible implications: The development of such highly efficient elution fluids can help to bypass complicated steps of filter pre-treatment and sample amendment which are currently in practice and have their own set of drawbacks. This system can be easily integrated to a whole host of detection devices as sample transfer is facilitated by simple syringe connections reducing the chance of sample contamination. Hence, through optimized protocols, this technique can address water quality issues through proper point-of-use monitoring.

The second objective of this thesis was to address the problem of water treatment by using the seed extract of the naturally available *Moringa oleifera* plant. In this part of the thesis, a nature inspired solution was chosen to focus towards an environment-friendly approach. *Moringa oleifera* seed extract contains the antimicrobial MOCP which causes bacterial cell death through the fusion of inner and outer cell membranes. We targeted towards reducing bacterial loads in non-turbid potable water by using the crude seed extract. A crude seed extract was produced by suspending powdered unshelled seeds in DI water and subsequently filtering the suspension through 0.45 μm membrane filter. An initial set of bacterial growth inhibition tests in solid (agar) and liquid media (LTB) were conducted using the seed extract and model bacteria *E. coli*, where parameters like extract concentration, extract volume and bacterial concentrations were var-

ied and their impacts identified. These were followed by simple jar tests to evaluate the temporal decay behavior of Gram-negative *E. coli* and Gram-positive *B. subtilis* in non-turbid water under the effect of different volumes and concentrations of extract. We observed that the MOCP rapidly took effect, causing bacterial cell death as early as 3 minutes from the start of the experiments. The decay characteristics for both bacterial species were found to follow exponential trends with three distinct regimes. Using a 10 mL volume of seed extract (5% concentrated), a maximum bacterial decay of 93.2% for *E. coli* and 96.2% for *B. subtilis* was observed over a span of 6 hours. The results indicated that bacterial decay was enhanced by the flocculating properties of MOCP as well, leading to an increased rate of decay in the initial regime for both the bacterial species under examination. The *Moringa* seed extract is a promising solution for cheap and sustainable water treatment with the added advantage of being harmless in nature. Although it is unable to remove bacterial contaminants completely, its ease of use along with the widespread availability of *Moringa oleifera* plants can address the preliminary household water treatment needs for limited resource communities.

Possible implications: Certain challenges such as residual turbidity in water after treating with *Moringa* seed extract need to be addressed as the organic residues may spur further contamination. The antibacterial properties of MOCP can be applied to create antibacterial surfaces and coatings which can replace the use of harmful chemicals. The development of methods and devices which can integrate natural substances like MOCP for point-of-use water treatment can lead to sustainable techniques and go a long way towards improving the quality of life in limited resource settings.

The third objective of this research was focused towards the development of a sustainable water treatment system targeted towards microbial contaminants.

We have demonstrated a novel and simple approach for rapid removal of *E.*

coli form water through a combination of 'fishing, trapping and killing' processes. Using paper strips laced with chemoattractant glucose molecules, the chemotactic response of bacterial cells is triggered whereby luring them onto the porous paper strips, away from bulk contaminated water. The inherent porosity of the paper helps in trapping the fished *E. coli* cells within the internal porous network. Further investigations have demonstrated that these trapped bacterial cells can be killed through prolonged contact (more than 3 minutes) by transferring them onto a paper strip laced with *Moringa oleifera* seed extract. The experimental results indicate that using an end-to-end combination of three blotting paper strips with the top and bottom coated with glucose and the middle one coated with *Moringa* seed extract, a maximum of 99.5% of the bacterial load can be removed in 90 minutes. Our results also indicate that the capillary action induced by the porous paper ensures the smooth transfer of the bacterial cells from the sample water and inside the paper strips. The main advantage of this process is that it uses easily available natural substances and bio-degradable paper, leaving no harmful residues in the treated water. This unique and environment-friendly process has tremendous potential as a rapid, point-of-use water treatment system and can be applicable towards broader implementations in communities.

Possible implications: The development of different combinations of suitable chemoattractants and antimicrobials can lead us to build a new generation of devices for simple and easy removal of pathogenic bacteria, virus and parasites from contaminated water sources.

5.1 Future work and recommendations

Antibacterial surfaces

The antimicrobial properties of MOCP can be implemented on any kind of surfaces in a very simple manner as shown in the current work. This can find potential application in devising contact killing surfaces which have a multitude of applications in hospitals and food packaging industry. MOCP can be adsorbed onto almost any surface with varying degree of effectiveness due to its cationic nature. By simply pouring requisite amounts of the concentrated seed extract on the concerned surface and drying it, a layer of antibacterial coating can be implemented in an inexpensive manner. To further improve its effectiveness, the MOCP must be extracted and purified from the seed extract through protein isolation and purification processes. The MOCP being water soluble, can also be harnessed onto the surface through a similar approach.

Targeted water treatment devices

Future work may be needed to quantify the viable and non-viable bacterial cells after the 'fishing trapping and killing' process, as described in Chapter 4. Further modifications to the device may be needed to improve and quantify the contact killing efficiency of the *Moringa* paper strips. As most bacterial species show chemotactic behavior, this general technique can be implemented to remove only specific pathogenic strains from water using a suitable chemoattractant corresponding to the target bacterial species. These targeted devices can be focused towards areas, where the water sources are contaminated by specific bacterial strains. Moreover, the fundamental principle remaining the same, these devices can be used to treat other contaminated liquids such as milk and fruit juice, for which further experiments are necessary.

Appendix A

Experimental conditions and control data

A1 Temperature conditions for experiments

Unless specifically mentioned, all experimental procedures in this research were conducted at an ambient temperature of 22°C. All *E. coli* cultures were grown at 37°C within an incubator. The *B. subtilis* strain used in the experiments were grown at 30°C.

A2 Control experiment for *E. coli* growth in LTB

Figure A1 shows the control growth curve of *E. coli* in the absence of *Moringa oleifera* seed extract at a temperature of 37°C. Lauryl Tryptose Broth (LTB) was used as the liquid nutrient media in this experiment.

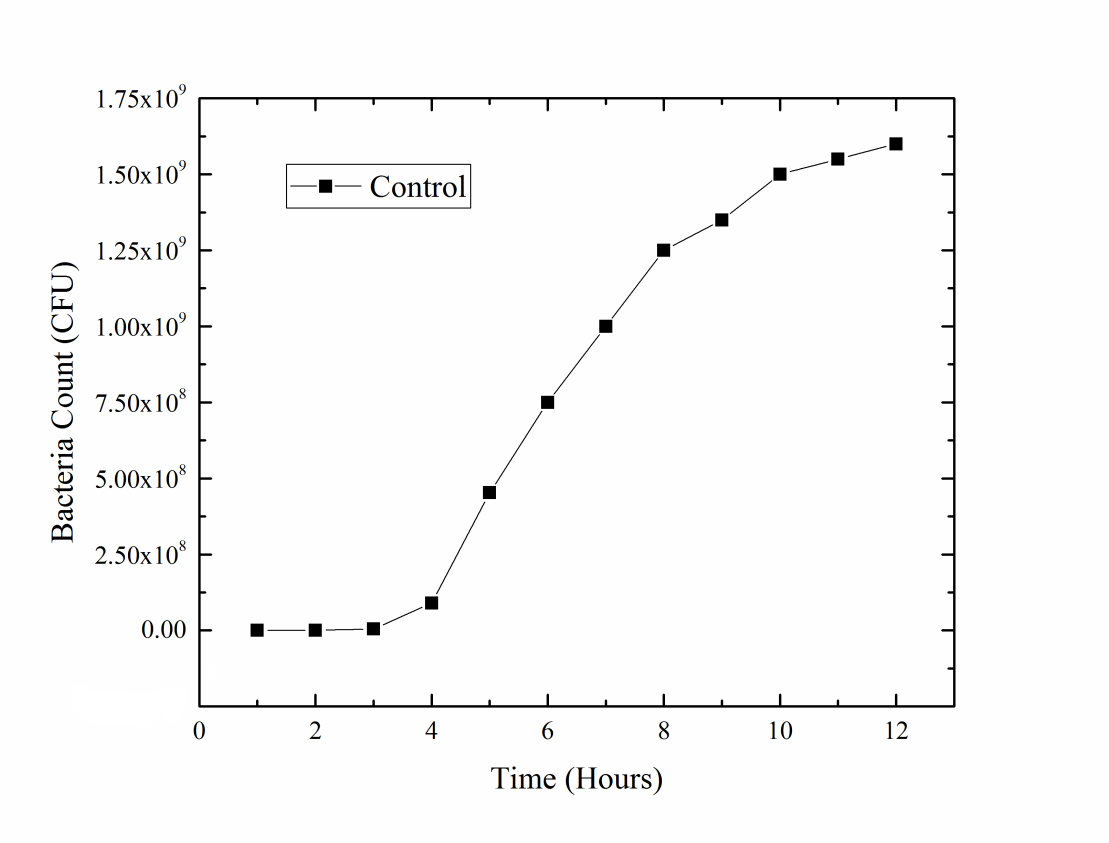

Figure A1: The control experiment where *E. coli* was grown in LTB only without any *Moringa oleifera* seed extract. Experiments were performed in triplicates for each data point.

Appendix B

Comparison Table

B1 Comparison between point-of-use water treatment products

Table B1: Comparison of dip and treat device with current products in the market.

Products	Technology Used	Targeted Contaminants	Price	Efficiency	Power Source
Filtration bottles, filtration straws, gravity filters	Microfiltration	Bacteria and protozoa	$20 - $150	>99%	Not required
Filtration bottles	Ultrafiltration and adsorption	Bacteria, virus, protozoa, chemicals, particulates, heavy metals	$60 - $400	>99%	Required in some cases
Chemical pills and solutions	Chemical treatment	Bacteria and protozoa	$10 - $ 20	Variable, depending on water sources	Not required
UV bottles and pens	Ultraviolet light	Bacteria, protozoa and virus	$70 - $150	> 99%	Required
DipTreat strips (present research)	Bacterial chemotaxis and contact-killing antimicrobial agent	Bacteria (*E. coli*)	$12 (per 100 strips, rough estimate)	> 99%	Not required

List of Publications

Journal Papers Published

1. **Saumyadeb Dasgupta**, Ravi Chavali, Naga Siva Kumar Gunda and Sushanta K. Mitra. Hollow fiber concentrator for water quality monitoring: role of surfactant based elution fluids. *RSC Advances*, 5(77):62439-6244, 2015.

2. **Saumyadeb Dasgupta**, Naga Siva Kumar Gunda, and Sushanta K. Mitra. Evaluation of the antimicrobial activity of *Moringa oleifera* seed extract as a sustainable solution for potable water. *RSC Advances*, 6(31):2591825926, 2016.

Journal Papers Submitted

1. **Saumyadeb Dasgupta**, Naga Siva Kumar Gunda, and Sushanta K. Mitra. Fishing, trapping and killing of *Escherichia coli (E. coli)* in potable water. *Environmental Science: Water Research and Technology*. Under Review, (August 3) 2016.

Report of Invention

1. Sushanta K. Mitra, Naga Siva Kumar Gunda and **Saumyadeb Dasgupta**, Paper based water treatment device, Invention Disclosure, IY2016-006, Innovation York, York University, Toronto, ON, Canada, 2016.

Conference Proceedings

1. **Saumyadeb Dasgupta**, Ravi Chavali, Naga Siva Kumar Gunda, and Sushanta K Mitra. Evaluating the Efficacy of Hollow Fiber Pre-Concentrator for Water Quality Monitoring. Paper # ICNMM2015-48192, Proceedings of

ASME 2015 13th International Conference on Nanochannels, Microchannels, and Minichannels collocated with the ASME 2015 International Technical Conference and Exhibition on Packaging and Integration of Electronic and Photonic Microsystems, ICNMM2015, San Francisco, CA, USA, July 6-9, 2015.

Oral Presentation

1. **Saumyadeb Dasgupta**, Ravi Chavali, Naga Siva Kumar Gunda, and Sushanta K Mitra. Evaluating the Efficacy of Hollow Fiber Pre-Concentrator for Water Quality Monitoring. Paper # ICNMM2015-48192, Proceedings of ASME 2015 13th International Conference on Nanochannels, Microchannels, and Minichannels collocated with the ASME 2015 International Technical Conference and Exhibition on Packaging and Integration of Electronic and Photonic Microsystems, ICNMM2015, San Francisco, CA, USA, July 6-9, 2015.