Biomolecular Phage Filter for the Detection of a Small Number of Pathogens in Large Volumes of Processing Water

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Abstract: Fresh specialty crop produce such as tomatoes, blueberries, strawberries, sprouts, cantaloupes, lettuce and leafy greens account for more instances of foodborne illness than any other food category. Recent announcements to consumers, by the United States (U.S.) Centers for Disease Control (CDCs), to discard all Romaine lettuce because of bacterial contamination has resulted in hundreds of millions of dollars in losses to growers and processors. Unfortunately, current microbiological testing of samples of specialty crops (whole fruits, leaves of spinach, etc.), as specified by FDA's Bacteriological Analytical Manual (BAM), requires at least 48 hours to perform the complicated, time-consuming and costly steps of soaking, pre-enrichment, concentration, enrichment, plate count or PCR to detect pathogens on these samples. Further complicating the BAM analyses are the realities that: 1) both PCR and ELISA are unable to distinguish between live and dead cells and 2) only a few samples out of as many as 100,000 fruits, vegetables or leaves of multi-ton batches of produce can be BAM tested. A Non-clogging Biomolecular Phage Filter has been developed to simultaneously capture, concentrate and isolate small numbers of pathogens from large volumes of produce wash water. This phage filter can then be evaluated to screen for live versus dead cells and ID the specific pathogen in minutes. Capture efficiencies of greater than 94% have been demonstrated.

1 INTRODUCTION

The U.S. Food and Drug Administration (FDA) and CDCs have, within the last year, taken the unprecedented step of warning the U.S. public to discard all Romaine lettuce because of possible bacterial contamination (CDCs, June 2018; CDCs, Dec 2018; Staff, 2018). This warning led to all Romaine lettuce in the food chain being discarded and a still to be determined loss to producers, processors and distributors estimated to be in the hundreds of millions of U.S. dollars. The cost of foodborne illness in the U.S. is enormous. A report in 2014 from the U.S. Department of Agriculture estimated that the direct and indirect costs associated with illnesses caused by major foodborne pathogens is more than $15.6 billion per year (Stephen, 2018). A more recent estimate from Ohio State University that covered all causes of foodborne illness, not just illness from the major foodborne pathogens, concluded the amount was at least $55.5 billion (Stephen, 2018). Each year in the U.S., about 48 million people get sick from foodborne illness. These foodborne illnesses result in approximately 128,000 hospitalizations and 3,000 deaths, as reported by the CDCs (CDCs, Feb 2018). Of the major foodborne pathogens, *Salmonella* causes about 1.2 million illnesses, 23,000 hospitalizations and 459 deaths in the U.S. each year (CDCs, Nov 2018).

Triple washed, ready-to-eat, salads have found a ready consumer market in the U.S. The produce that goes into these ready-to-eat salads typically comes from numerous farms and are processed in multi-ton batches. The produce is washed in a series of cleaner and cleaner water, finally being drip dried on a vibrating conveyor followed by centrifugal drying. It is common practice for a few ml samples of the

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final wash water to be tested for pathogens using off-site commercial testing laboratories following FDA accepted BAM procedures using PCR. Despite this testing, contaminated batches of Romaine lettuce have reached consumers and resulted in illness. The U.S.'s recent failures to identify contaminated ready-to-eat salads, demonstrates the need for new technologies that are capable of capturing and concentrating small numbers of pathogens from large volumes of produce wash water. To meet these needs, the authors of this paper are developing a non-clogging, biomolecular phage filter. This paper presents the concept and design of the phage filter, and capture efficiencies of two different designs of the filter system.

2 PHAGE FILTER DESIGN

2.1 Concept

Magnetoelastic (ME) biosensors immobilized with filamentous E2 phage have been widely studied and reported in our previous publications (Nambi and Nyalamadugu, 2003; Guntupalli and Lakshmanan, 2007; Huang and Yang, 2008a; Huang and Yang, 2008b; Shen and Mathison, 2010; Park and Wikle, 2012; Chai and Li , 2012; Li and Horikawa, 2012; Guntupalli and Sorokulova, 2012; Chai and Horikawa, 2013a; Park and Park, 2013a; Park and Li, 2013b; Chai and Horikawa, 2013b; Chai and Wikle, 2013c; Horikawa and Chai, 2015). These ME biosensors are typically rectangular strips made of a magnetoelastic material, 1000 x 200 x 30 microns in size. As the specific pathogens are captured by the phage coated on the ME biosensor, the added mass of the captured pathogens, causes the resonance frequency of the ME resonator to decrease as a direct function of number of captured bacteria. E2 phage has been specifically engineered to bind *Salmonella typhimurium*. Because of the selective and specific binding affinity of E2 phage, *Salmonella* pathogens can be captured on surfaces of ME biosensors and are removed from the test liquid, when the sensors are collected. The biomolecular phage filter is composed to many individual ME biosensors, each biosensor serving as an individual filter element. Each filter element is held in the filter by a controlled magnetic field.

The phage filter consists of phage immobilized ME biosensors and supporting frames. Many layers of frames (arranged at different orientations) can be combined together to form a single filter. The ME filter elements are held by a controlled magnetic field that is applied to align individual ME elements. Either electromagnets or permanent magnets can be used to generate the magnetic field. Openings of supporting frames are close packed with ME filter elements each immobilized with E2 phage. The surfaces of ME filter elements are held perpendicular to the direction of the flowing liquid. *Salmonella typhimurium* that comes into contact with a filter element immobilized with E2 phage will bind to that element. Figure 1 shows how the phage filter works. Each filter element is held at one end to the supporting frame by the magnetic field. The field also holds the ME filter elements in the magnetic plane. *Salmonella typhimurium* shown in red, is captured and bound to the filter element by the E2 phage, once collision occurs. At the same time, debris, such as fruit pulp and sand, will not be captured by the phage and pass through the ME filter elements. Large debris (shown in white) will cause the ME filter elements to open like a gate, passing through the phage filter. Once the debris has passed, the ME filter elements will realign due to the planar magnetic field. Therefore, this phage filter is pathogen specific and is non-clogging. Auburn University has engineering different phages that are designed to capture different pathogens such as *Salmonella enterica*, *Salmonella typhimurium*, *Campylobacter spp.*, *Listeria spp.*, and *E. coli* O157H7, etc.

Figure 2 compares the differences between the Biomolecular Phage Filter and conventional packed magnetic bead filters. As shown in the figure, the antibody immobilized beads capture the specific pathogen, but become clogged with larger debris that cannot pass through the interstitials of the packed spheres. Debris builds up ahead of the packed bead column and prevents further flow of liquid and capture of pathogens by the antibody immobilized beads.

2.2 Design and Simulation

Magnetic fields were used to attach, align and release the ME filter elements from the filter and supporting frames. In the research, two different methods of filtering were investigated: 1) stationary filter with gravity-driven, vertical liquid flow and 2) rotational moving filter driven by electric motor.

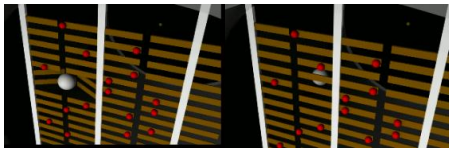

Figure 1: White debris passing through phage filter.

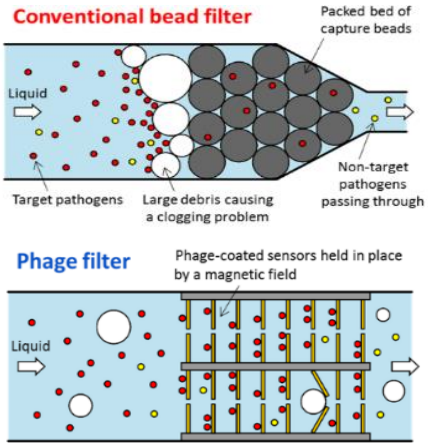

Figure 2: Difference between phage filter and conventional bead filter.

2.2.1 Vertical Flow Model

For the vertical flow model, the magnetic field was created by electromagnetic coils that were wound around supporting frames. The solenoid coils were made of plastic coated electrical wire (28GA). The ME filter elements were aligned and held to the frame when current was applied to the coils. The ME filter elements could therefore be easily released by turning off the current. The supporting frames were fabricated from one kind of soft magnetic material, Permalloy 80, by electric discharge machining (EDM). A grooved structure of supporting frame (Figure 3) was selected to concentrate the magnetic field between the supporting frames. The distance between frame members was determined by the length of ME filter elements and the size of the pipe. Figure 3 shows a 3D model and photo of the phage filter, including supporting frames and electromagnetic coils. The ME filter elements were attached by the magnetic field to the supporting frames perpendicularly to cover the opening of supporting frame.

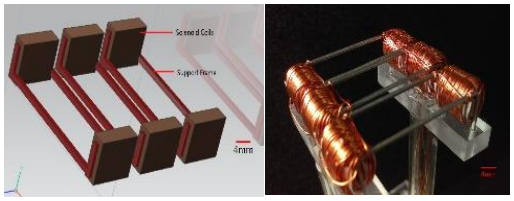

Figure 3: 3D model & photo of vertical model phage filter.

The magnetic field was modeled using ANSYS Maxwell. The 3D model was designed using NX Unigraphics software. The predicted magnetic field is shown in Figure 4. ME filter elements align parallel to the magnetic field vectors. Iron powder was used to experimentally verify predicted magnetic flux lines. In addition, the magnetic force was measured by Gauss meter. A value of 69.72 gauss generated by an electrical current of 1.5A was enough to hold ME filter elements to supporting frames.

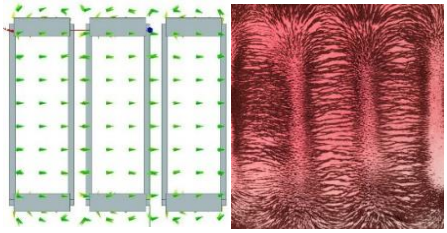

Figure 4: Simulation and iron powder test of magnetic flux vectors of vertical model phage filter.

2.2.2 Rotating Filter Model

For the rotating model, permanent magnets (Grade N52) with diameter of 3/16" were used to generate the magnetic field instead of electromagnetic coils. Figure 5 (left) shows the 3D model of the rotating phage filter with ME filter elements attached. Supporting frames were made of Permalloy 80 wire with a diameter of 1mm. Two Permalloy 80 sheets were fixed on the end of magnets, in order to improve uniformity of the magnetic field. The modified 3D model (improved) of the phage filter is shown in Figure 5 (right). Simulation results are shown in Figure 6. The magnetic flux vectors were also perpendicular to the supporting frames of phage filter, especially for the corners of the supporting frame. Therefore, the ME filter elements can be coupled to the supporting frames with the same direction of magnetic flux vector, passing parallel to the long axis of ME biosensors.

2.3 Testing of Vertical and Rotating Filter Systems

Tests of both the Vertical Flow and Rotating Filter Systems were conducted. The systems were both constructed of transparent food-grade plastic.

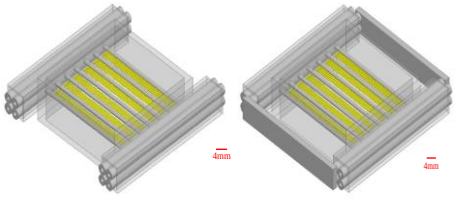

Figure 5: 3D model of phage filter of rotating model before & after modification.

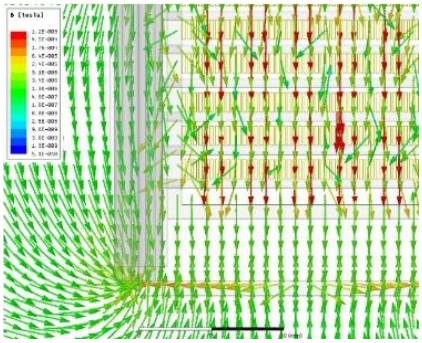

Figure 6: Simulation of magnetic flux vectors of rotating phage filter.

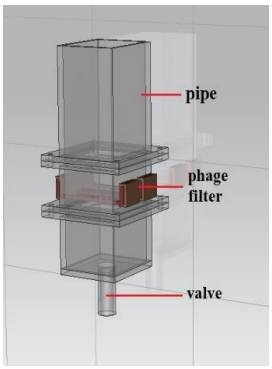

Figure 7: 3D model of vertical flow system.

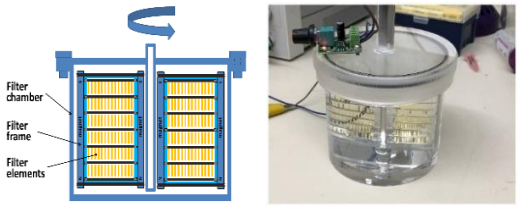

Figure 8: 2D model and photo of rotating flow system.

2.3.1 Vertical Flow System

The vertical gravity-fed system consisted of a square pipe, phage filters and a valve. The valve was used to control the flow of pathogen solution. For the phage filter, solenoid coils were placed outside the pipe to guarantee all the pathogen solution could pass through the phage filter plane containing the ME filter elements. ME filter elements with size of 1mm x 0.2mm x 0.03mm were used. Figure 7 shows the 3D model of vertical system. For multiple layers of phage filters, each layer was horizontally rotated 90-degree relative to the previous layer. In this way, a higher packing density of ME filter elements was obtained, in addition to increasing the probability of impact and hence capture by phage of the target pathogen.

2.3.2 Rotating Filter System

The rotating system was constructed using a transparent food grade plastic chamber, phage filter on a spindle and a motor with speed control unit. Figure 8 shows the 2D model and photo of rotating filter system. ME filter elements of size 4mm x 0.8mm x 0.03mm were used. All the phage filter layers can be rotated along the spindle, which was driven by a speed-controlled electric motor. The pathogen containing solution was filtered by the phage filter when rotated.

3 MATERIAL AND METHODS

3.1 ME Filter Elements

ME filter elements were fabricated from Metglas 2826MB as cast ribbon. The raw material was diced to form individual ME filter elements that were rectangular in shape. An Ultraviolet (UV) sensitive film was used to hold the Metglas material during dicing with an automated saw (Disco 3220). Acetone was used to wash the UV sensitive film with the ME filter elements prior to exposure to UV radiation to release the ME filter elements from the film. After release, ME filter elements were ultrasonically cleaned in acetone followed by methanol. The ME filter elements were then vacuum annealed at 220°C for 3 hours to remove residual stresses caused by the dicing process. Chromium (30µm) and then gold (150µm) were sputtered onto all surfaces of the ME filter elements. The Cr layer was used to improve the adhesion of Au on surfaces of ME filter elements. The Au layer provided a bioactive surface for phage immobilization and corrosion resistance.

3.2 E2 Phage and Surface Blocking

The filamentous E2 phage was prepared and provided by the Department of Biological Sciences. E2 phage is a genetically engineered landscape phage with bio-receptors designed to capture *Salmonella typhimurium* bacteria. The E2 phage solution was diluted to $5x10^{11}$vir/ml with Tris-Buffered-Saline (TBS) solution. ME filter elements were incubated in a 1.5ml centrifuge tube for 1 hour using a 3D rotating incubator at a speed of 8 rpm. After attaching the phage to the ME filter elements, the ME filter elements were washed in TBS solution. The ME filter elements were then incubated in super

blocking buffer (1x, Thermo Fisher Scientific) for 40mins, in order to reduce non-specific binding. After blocking, the ME filter elements were washed with TBS solution and filtered deionized water one time. After final washing, the ME filter elements were ready for placement into the filters to capture *Salmonella* pathogen.

3.3 Phage Filter Performance

The *Salmonella* pathogen with an original concentration of 5 x 10^8cfu/ml was prepared and provided by the Department of Biological Sciences. The concentration of *Salmonella* solution was diluted to 5 x 10^4 cfu/ml and 5 x 10^3 cfu/ml with filtered deionized water. The total theoretical number of *Salmonella* pathogen input were 1650cfu in the vertical gravity-fed system and 5 x 10^4 cfu in the rotating flow system, respectively. The phage-immobilized ME filter elements were aligned compactly on phage filters and placed into the test system. For the vertical gravity-fed system, a liquid velocity of 3mm/s was controlled by the valve. The tested *Salmonella* solution was passed through phage filter one-time in. For the rotating flow system, single and double layers were tested, separately. A rotational speed of 12 rpm was maintained for 30mins.

4 RESULTS AND DISCUSSION

4.1 Vertical Flow System

The stability of the vertical flow system was tested using filtered deionized water. Experiments showed that the ME filter elements could be suspended perpendicular up to the maximum obtained volumetric flow rate of 20 l/min (bottom exit valve wide open). In all tests, no ME filter elements detached from the filter frame elements, except when the current was removed.

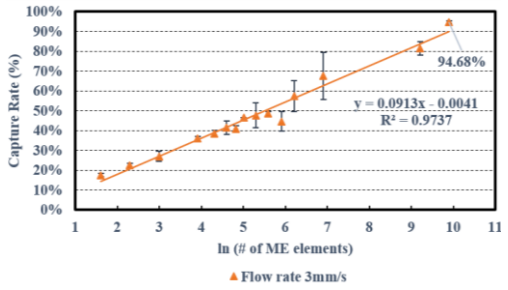

Figure 9: Capture efficiency of the vertical flow system.

Plate counts were conducted on input and exit solutions to determine the capture efficiencies. For fluid velocities of 3mm/**s**, capture efficiencies of 94%+ were obtained, which was shown in figure 9.

Figure 9 demonstrates that the capture rate of *Salmonella* increases with an increase in number of ME filter elements.

4.2 Rotating Flow System

Figure 10 shows the capture efficiency of single layer and double layers for the rotating flow filter system in 30mins. The capture efficiency increases with increasing the number of filter layers.

In addition, the capture efficiency was measured as a function of time for double filter layers. Figure 11 shows the capture efficiency of the rotating filter also increased with time. A capture efficiency of nearly 80% was obtained for a two-layer system after 75 minutes.

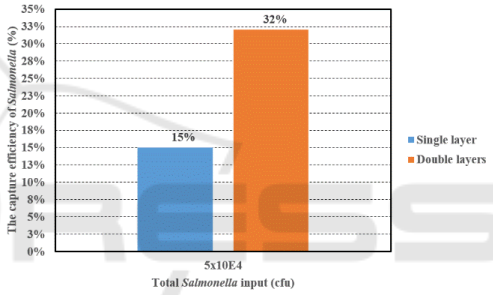

Figure 10: Capture efficiencies of single and double layers rotating systems.

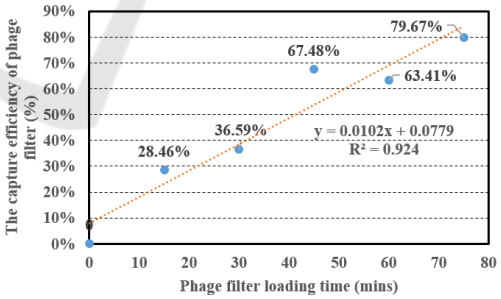

Figure 11: Capture efficiency increases with time, rotating filter.

5 CONCLUSIONS

The concept, design and testing of a Non-clogging Biomolecular Phage Filter has been presented in this paper. This phage filter when deployed will be able to simultaneously capture, concentrate and isolate small numbers of pathogens from large volumes of

produce wash water. Two different filter designs (vertical, gravity driven and rotating system) are presented and tested. Adding multiple layers of ME filter elements increases capture efficiencies. Capture efficiencies of greater than 94% have been demonstrated for flow velocity of 3mm/s.

Future research will address the effects of temperature, flow rate, and water organic content on capture efficiencies.

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REFERENCES

CDC, June 2018, https://www.cdc.gov/ecoli/2018/o157h7-04-18/index.html.

CDC, December 2018, https://www.cdc.gov/ecoli/2018/o157h7-11-18/index.html.

CDC, February 2018, https://www.cdc.gov/foodsafety/foodborne-germs.html.

CDC, November 2018, https://www.cdc.gov/ *salmonella*/index.html.

Chai Y, Li S, (2012) Rapid and sensitive detection of *Salmonella* typhimurium on eggshells by using wireless biosensors. *Journal of Food Protection*, 75 (4), 631-636.

Chai Y, Horikawa S, (2013a) A surface-scanning coil detector for real-time, in-situ detection of bacteria on fresh food surfaces, *Biosens. Bioelectron*, 50, 311.

Chai Y, Horikawa S, (2013b) Surface-scanning coil detectors for magnetoelastic biosensors: A comparison of planar-spiral and solenoid coils, *Appl. Phys. Lett*, 103, 173510.

Chai Y, Wikle H. C, (2013c) Design of a surface-scanning coil detector for direct bacteria detection on food surfaces using a magnetoelastic biosensor, *J. Appl. Phys*, 114, 104504.

Guntupalli R, Lakshmanan R, (2007) Magnetoelastic biosensor for the detection of *Salmonella typhimurium* in food products, *Sensing and Instrumentation for Food Quality and Safety*, Volume 1, Issue 1, Pages 3-10. doi:10.1007/s11694-006-9003-8.

Guntupalli R, Sorokulova I, (2012) Detection and identification of methicillin resistant and sensitive strains of Staphylococcus aureus using tandem measurements, *J. Microbiol. Methods*, 90(3), 182-91.

Huang S, Yang H, (2008a) The Effect of Salt and Phage Concentrations on the Binding Sensitivity of Magnetoelastic Biosensors for *Bacillus anthracis* Detection, *Biotechnol. Bioeng*, 101, 1014-1021.

Huang S, Yang H, (2008b) Optimization of Phage-Based Magnetoelastic Biosensor Performance, *NSTI nanotech 2008 conference, symposium on Phage Nano-biotechnology*, pp. 642-645.

Horikawa S, Chai Y, (2015) Direct detection of *Salmonella* on fresh produce. *ECS Transactions* 69 (38), 25-31.

Li S, Horikawa S, (2012) Amorphous metallic glass biosensors, *Intermetallics* 30, 80-85.

M. K. Park, J. W. Park, (2013a) Evaluation of phage-based magnetoelastic biosensors by comparison with TaqMan-based quantitative PCR, *Sens. Actuators, B: Chem*, 176, 1134-1140.

Nambi S, Nyalamadugu S, (2003) Radio Frequency Identification Sensors for Food Safety, *Wireless Communications Systems and Circuits Design in the 7th World Multi-Conference on Systemics, Cybernetics, and Informatics*, pp. 386-390.

Park M.K, Wikle H. C, (2012) The effect of incubation time for *Salmonella* typhimurium binding to phage-based magnetoelastic biosensors, *Food Control*, Vol.26 (2), pp 539-545.

Park M. K, Li S, (2013b) Detection of *Salmonella typhimurium* grown directly on tomato surface using phage-based magnetoelastic biosensors, *Food Bioprocess Tech*, 6(3), 682-689.

Staff, November 2018, FDA Update on Romaine Lettuce Outbreak as Yuma, AZ, Growing Season Begins, *Food Safety Magazine*.

Stephen Ostroff, M.D. June/July 2018. The Costs of Foodborne illness, Product Recalls Make the Case for Food Safety Investments. *Food Safety Magazine*.

Shen W, Mathison L.C, (2010) Design and characterization of a magnetoelastic sensor for the detection of biological agents, *Journal of Physics D: Applied Physics*, 43 (2010) 015004. DOI: 10.1088/0022-3727/43/1/015004.