Mechanistic Study of Membrane Concentration and Recovery of *Listeria monocytogenes*

Wan-Tzu Chen,1,2 Richard L. Hendrickson,2 Chia-Ping Huang,3 Deb Sherman,5 Tse Gong,3 Arun K. Bhunia,3 Michael R. Ladisch1,2,4

1Department of Biomedical Engineering, Purdue University, West Lafayette, Indiana 47907
2Laboratory of Renewable Resources Engineering, Potter Engineering Center, 500 Central Drive, West Lafayette, Indiana 47907-2022; telephone: 765-494-7022; fax: 765-494-7023; e-mail: ladisch@purdue.edu
3Department of Food Science, Purdue University, West Lafayette, Indiana 47907
4Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, Indiana 47907
5Life Science Microscopy Facility, Purdue University, West Lafayette, Indiana 47907

Received 7 October 2003; accepted 6 July 2004

Published online 21 December 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/bit.20256

Abstract: Detection of the foodborne pathogen *Listeria monocytogenes* requires that food samples be processed to remove proteins and lipids, concentrate microorganisms to a detectable concentration, and recover the concentrated cells in a small volume compatible with micro-scale biosips. Mechanistic considerations addressed in this research include the roles of membrane structure, pore size, and detergents in maximizing recovery of cells from a complex biological fluid. The fluid in this case was a food sample (hotdog extract) inoculated with *L. monocytogenes*. This study showed how membrane filtration using a syringe filter is able to concentrate *L. monocytogenes* by 95× with up to 95% recovery of living microorganisms by concentrating 50 mL of food sample into a volume of 500 μL. Tween 20 was added to the sample to prevent irreversible adsorption of the microorganism to the membrane and thereby help to ensure high recovery. Comparison of polycarbonate, mixed cellulose, nylon, and PVDF membranes with 0.2 to 0.45 μm pores showed that the 0.2 μm polycarbonate membrane with straight through, mono-radial pores gives the highest recovery of living microorganisms. The mixed cellulose, nylon, and PVDF membranes have a fibrous structure whose characteristic openings are much larger than their effective pore size cut-offs of 0.22 or 0.45 μm. We define conditions for rapid membrane-based cell concentration and recovery that has the potential to supplant enrichment steps that require a day or more. This approach has the added benefit of facilitating examination of a large amount of fluid volume by reducing its volume to a range that is compatible with the microliter scales of biochip or other biosensor detection systems. © 2004 Wiley Periodicals, Inc.

Keywords: *Listeria monocytogenes*; membrane filtration; microorganism concentration; pathogen detection; biochip; microfluidics; food safety

INTRODUCTION

Rapid sample preparation is needed to reduce the time between sampling the food and detecting presence of pathogenic bacteria. Our goal is to achieve an overall time to result of 3 hours so that monitoring may occur at a timescale compatible with the processing time associated with food manufacture. A rapid cell concentration step that replaces culture methods will contribute to decreasing the time to result and when coupled with biochip detection system could enable a 3-hour assay to be achieved.

Detection of a food pathogen also requires quantitative recovery of viable organisms in a concentrated form. In this article we show protein removal and 95-fold concentration with 95% recovery of living microorganisms can be achieved within 15 minutes. The proper choice of membrane and the use of blocking agents is as important to the success of this approach as is the ability to test for presence of *L. monocytogenes* in a small volume of liquid (less than 0.1 μL) on a microfluidic device whose surfaces are chemically modified to block nonspecific adsorption of microorganisms (Bashir et al., 2001; Huang et al., 2003). We report membrane microfiltration for the rapid recovery, fractionation, and concentration of living microbial cells from an aqueous extract of hotdogs. The starting point of
this work was membrane filtration used in microbiological analysis of water and dairy products (Besse and Lafarge, 2001; Carroll et al., 2000; Ennis and Lerner, 2000; Peterkin and Sharpe, 1980).

The technical challenges of recovering microorganisms from fluids obtained from meats are not unlike those of other liquids derived from biological samples, including blood. Hotdog Meat (HDM) broth, is a complex, turbid fluid consisting of salts, proteins, lipids, colloidal particles, and mono- and oligosaccharides. The chemically and physically complex nature of this material requires that potentially interfering substances and molecules be removed and cells concentrated before a meat extract is tested. While colloidal particles, at 10 to 50 microns, are generally much larger than microbial food pathogens (at 1 micron) the other components are soluble and/or much smaller. Sieving removes submillimeter-sized colloidal materials while microfiltration retains cells whose characteristic dimension is 1 micron. Soluble constituents, that otherwise cause fouling of membranes are small enough to pass through the filters with permeate and thus are removed (Ladisch, 2001; Palacio et al., 2002). The properties of mixed cellulose, nylon, PVDF, and polycarbonate membranes with 0.2-0.45 micron cut-offs are reported here for filtering and rapidly concentrating microbial cells.

The focus of this work is on Listeria monocytogenes, a food pathogen found in dairy and ready-to-eat foods, including hotdogs. This pathogen causes 20% mortality among immunocompromised populations and spontaneous abortion and stillbirth in pregnant women. Its presence is currently detected by extracting a liquid from the hotdog or other food and then enriching the microbes in the sample by culture steps that take 3 to 7 days. After enrichment is completed, biochemical, Christie-Atkins-Munch-Peterson (CAMP), or serological tests are carried out (Hitchens, 1998). Thus, our study shown here presents a better method to replace this lengthy culturing and enrichment step and can reduce total time-to-result greatly.

MATERIALS AND METHODS

Bacterial Strains

Listeria monocytogenes, strain V7 was obtained from Dr. Arun Bhatia’s laboratory in the Department of Food Science at Purdue University. Listeria monocytogenes is a rod-shaped, gram-positive bacterium with a net negative charge at pH 7.4, which measures about 1 x 0.5 microns. A stock culture was maintained at refrigerator temperature (4°C) for at most 3 months before undergoing serial transfers. Before each experiment, cultures of 10^9 cells/mL were freshly prepared by incubating 20 μL of L. monocytogenes in 3 mL of Brain Heart Infusion (BHI, Difco, Cat #D0418177) broth for 18 h at 37°C. The enumeration of L. monocytogenes was carried out on selective modified Oxford agar (MOX, Difco, Cat #222530) in which the colonies of these bacteria were black.

Sterilization of Reagents and Sterile Filtration of HDM Broth

The experiments described here are based on correlating the initial and final bacterial cell counts in the HDM broth, the filtrate, and the retentate. Consequently, all reagents except for HDM broth were sterilized at 121°C for 15 min in an autoclave in sealed bottles. HDM broth was sterile filtered through 0.22 μm cellulose membrane (Nalgene, Cat #121-0020) to avoid heat denaturation of its biological constituents.

Preparation of Hotdog Meat (HDM) Broth

Hotdogs were purchased from local vendors. A volume of 250 mL of phosphate buffered saline (PBS, pH = 7.4) buffer mixed with each package of hotdogs containing 250 g of meat. The PBS consisted of 0.2M NaCl in 10 mM phosphate buffer. The buffer was gently contacted with the hotdogs in a stomacher bag that consisted of two chambers. The hotdogs were held in an inner chamber and massaged at 10 min intervals for 2 hours while immersed in PBS. The sample inside the stomacher bag separated large food particles from a turbid fluid containing proteins, lipids, carbohydrates, and microbes. The hotdog solution was then decanted from the bag into a filtration unit and filtered across a 0.22 μm cellulose membrane to remove food particles and microbes so that controlled experiments with L. monocytogenes could be carried out. The filtrate (i.e., HDM broth) was cultured on BHI agar and gave no colony forming units, showing that the initial HDM is free of microorganisms (Chen, 2003).

Culture of Listeria monocytogenes in HDM Broth and Determination of Colony Forming Units

Freshly cultured L. monocytogenes in BHI was inoculated into sterile filtered HDM broth. Serial dilution with HDM

![Figure 1. Listeria monocytogenes growth curve in HDM broth at 37°C.](image-url)
broth was carried out to a final concentration of 50-70 CFU/100 µL. Colony counts were determined by plating out 100 µL of the diluted HDM broth on MOX agar (selective media for Listeria spp.) using a spreader. The plates (3 inch in diameter) were incubated at 37°C and the number of colonies counted after 24 h, with each colony apparently formed from a single microbial cell. A volume of 100 mL HDM broth (pH 7.4) was inoculated with

Table 1. Summary of characteristics of membranes used in this study.

<table>
<thead>
<tr>
<th>Type</th>
<th>Functionality</th>
<th>Nominal Pore Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nylon</td>
<td></td>
<td>0.22, 0.45</td>
</tr>
<tr>
<td>Mixed cellulose ester</td>
<td>Cellulose acetate and cellulose nitrate</td>
<td></td>
</tr>
<tr>
<td>Cellulose nitrate</td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td>Cellulose acetate</td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td>Polyvinylidene fluoride</td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td>Polycarbonate</td>
<td></td>
<td>0.2, 0.4</td>
</tr>
</tbody>
</table>
*L. monocytogenes* at a concentration of 400 cell/μL and incubated in a shaker for 12 h. Samples were taken every hour, appropriately diluted, and 100 μL was plated on MOX agar. A lag phase of about 2 h was noted during which the bacteria adjusted to the new environment (Fig. 1). A typical log-phase growth was observed after 4 h. On this basis, the filtration experiment was carried out within 2 h, where the bacteria number remained approximately constant.

**Membrane Filtration Procedure**

The procedure used to concentrate cells in HDM broth is summarized in Scheme 1. Polypropylene syringe filter holders (13 mm, Fisher Scientific, Cat #SX0001300) sealed with a silicone gasket were used to hold the membranes. Preliminary tests were carried out in the absence of Tween 20. The membranes were wetted in FBS buffer (pH 7.4) and different volumes of inoculated HDM broth were manually forced through the membranes at room temperature by applying pressure on the syringe plunger. The membranes were then removed and soaked in 500 μL PBS in 1.5-ml microcentrifuge tubes for 30 min with gentle shaking (Eppendorf, Cat #05402-24c, polypropylene). An aliquot of the resulting solution (100 μL) was plated out in triplicate on modified Oxford agar (MOX) and incubated for 48 h at 37°C. Filtrates were also sampled and plated out on MOX agar to check for presence of bacteria but no significant number was found.

**Membranes**

*Listeria monocytogenes* adsorbs on hydrophobic surfaces (Huang et al., 2003). Thus, membranes with hydrophilic

![Figure 2](image1.png)

**Figure 2.** Microbial imprint assay based on hydrolysis of esculin to esculin by *L. monocytogenes* resulting in black color formed when esculin-formed complex with ferric ions. Circles 1, 2, 3, 4, and 5 represent microfiltration of 1, 5, 10, 25, and 50 mL, respectively, of *L. monocytogenes*-inoculated HDM broth containing 76 CFU/100 μL through (A) 0.45 μm PVDF membranes before washing, 0.4 μm polycarbonate membranes (B) before washing with buffer, and (C) after washing with buffer.
surfaces were selected to minimize adsorption and maximize recovery of microorganisms. However, hydrophilic character alone was found to be insufficient to ensure 95% recovery of the microorganisms. Since the organism of interest, L. monocytogenes, has dimensions of 0.5 × 1.0 micron and does not aggregate at the pH of the buffer (pH 7.4), the membrane cut-off needed to be smaller than this. Hence, 0.2 to 0.45 μm pore size cut-offs were selected.

The membranes tested in this study were made of nylons (Magna Nylon, 0.45 μm, Cat #R04SP01300, Biech #163519, Osmonics), mixed cellulose esters (MiconSep, 0.45 and 0.22 μm, Cat #EO4WP01300, Lot #78317,Osmonics; possible structure in Table I although degree of substitution may be higher), polyvinylidene fluoride (PVDF, 0.45 μm, Cat #HVHP01300, Lot #R1HN96852, Millipore) and polycarbonate (Nucleopore 0.4 and 0.2 μm, Cat #0930053, Lot #2134023, Whatman) (Table I). The membranes were gently dipped in either PBS buffer (pH7.4) or PBS with 1% v/v Tween 20 (PBST) in order to pre-wet them before testing.

PVDF and nylons membranes are hydrophilic and chemically robust, while mixed cellulose membranes consist of mixed cellulose esters (cellulose nitrate and cellulose acetate). Polycarbonate membrane is made by track-etching techniques giving straight through pores with defined and relatively uniform radii (Ladiesh, 2001). Pores on polycarbonate membrane filter have defined path and sizes, which made it useful in aerosol filtration (Palmgren et al., 1986; Shaw, 1985). Depth membrane filters (nylon, mixed cellulose, and PVDF), on the other hand, are good candidates for removing unwanted substances, but less attractive for cases where the solids are to be recovered from the filter since these are entrapped in the filter.

Membrane Imprint Assay

A membrane imprint assay was used as a rapid way to screen the concentration of L. monocytogenes by different membranes. Moreover, the ability of L. monocytogenes to hydrolyze the esculin on MOX agar can help differentiate L. monocytogenes from other bacteria that are incapable of hydrolyzing esculin.

Figure 3. Bradford protein assay of filtrates compared to starting ERM broth.

After the filtration step was carried out, the membranes were removed from the filter holder and then directly placed on MOX agar, retenate side down, and incubated at 37°C for 48 h. The agar contained esculin and ferric ammonium citrate to give blackening of the colonies while lithium chloride offered selectivity for L. monocytogenes because this microorganism has high tolerance for salt (Fraser and Saperber, 1988). The nutrients in the agar diffused into the membranes and dark-colored colonies of bacteria, if present, developed on the membrane surface after 24 h. This gave a first indication of whether L. monocytogenes had been retained by the membrane. Figure 2A shows an imprint assay for the PVDF membrane before washing as well as results for polycarbonate membranes before and after washing (Fig. 2B, C). Color formation is related to the reaction given in Equation (1):

![Scheme 2.](image)

Esculetin can then react with ferric ions (III) in the agar and form the complex Fe(esculetin)₃³⁻ that gives the black color (Griffith and Mostafa, 1992). Studies have shown that the lack of iron ions will decrease the growth and reduce the virulence of L. monocytogenes (Coulanges et al., 1996). The membrane imprint assay on MOX agar not only screens different membrane filters, but is selective for Listeria species over E. coli (Difco Laboratories, 1998).
However, it is crucial to maintain the MOX agar at 37°C where *L. monocytogenes* colonies can be fully developed (Fraser and Sperber, 1988).

**Bradford Protein Assay**

Filtrates were examined for protein concentration using the Bradford protein assay (Cat. No. 500-0006, Bio-Rad). One part of the protein staining solution was mixed with four parts of deionized water. A volume of 100 μL sample was added to 5 mL of the pre-mixed staining solution. The mixture was gently shaken and incubated for 5 min at room temperature. Its absorbance at 595 nm was read with spectrophotometer.

**Scanning Electron Microscopy (SEM)**

Scanning electron microscopy of membrane filters and *L. monocytogenes* solutions on membrane surfaces were taken after the samples were fixed to the membrane with 2% of glutaraldehyde in PBS. Subsequent washing was carried out and followed by dehydration with increasing concen-

![Graph](image)

**Figure 4.** Comparison of theoretical recovery of all volumes (solid line) with actual cells recovered from different membrane filters: polycarbonate (○), nylon (□), mixed cellulose (△), and PVDF (X). Volumes filtered are 1 mL, 10 mL, 25 mL, and 50 mL.
trations of ethanol. Samples were then dried in critical point drying (CPD) where ethanol is replaced with liquid CO₂. Temperature and pressure were then changed to 34°C and 1100 psi, respectively, where CO₂ vaporized so that the samples were dried. Each membrane sample was mounted to a stub and sputter-coated with AuPd. The images were obtained under 5 kV, with a working distance (WD) of 10 mm or 22 mm in a vacuum chamber. The magnification was 7000×.

RESULTS

Initial assessment of membrane performance for cell concentration was based on a 48-h membrane imprint assay to quickly screen membrane candidates. This was followed by the more labor-intensive but quantitative culture methods that assessed not only microbial retention by the membrane but also the extent of recovery of viable microorganisms. When complete retention but low recovery of the microorganisms was encountered, the potential role of membrane structure on microbial entrapment was examined using SEM, and the role of a surfactant (Tween 20) in minimizing nonspecific adsorption of the microorganism on the retentate side of the membrane was investigated.

Membrane Imprint Assay

Filtration of L. monocytogenes in HDM broth was first carried out over nylon, mixed cellulose ester, and PVDF membrane that had 0.45 μm pore size cut-offs, and a polycarbonate membrane with 0.4 μm pore size cut-off. Volumes of 1, 5, 10, 25, and 50 mL, respectively, of HDM broth (containing 700 cfu L. monocytogenes per mL) were filtered using the syringe to push the fluid through the membrane. The membrane imprints 1 through 5 became increasingly darker thus indicating increasing concentrations of L. monocytogenes (Fig. 2). Once the membrane imprint assay had confirmed the cells could be concentrated on all four membranes in a viable state, the recovery of the cells from these membranes was examined. The first attempt using washing with PBS buffer showed some retention of microorganisms by the membrane occurred (Fig. 2C). Consequently, Tween 20 was added to the buffer to enhance release.

Protein Assays

Bradford protein assay was carried out to identify HDM proteins from microorganisms. Filtrates were tested for their protein concentrations compared to unfiltered HDM broth. Figure 3 shows that proteins passed through both the polycarbonate and cellulose membranes as indicated by protein analysis for both initial solution and permeate were the same at 0.12 ± 0.002 mg/mL.

Quantitation of Microorganism Concentration and Recovery Using Culture Method

The experiments were repeated using the same membranes. Instead of using the imprint assay, we placed the membranes in PBS to desorb the bacteria in microcentrifuge tubes, and the resulting solution was cultured as described in the

Figure 5. Comparison of electron micrographs at 7000 magnification (scale bars = 2 μm), for different membranes (A) PVDF, 0.45 μm; (B) mixed cellulose, 0.45 μm; (C) polycarbonate, 0.4 μm. Arrow indicates where the bacteria are buried inside tortuous and non-uniform structure of (A) PVDF or (B) cellulose, but not (C) polycarbonate.
Table III. Effect of Tween 20 on recovery percentage for polycarbonate and mixed cellulose. Each set of experiments was done in triplicate. Recovery was compared in the presence and absence of Tween 20. Larger than 10-fold recovery percentage was obtained by using Tween 20.

<table>
<thead>
<tr>
<th>Volume of HDM broth filtered</th>
<th>Polycarbonate 0.4 μm</th>
<th>Recovery percentage (%)</th>
<th>Mixed cellulose 0.45 μm</th>
<th>Recovery percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial bacteria number</td>
<td>Without Tween 20</td>
<td>With Tween 20</td>
<td>Initial bacteria number</td>
</tr>
<tr>
<td>1 mL</td>
<td>727</td>
<td>5.3 ± 1.1</td>
<td>65.4 ± 0.7</td>
<td>727</td>
</tr>
<tr>
<td>5 mL</td>
<td>3654</td>
<td>5.3 ± 0.5</td>
<td>58.4 ± 0.8</td>
<td>36334</td>
</tr>
<tr>
<td>10 mL</td>
<td>7267</td>
<td>8.4 ± 0.9</td>
<td>67.7 ± 2.1</td>
<td>7267</td>
</tr>
<tr>
<td>25 mL</td>
<td>18168</td>
<td>9.0 ± 1.6</td>
<td>48.4 ± 3.0</td>
<td>18168</td>
</tr>
<tr>
<td>50 mL</td>
<td>36335</td>
<td>10.8 ± 1.5</td>
<td>72.2 ± 0.9</td>
<td>36335</td>
</tr>
</tbody>
</table>

Materials and Methods. This gave colony counts from which the ratio of cell count in the retentate to the initial cell count in the HDM broth was determined. The result is reported as the ratio of concentration after filtration to concentration before filtration (Concentration factor, Table II) for an initial cell concentration of about 450 cells/mL. Comparison of these data with the theoretical maximum (i.e., total numbers of cells in the starting HDM broth as indicated by the line Fig. 4) showed that over half of the cells were unaccounted for.

Mechanisms of Cell Retention by Membranes

Three possibilities for lack of full recovery of concentrated cells were considered: (1) passage of cells through the membranes with the filtrate or permeate; (2) loss of viability of cells; and/or (3) nonspecific adsorption of cells onto the membrane. Plating out of the filtrates eliminated possibility (1) since the number of cells in the filtrate was statistically insignificant after 24 hours of culture on MOX. Plating out of the starting material also showed cells retain viability during the 2-h time period over which membrane filtration was done (Fig. 1), hence suggesting possibility (2) was unlikely as the only explanation. Fluorescent microscopy (data not shown) and microbial imprints assay images (Fig. 2) suggested that possibility (3) offered an explanation since cells remain on the membrane's surface after washing with buffer.

PVDF, nylon, and cellulose are known as depth filters, whose paths are tortuous. Pore structure is the result of stacking layers of porous materials. By design, the interconnected pore structures of these types of membranes retain cells by entrapment, thus ensuring effective removal of microbes, and a sterile permeate. However, entrapment of a microbial pathogen such as L. monocytogenes is not desirable for our purposes since entrapped cells may be difficult to recover and analyze (Fig. 5 A, B).

An assay designed to interrogate a microbial sample for presence of pathogens requires that as many cells as possible be recovered for subsequent processing. The polycarbonate membrane filter is a screen filter that has a more uniform pore size distribution and straight pathways through the membrane. This type of membrane is less likely to retain cells larger than the average pore size (Fig. 5C). Recovery is further hindered when cells are adsorbed due to weak hydrophobic, van der Waals force and/or physical interaction with the membranes' surface. De-

![Figure 6](image_url)

Figure 6. Growth of L. monocytogenes in PBST. Isolated PBST with initial bacterial count of 300 cell/100 μL was incubated in 37°C in the shaker and samples were plated out on MOX agar periodically. The plates were incubated for at least 24 hours at 37°C.
sorption of all cells from the polycarbonate membrane using buffer alone was found to be insufficient. Addition of surfactant (Tween 20) to the buffer promoted desorption perhaps by reducing the surface tension of the membrane.

**Effect of Tween 20 (Surfactant)**

Tween 20, a non-ionic detergent known to eliminate non-specific binding for immunoassays, significantly enhanced *L. monocytogenes* recovery by a factor of between 5 to 20 (Table III) when the initial solution had counts of 700 cells (as cfu)/mL in HDM broth. After filtration, the highest recovery rate attained was 86%.

Previous studies showed that 1% surfactant (Twee 80) in cheese has little effect on growth of *L. monocytogenes* after the detergent was incubated with the bacteria for 10 min (Besse and Lafarge, 2001). However, *L. monocytogenes* growth in 1% Tween 20 (PBST) may give inconsistent results (Fig. 6) because the PBS with 1% Tween 20 (PBST) has no carbon source. Nonetheless, the viable cell count is stable over 30 min after which the cells die gradually over a several hour period. The cells, once concentrated, were therefore recovered and processed within 30 min.

**Comparison of Polycarbonate and Mixed Cellulose Membranes**

Polycarbonate and mixed cellulose membranes were chosen to further test their effectiveness for recovery of cells in

---

**Figure 7.** Concentration factors for microfiltration of 70 cell/100 μL *L. monocytogenes* on (A) polycarbonate (0.4 μm) and mixed cellulose (0.45 μm), (B) polycarbonate (0.2 μm) and mixed cellulose (0.22 μm) membrane filters in the presence of PBS buffer containing Tween 20. Volumes represent the amount of HDM broth being filtered. The white bars depict the theoretic maximum concentration factors if all *L. monocytogenes* on the membrane filters were washed off. Numbers on top of each bar denote the average percentage of viable cells recovered based on triplicates.
PBST. The polycarbonate membrane gives 70% recovery of *L. monocytogenes*, with an overall concentration factor of 72 (Fig. 7A) while mixed cellulose membrane gave 58-fold concentration starting from around 35,000 microorganisms in 50 mL (or 700 cell/mL) which were collected in 500 µL retentate. It should be noted that all bacteria are retained, as there were none detected in the filtrates. However, plating out gives only a count of the living organisms, and it is on this basis that recovery is reported. This is a key parameter since living bacteria (not dead ones) are pathogenic in our case.

**Effect of Pore Sizes for Polycarbonate and Mixed Cellulose Membranes**

The recovery of cells was improved from less than 10% (membranes suspended in PBS) to higher than 70% (in PBS with 1% Tween 20). However, a higher recovery was still the goal. Since micrographs of the membrane surfaces showed entrapment of microbes (Fig. 5), membranes with 0.2 micron nominal pores were tested. Under the same experimental conditions, polycarbonate (0.2 µm pores) and mixed cellulose (0.22 µm) were more efficient in capturing microorganisms and concentrating them than the 0.4 or 0.45 µm membranes (Fig. 7). The concentration factor was 95× for polycarbonate and 60× for mixed cellulose. The concentration factor, which gives the ratio of the total number of living cells in the retentate to the total living cells in the starting solution, increases with increasing volume of solution that is filtered for both the 0.2 µm pore cut-off polycarbonate membrane and the 0.22 µm mixed cellulose membrane.

Entrapment of the microorganisms is likely to be greater for the mixed cellulose membrane due to its broader pore size distribution (Fig. 5B) than for the polycarbonate membrane (Fig. 5C). Evidence supporting this hypothesis comes from measurement of the change in the concentration factor. For the polycarbonate membrane, (0.2 micron) a 157% change occurs between 10 and 25 mL of filtered volumes (Figure B). A similar transition is noted for the 0.4 µm polycarbonate, but it occurs between 25 and 50 mL of filtered volumes instead of 10 to 25 mL. A probable explanation is given by the capacity of the membranes to entrap particles (in this case, microorganisms) within the membrane structure. The pores that rod-shaped organisms are able to explore are defined by its hydrodynamic volume; *V*<sub>H</sub> and radius of gyration. As summarized by Ladisch (2001), these can be calculated from:

\[
V_H = 2\pi R^2 h = 2\pi \left(\frac{5 \times 10^{-15} \text{cm}^3}{2}\right) (10^{-4} \text{cm})
= 3.93 \times 10^{-13} \text{cm}^3 = 3.93 \times 10^{-7} \text{nL (for rod)} \quad (1)
\]

\[
V_H = \frac{4}{3} \pi R^3 = \frac{4}{3} \pi \left(\frac{5 \times 10^{-4} \text{cm}}{2}\right) = 6.5 \times 10^{-14} \text{cm}^3
= 6.5 \times 10^{-14} \text{nL (for sphere)} \quad (2)
\]

The radii of gyration are:

\[
R_k = \left(\frac{3 \cdot 3.93 \cdot 10^{-13} \text{cm}^3}{4\pi}\right)^{1/3} = 4.6 \times 10^{-5} \text{cm}
\approx 0.46 \mu \text{m (for rod)} \quad (3)
\]

![Figure 8. Number of bacteria recovered vs. filtered volumes. Lower initial concentration (15 cell/mL) and higher filtered volumes (up to 90 mL) was used. Straight line represents linear regression between two axes.](image)

**Figure 8.** Number of bacteria recovered vs. filtered volumes. Lower initial concentration (15 cell/mL) and higher filtered volumes (up to 90 mL) was used. Straight line represents linear regression between two axes.
Membranes with average pore sizes of 0.2 and particularly 0.4 μm are therefore likely to capture some organisms given the range of their pore size distributions. This is consistent with both the data and microscope images (Figs. 5 and 7). As more volume is passed through the membrane, the latent capacity of the membrane to entrap cells is postulated to be approached. Ultimately, the percentage recovery and concentration of the cells increases with increasing filtered volume after the retention capacity of the membrane’s pores has been reached, i.e., when a critical micro-filterate volume is attained.

Samples containing 15 cell/mL of HDM broth were tested to mimic a sample that might be derived from hogs’ guts. Different volumes were filtered through the same membrane apparatus. A linear relationship between filtered volumes and recovered cells with 65 to 80% recovery results (Fig. 8). Hence, cell concentration and recovery using membrane filtration has the potential to supplement lengthy enrichment culture steps for pathogen detection systems that require only very small sample volumes.

CONCLUSIONS

The concentration and recovery of L. monocytogenes using 0.2 or 0.4 μm pore size cut-offs is subject to the pore size, structure, and adsorption characteristics of the membrane. Filtration through a polycarbonate membrane and use of a wash buffer containing Tween 20 enables the bacterium L. monocytogenes to be concentrated 100× with up to 95% recovery of living microbes in 15 minutes.

We thank Dr. Rashid Bashir and Dr. Nate Mosier for their review of this paper and helpful suggestions. We thank Anna Burgner for initiating the research by characterizing components in hogd meat broth, Randy Woodson, Director of Agricultural Research, and Dr. Richard Linton, Director of the Center of Food Safety Engineering for providing the resources and environment that catalyzed this type of multidisciplinary research.

References


