Bacterial Transport in Rolled Stationary Phase Monoliths

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Outline

Background
  Rolled cotton (cellulose) monoliths
  Rigidity and robustness
Methods
  Preparing monolith
  Separation characteristics: tangential and radial flow
Results
  Fit with pathogen detection
Conclusions
Stationary Phase Monolith

Textile being rolled and packed

Chromatography column
## Fabrics Tested

<table>
<thead>
<tr>
<th>Fabric type</th>
<th>Yarn diameter (mm)</th>
<th>Fabric thickness (mm)</th>
<th>Fabric count (/inch)</th>
<th>Weave structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea island cotton</td>
<td>0.03</td>
<td>0.16</td>
<td>158</td>
<td>Plain</td>
</tr>
<tr>
<td>Cotton batiste</td>
<td>0.05</td>
<td>0.22</td>
<td>88</td>
<td>Plain</td>
</tr>
<tr>
<td>Cotton print cloth</td>
<td>0.06</td>
<td>0.26</td>
<td>80</td>
<td>Plain</td>
</tr>
<tr>
<td>Mercerized CPC</td>
<td>0.07</td>
<td>0.31</td>
<td>90</td>
<td>Plain</td>
</tr>
<tr>
<td>Cotton velveteen</td>
<td>0.10</td>
<td>1.12</td>
<td>71</td>
<td>Pile</td>
</tr>
<tr>
<td>Cotton flannel</td>
<td>0.12</td>
<td>0.78</td>
<td>55</td>
<td>Plain, napped</td>
</tr>
<tr>
<td>Microdenier rayon</td>
<td>0.13</td>
<td>0.44</td>
<td>45</td>
<td>Twill</td>
</tr>
<tr>
<td>Mercerized flannel</td>
<td>0.16</td>
<td>0.64</td>
<td>33</td>
<td>Plain, napped</td>
</tr>
<tr>
<td>Double napped blanket</td>
<td>0.20</td>
<td>1.07</td>
<td>76</td>
<td>Basket, napped</td>
</tr>
</tbody>
</table>
Continuous Stationary Phase

Woven Yarns
Interyarn, $\varepsilon_b$

Yarn of Fibers
Interfiber (Intrayarn), $\varepsilon_p$

Corresponds to

Cellulose Fiber Pore
(Intrafiber), $\varepsilon_i$

Column Evaluation

2 to 5 columns each fabric

Column dimensions
~180mm x 10mm

Probes
D₂O, NaCl, Glucose, BSA
BSA + NaCl, 3 replicates

Mobile phase buffer
50mM pH8.0 Tris

Sample concentration
5mg/ml

Sample size
50µl

Flow rate
2ml/min, 8ml/min
Chromatogram: Protein Desalting

flannel columns 511mm x 10mm at 4ml/min

BSA
NaCl

Li et al, 2000
Pore Volume Distribution

![Graph showing pore volume distribution](image)

- **Differential pore volume (ml/g Å)**
- **Pore diameter D (Å)**

- **Inaccessible pore volume (ml/g)**
  - Untreated
  - Enzyme 0.82
  - Enzyme 4.1
Rapid Chromatography over Cellulose Monoliths

Separations based on size
Pores less than 100 Angstroms in size
Monoliths are structurally stable
Bacteria pass through
Foodborne Pathogens

Example: *Listeria monocytogenes*

- Infectious number unknown
- Zero tolerance by USDA

Challenges in detection

- Ingredients
- Indigenous microflora
- Low numbers in foods

Swaminathan and Feng, *Annual Reviews in Microbiology*, 1994

Stevens and Jaykus, *Critical Reviews in Microbiology*, 1994
Conventional methods

- Preenrichment
- Selective enrichment
- Selective differential plating
- Presumptive identification
- Biochemical identification
- Sequester and amplify target bacteria
- Rapid detection
- Rapid identification
- Serotyping

Food + Buffer

Protein Biochip for Detecting Microorganism

Well = 5 nanoliters
30 to 50 wells/chip

Bashir, Gomez et al, 2001
**Cross-section of a well on a Biochip**

- **Protein receptor**
  - 20 nm size

- **Target molecules bound to receptors**

- **Glass cover**

- **Receptors immobilized on electrode**

- **Pt electrode**

- **SiO₂ insulation**

- **Silicon substrate**
Listeria monocytogenes

*L. monocytogenes* has 66 kD cell surface protein. Monoclonal antibody, gold labeled, binds to it.

**Objective of research:**
Capture/detect *L. monocytogenes* with antibody immobilized on a surface (bead, chip, other), *i.e.*, proteins at surfaces

Bhunia, et al, 2003
Benefits: Microfluidic Systems

Miniaturization
Consumes less reagents
Enables higher sensitivity
Shorter analysis time
Food Sample

Sample Volume = 100 mL

Detection volume = 100 nL

ERRC, 2000
First Step: Clarify Sample

Must be rapid (minutes)
Retain particles but not microorganisms
Maintain viability of microorganisms
Process at least 100 mL sample volume
Rolling Textile into Monolith

To Begin:

1. The fabric is cut into a rectangular shape.

2. The wet fabric is laid out on a hard smooth surface.

3. A small initial roll is begun at one end of the swatch.

4. The fabric is rolled by applying even pressure with the palms of the hands.

5. The roll is repeatedly pressed and rolled still using the palms of the hands.

6. The finished product is secured using cable ties.

Hamaker et al, Biotechnology Progress, 1998
Packed Filtration Holder
Experimental Setup

- Vacuum Trap
- Vacuum Pump
- Filtration Holder
- Air
Normal Flow Filtration

Millipore, Protein Concentration and Diafiltration by Tangential Flow Filtration, 2003,
Tangential Flow Filtration

Millipore, Protein Concentration and Diafiltration by Tangential Flow Filtration, 2003,
Particulate Removal Mechanism

Pressure

Flow Directions

Surface of packed RSP
Particulate Removal

Turbidity (Klett Units)

- Homogenate
- HSH solution
- RSP filtered HSH solution
SEM of Polycarbonate Membrane after filtration with clarified broth
Estimated Shear Stress in Monolith

\[ \tau_{\text{max}} = \frac{D}{4} \frac{\Delta P}{L} \]

where

- \( \tau_{\text{max}} \) = maximum intensity of shear stress
- D = capillary diameter
- \( \Delta P \) = Pressure drop over capillary ends
- L = capillary length
Effect of Shear Stress on Bacteria

E. Coli disrupted at about 1810 Pa\(^1\)
Calculated Shear stress in monolith is 9 Pa
Cells likely to survive filtration through monolith
Cells swept tangentially across fibers

\(^1\) Lange et al, 2001
Cells pass through monolith and remain viable

<table>
<thead>
<tr>
<th>Starting Cells CFU / mL</th>
<th>% Cells in Filtrate</th>
<th>% Cells Retained</th>
<th>% Recovered from monolith</th>
</tr>
</thead>
<tbody>
<tr>
<td>773 ± 40</td>
<td>40.5 ± 24</td>
<td>59.5 ± 24</td>
<td>21.7 ± 11</td>
</tr>
</tbody>
</table>
Conclusions: Monolith Filtration

1. Clear solution obtained from a turbid homogenate
2. Clear solution must be processed further before introduction to biochip
3. Microorganisms recovered in viable form