Rapid Sample Processing for Pathogen Detection

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Rapid Sample Processing for Pathogen Detection

Outline

1. Cell concentration and recovery (proteins at interfaces)
2. Cost constraints
3. Engineering of “hands-off” systems

Work supported by USDA Cooperative Agreement OSQR 935-42000-049-00D
The need and goal

Detect Presence of Food Pathogens, Identify Source, Reduce Public Health Risks

Rapidly Bring Microbial Concentrations to a Detectable Level

Enrichment Culture
Cell Concentration and Recovery

Challenge is one of biomaterials science and bio-separations engineering
Options to get needed number of cells time to result and mitigating membrane fouling drive prototype development

Enrichment Culture or Cell Concentration and Recovery

Elapsed Time hrs

Step

Sample Preparation

Sample from Food

Detection/Identification

Reporting

Time Consuming

Automated, Time < 1 hr

Foster, Vibbert, Ximenes et al, 2010, 2011
Rapid Pathogen Detection Metrics

Sensitive  1 - 100 cells/mL
Fast       1 hour
Low cost   $1 to 5
On site (farm, plant, store)
  low throughput, detection
Lab (in-house, regulatory)
  high throughput
  rapid screening

Tu et al, 10/25/2000
Sample Characteristics

Target cells present against background of:
- nonpathogenic cells,
- lipids,
- proteins,
- nucleic acids,
- salts, sugars,
- polysaccharides,
- vesicles,
- chlorophyll, etc.

sometimes on surface, sometimes internalized

Need to design / select bio-compatible capture materials with appropriate surface chemistry; package into automated and robust prototypes

Flat Membrane CCR Process

Sample, Concentrate, Recover
(in early days of this project, these were manual operations)

Limit of <100 mL before membranes clog

Liu et al, 2004, 2005
2500 viable cells are required from a food sample to detect a single cell at the endpoint of an assay procedure. Estimate from plot of cfu/g food vs. cfu/mL microfiltered concentrate.

3.4 log_{10} CFU/g = 2500 microorganisms in 100 mL of wash buffer required to detect a single pathogenic microorganism.

Cells, log_{10} cfu/100 mL wash buffer contacted with 1 g food material vs. number of cells (log_{10} cfu/mL) detected in micro-filtered concentrate.

Banada et al, 2011
Cross Flow Hollow Fiber Membrane Enables Processing of Larger Volumes

Feed → Pressure → PERMEATE → RETENTAT

Sample Solution volume decreases with time

Pressure Gauge

Pump

Valve

Hollow Fiber

Retentate is recirculated

Sample Solution volume decreases with time

Huang, Kreke et al, 2005-2009
OVERCOMING MEMBRANE FOULING

Chicken extract: treated initially with Lipase (1% (v/v)) at 30°C for 1 h
Control: water added (1% (v/v))

<table>
<thead>
<tr>
<th>STEP</th>
<th>Membranes</th>
<th>Time for Filtration</th>
<th>Volume Applied</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Microfiber Filter</td>
<td>1 min</td>
<td>100 mL</td>
</tr>
<tr>
<td>2</td>
<td>Polyethersulfone</td>
<td>2 min</td>
<td>~100 mL</td>
</tr>
</tbody>
</table>

Before Filtration

After Filtration

Vibbert, Liu, Ximenes, 2010
Hollow Fiber Membrane CCR Process: Engineering (4th Prototype)

<table>
<thead>
<tr>
<th>Key Components</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber module</td>
<td>0.2 µm hollow fiber 11 inch, Polysulfone</td>
</tr>
<tr>
<td>Pressure Transmitter</td>
<td>60 PSI max</td>
</tr>
<tr>
<td>2 Peristaltic Pumps</td>
<td>Rainin Rabbit Plus</td>
</tr>
<tr>
<td>Flow Meter</td>
<td>0-50 mL/min</td>
</tr>
<tr>
<td>Software</td>
<td>Labview 2009f3</td>
</tr>
</tbody>
</table>

Second pump passes liquid through the permeate side of the membrane in order to achieve a constant pressure gradient and increase transmembrane flux.
Concentrated GFP Cells are Visible

10^7 cfu/ml 10^5 cfu/ml

Demonstration that illustrate concentration of cells
- 100 X Concentration of *E. coli* (GFP)
- Average Time : 18 min

Concentrate Cells (Salmonella sp, Listeria sp, E.coli sp) Against a Background of Microorganisms

Identification by Different Methods

Multifluidic detection Antibody PCR Bacteriophage reporter Ramon Spectroscopy Light Scattering

Other parts of technology: capture antibodies (HSP 60), “block and anchor” chemistry, di-electrophoretic capture and impedance based detection. microfluidic approach enables other applications: water, blood, pharma