Microbial ingress through breaches in aseptic manufacturing systems

Experimental investigation of pressure-driven leaks

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OVERVIEW

- Aseptic processing and microbial ingress
- Mechanisms of microbial ingress
- Hydrodynamic principles
- Criterion for preventing microbial ingress
- Experimental verification of criterion for ingress
- Fluid flow model
Can a microbe migrate through a pin-hole leak?

Will the outflow of fluid prevent ingress of the leak?

(Not to scale)
Aseptic Processing and Microbial Ingress

- It’s all about keeping the microbes out
- Pressurized systems will have leaks
- Can ingress occur *against* the outflow of fluid?
- If so, *how long would it take?*
## Mechanisms of Ingress (1)

<table>
<thead>
<tr>
<th>MECHANISM</th>
<th>RATE ($\mu$m/sec)</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Convection</td>
<td>&gt; 1000</td>
<td>Typical</td>
</tr>
<tr>
<td>Swimming/Swarming</td>
<td>10 - 100</td>
<td>Individual / Group</td>
</tr>
<tr>
<td>Gliding/Twitching</td>
<td>10</td>
<td>Surface translocation</td>
</tr>
<tr>
<td>Sedimentation</td>
<td>&lt; 1</td>
<td>When oriented along gravity vector</td>
</tr>
<tr>
<td>Biofilm</td>
<td>&lt; 0.1</td>
<td>~ 24 hours to develop</td>
</tr>
<tr>
<td>Brownian Motion speeds</td>
<td></td>
<td>Random walk; factored in swimming/swarming</td>
</tr>
</tbody>
</table>
### Highest reported swimming speeds for bacteria*

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>MOTILITY ($\mu$m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>55</td>
</tr>
<tr>
<td><em>Chromatium okenii</em></td>
<td>45</td>
</tr>
<tr>
<td><em>Thiospirillum jenense</em></td>
<td>86</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>16</td>
</tr>
<tr>
<td><em>Bacillus licheniformis</em></td>
<td>21</td>
</tr>
<tr>
<td><em>Sarcina ureae</em></td>
<td>28</td>
</tr>
<tr>
<td><em>Salmonella enterica</em></td>
<td>55</td>
</tr>
<tr>
<td><em>Pseudomonas species</em></td>
<td>~70</td>
</tr>
</tbody>
</table>

* Does not include marine and sulfur bacteria
Schematic of a Typical Leak

Schematic showing a sterile boundary and PIPE/Tubing with inlet pressure $P_{in}$ and outlet pressure $P_{out}$, not to scale.
A common practice for preventing microbial ingress into a sterile system is to maintain a positive pressure differential across the sterile boundary.

\[ P_{\text{in}} > P_{\text{out}} \]

E.g. Laminar flow hoods and bioreactors
Profile of Fully Developed Laminar Flow
Plug flow at the entrance due to minimalized friction force
For a cell to enter the sterile boundary it must overcome the drag force imposed by the opposing fluid flow.
In other words, the motility of the cell \( (u) \) must exceed the convective velocity of the fluid at the sterile boundary \( (V) \).

However, we know that \( u \) is orders of magnitude smaller than \( V \).
Criteria for preventing microbial ingress (2)

Most conservative estimate of fluid velocity in a breach is on the order of one cm/sec

Microbial motility is on the order of one micron/sec

Breaches are routinely simulated during media challenges, clean-in-place operations, and sampling
Experimental Verification of Results - Beads

microscope/video camera

filter
Experimental Verification of Results - Beads
Experimental Verification of Results
- Beads
Experimental Setup – Liquids

Site of simulated leak
(0.75 inch incision in thin walled tubing)

Pump

Vent

Trypticase Soy Broth (TSB)

*Pseudomonas aeruginosa*
in TSB (growth promoting media)
Experimental Design - Liquids

- Flow Rate 0.6 mL/min (worst-case)
- Thin Tubing (worst-case)
- Highly motile organism (*Pseudomonas aeruginosa*)
- High Inoculum Concentration
  (3.5 x 10^6 – 8.0 x 10^6 per inoculum)
- 24 Inoculations of 10 μL every 15 minutes
  (140 million cells over a period of 8 hours)
- 7 Day Incubation at 37°C
Experimental Results - Liquids

Results: After 7 days of incubation:

- No growth was observed in media (media was clear);
- Control media (inoculated with *Pseudomonas aeruginosa*) was murky.
Experimental Results - Liquids

Turbid

Transparent

POSITIVE CONTROL  EXPERIMENTAL
Experimental Verification of Results - Gases

- Sterile bag (TSB)
- Pump
- Vent
- Sterile Filter
- Leak Point 1 (thin tubing)
- Aerosolized Pseudomonas aeruginosa
- Nebulizer
- Leak Point 2 (thick tubing)
Experimental Verification of Results - Gases

Laser drilled slit in disc (3 mm x 1 mm) [Video]
Results – Gases

• Worst-case Scenario
  ▪ Relatively large leak (3mm by 1 mm)
  ▪ Small internal pressure (0.03 PSI)
  ▪ High Enumeration: 38,000 cfu/cm²
  ▪ High Concentration: $4.0 \times 10^9$ CFU/m³:
    7 orders of magnitude higher than Class D (200 CFU/m³)

• 17 Day Incubation
  ▪ Growth in positive control
  ▪ No growth in experimental media
Results – Gases

- Air velocity mainly dependent upon pressure
  - At 0.03 psi, velocity is about 35 mph
  - At 1 psi, velocity rises to 200 mph!
  - Typical operating pressures are well over 1 psi
Fluid Flow Model

Bernoulli Equation modified to account for friction.

**Average fluid velocity in breach (V) as a function of:**

- Pressure drop across breach
- Dimensions of breach
- Viscosity of fluid
- Friction factors (empirical)
Experimental verification of model

Volumetric Flow Rate Through Smart Gasket Valve (Pore Simulation)

- Flow Rate (mL/min)
- Gauge Pressure (PSI)

- Measured
- Bernoulli (Acct for Friction)
Model Parameters

Ingress is only possible if fluid velocity $V < \text{microbial motility } u$

Breach is only detectable if outflow $Q > \text{evaporation rate } E$

\[
\begin{align*}
  u &= \text{Maximum microbial motility} = 100 \text{ μm/s (literature)} \\
  V &= \text{Average fluid velocity in breach} = 200 \text{ μm/s} \\
  d &= \text{Critical size of water droplet} = 3 \text{ mm (expt)} \\
  E &= \text{Evaporation rate} = 0.4 \text{ mL/day (expt)}
\end{align*}
\]
Predicted criterion for preventing ingress

**Approach:**
Set $V$ to 200 μm/s

Compute minimum pressure required for $Q > 0.4$ mL/day.

If operating pressure is below minimum pressure, breach is undetectable;
if not, the implication is that ingress is not possible.
Criterion for preventing ingress

Flexible Tubing

- Pinhole (Pore): ~ 0.0004 psig ~ 170 µm
- Slit (Parallel Plates): ~ 0.04 psig ~ 7 µm

Flange/Gasket Assembly (Hard Pipe)

- Pinhole (Pore): ~ 0.06 psig ~ 170 µm
- Slit (Parallel Plates): ~ 0.35 psig ~ 20 µm

These pressures are much lower than typical operating pressures.
Questions?