E. Coli Lysate Clarification – Scale up

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Abstract

This application note is showing the performance for cell debris retention and 75 kD GFP protein recovery using filters with different fluid paths and the same fiber (750 kD MWCO, 0.5 mm lumen ID). It demonstrates an equivalent scale-up performance between modules, a Reuse line Explorer 12-inch and a Reuse line Explorer 24-inch filter using the 3× concentration and 3 diavolumes (DV) at 9,000 sec⁻¹ shear. A permeate flux rate of 20 LMH during concentration produced a desirable low and stable (flat) TMP profile between 2 – 3 psig when operating at a crossflow shear rate of 9,000 sec⁻¹, a membrane mass loading of 1.7 – 2.0 Kg/m² and volume loading of ~ 30 L/m² both trials. The equivalence of the TMP profiles confirms a linear scale-up going from a 12-inch module to a 24-inch module.
**Introduction**

Clarification of E. coli lysate focused on separating cell debris of the E. coli homogenate to recover the 75 kD target protein in the permeate.

**Materials**

For this clarification process two Green Line Explorer with a length of 12-inch and 24-inch and a molecular weight cut-off (MWCO) of 750 kD with 0.5 mm fiber ID were used. The filter area of each module is 0.0321 m². Like all our Hollow Fiber Modules the membrane consisted of modified Polyethersulfon (m-PES).

**Details of used Hollow Fiber Module**

<table>
<thead>
<tr>
<th>Family</th>
<th>Reuse</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Product Size</td>
<td>Explorer</td>
<td>Explorer</td>
</tr>
<tr>
<td>MWCO</td>
<td>750 kD</td>
<td>750 kD</td>
</tr>
<tr>
<td>Pore Size</td>
<td>0.5 mm</td>
<td>0.5 mm</td>
</tr>
<tr>
<td>Fiber ID</td>
<td>12 inch</td>
<td>24 inch</td>
</tr>
<tr>
<td>Length</td>
<td>0.0155 m²</td>
<td>0.0321 m²</td>
</tr>
<tr>
<td>Filter Area</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>No. of Fibers</td>
<td>150 - 750 mL</td>
<td>250 - 1,500 mL</td>
</tr>
<tr>
<td>Recommended batch volume per module</td>
<td>1.3 cm</td>
<td>1.3 cm</td>
</tr>
<tr>
<td>Diameter Module (cm)</td>
<td>½-inch TC</td>
<td>½-inch TC</td>
</tr>
<tr>
<td>Feed</td>
<td>Retentate connectors</td>
<td>¾-inch Hose Barb</td>
</tr>
<tr>
<td>Permeate connector</td>
<td>WA75005EXP12S0 (1-pack)</td>
<td>WA75005EXP24S0 (1-pack)</td>
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**Methods**

- Duplicate the performance for cell debris retention and 75k D GFP protein recovery using filters with different fluid paths and the same fiber (750k D MWCO, 0.5 mm lumen ID).
- Demonstrate equivalent scale-up performance between modules, a Reuse line Explorer 12-inch and a Reuse line Explorer 24-inch filter using the 3× concentration and 3 diavolumes (DV) at 9,000 sec⁻¹ shear.

**Results**

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<tr>
<th>Homogenization Process</th>
<th>1 pass at 900 bar</th>
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<tr>
<td>Lysate cell density (LCD)</td>
<td>60 g/L</td>
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<tr>
<td>Initial Feed Turbidity</td>
<td>1,650 NTU</td>
</tr>
<tr>
<td>Initial Feed Volume &amp; Loading</td>
<td>34 L/m²</td>
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<tr>
<td>Membrane Mass Loading</td>
<td>~2 Kg/m²</td>
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<tr>
<td>Explorer 12-inch = 30 g/155 cm²</td>
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</tr>
<tr>
<td>Explorer 24-inch = 60 g/321 cm²</td>
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<tr>
<td>Process Flux Rate (constant)</td>
<td>20 LMH - Concentration</td>
</tr>
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**Process Step:**
E. coli Lysate Clarification (60 g/L)

**Element & Membrane:**
Explorer 12, 750k D PES, 150 cm², 0.5 mm ID

**Cell Density & Lysis:**
2.0k g/m², 32 L/m² (post centrifuge)

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**Figure 1:** Pressure profiles and permeate flux during processing (concentration & diafiltration) with an Explorer 12-inch

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**Process Step:**
E. coli Lysate Clarification (60 g/L)

**Element & Membrane:**
Explorer 24, 750k D PES, 310 cm², 0.5 mm ID

**Cell Density & Lysis:**
1.7k g/m², 28 L/m² (post centrifuge)

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**Figure 1:** Pressure profiles and permeate flux during processing (concentration & diafiltration) with an Explorer 24-inch
Conclusion

A permeate flux rate of 20 LMH during concentration produced a desirable low and stable (flat) TMP profile between 2 – 3 psig when operating at a crossflow shear rate of 9,000 sec⁻¹, a membrane mass loading of 1.7 – 2.0 Kg/m² and volume loading of ~ 30 L/m² both trials.

The equivalence of the TMP profiles confirms a linear scale-up going from a 12-inch module to a 24-inch module. The fitting hollow fiber module for this E.Coli process would have a module length of 24 inches.