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Tangential Flow Filtration Membranes For the Washing of *Escherichia coli* Cells

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Background

Microbial fermentation is used to produce such products as therapeutic proteins, antibiotics, hormones, enzymes, amino acids, blood substitutes and alcohol. These products may be expressed by the microbial cells themselves, expressed intracellularly in the cytoplasm or, in the case of bacterial cells, in the periplasmic material.

Before any intracellularly-produced molecules can be used and further purified, the cells (*i.e.*, *E. coli*) must be concentrated and set in an appropriate buffer (cell washing) before being lysed. Then after lysis, molecules of interest have to be separated from the parent cells and then clarified to remove cellular debris and other contaminants. Depending on the process used, additional clarification steps may be necessary (Figure 1).

Objectives and Criteria for Success

During the washing of *Escherichia coli* cells, the objective is to reach a sufficiently high concentration of the *E. coli* in the correct buffer before cell lysis and/or cell solubilization, which then allows for recovery of the particular expression protein.

The vast majority of *E. coli* cells are concentrated to achieve a final dry material content of approximately 200 g/L dry material. The cells are then diafiltered with 5–10 dialfiltration volumes (Tris 50 mM pH 7.4 buffer or PBS buffer + EDTA) before the required formulating product is obtained and is ready for cell lysis.

The following are the main success criteria for the washing step of *E. coli* through concentration/diafiltration:

- Maintain a standard UF/DF process time of approximately three hours, not including setup and cleaning. This can be achieved by limiting the fouling effect of the cells, thereby maintaining a constant filtrate flux throughout the step.
- Create efficient procedures by removing culture media quickly, leaving the *E. coli* cells ready for lysis.
- Obtain a product yield > 80%.
- Obtain a minimum volume of *E. coli* cells in order to limit the need for lytic solutions.

Process Parameters

Process Considerations

A two-pump system is used to maximize productivity and effectively manage all success criteria points. This system allows an accurate control of the permeate flow rate (limiting the fouling effects) and consequently achieving the desired process time of less than three hours. Figure 2 shows the main setup that can be used for running two-pump system applications.

The process of washing *E. coli* cells requires that the cells must first be concentrated, then diafiltered with a buffer

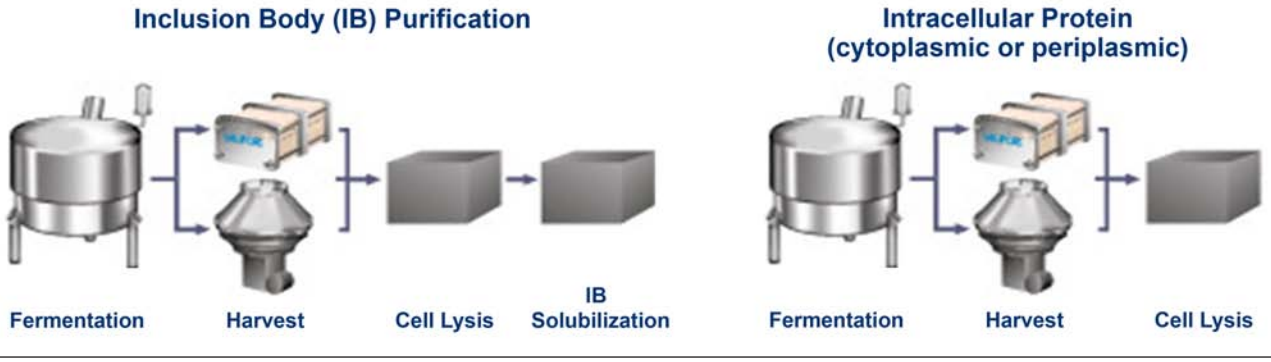

FIGURE 1. Two purification examples.

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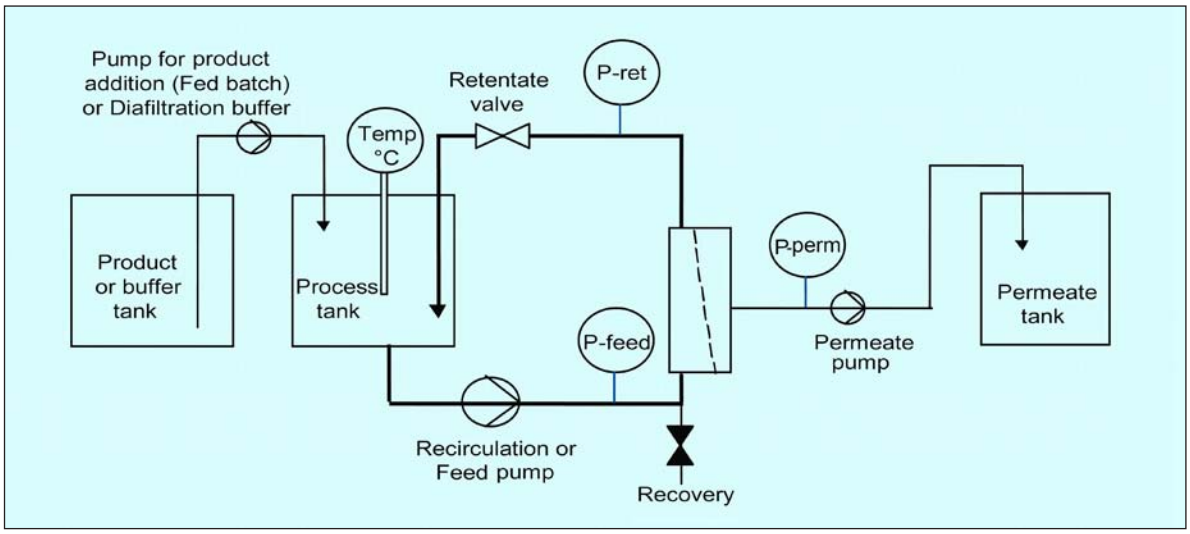

FIGURE 2. Setup for a two-pump system.

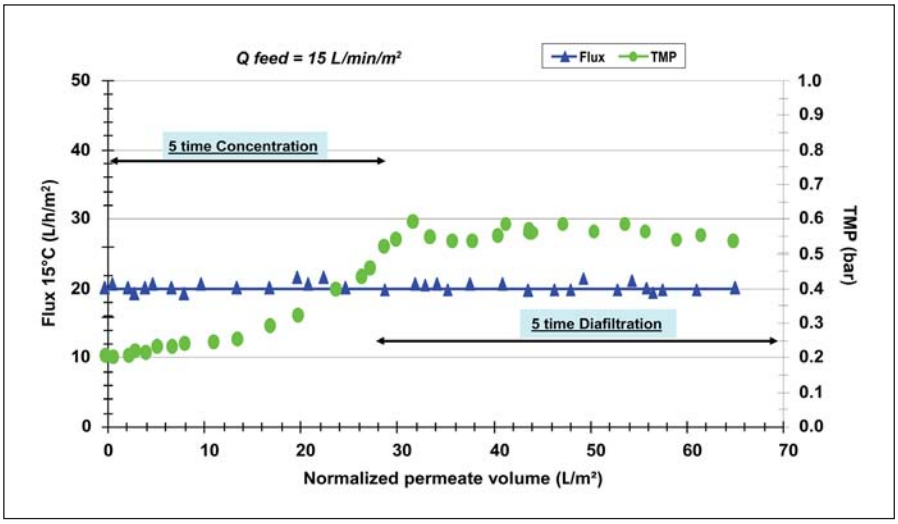

FIGURE 3. Flux and TMP vs. permeate volume.

TABLE 1. Process parameters.	
Membrane	Biomax 1000 kDa – V
Initial Dry Material (g/L)	15–20
Process Temperature (°C)	10–15
Average Flux (LMH)	20–25 (permeate flux-controlled, double-pump system)
Cross-Flow (L/m²/min)	10–15
TMP (bar)	Minimum for the lowest fouling effect
Concentration Factor	5–10
N Diafiltration Volume	3–5
Yield (%)	> 80
Process Time	< than 3 hours

suitable for subsequent lysis of the cells, and recovery of recombinant proteins out of the cells. As shown in Figure 2, the control of hydraulic parameters obtained during these successive steps is key for optimizing this step.

It is important to note that during procedures involving concentration and dialfiltration of *E. coli*, flux may be greatly impacted by the presence of antifoam in addition to the temperature and oxygenation of the culture. Antifoam and culture conditions are thus, key parameters to control. (See Figure 3.)

Table 1 summarizes process parameters that are obtained during development work (usually done on 0.1 m² membrane area), and assessed during pilot work (usually performed on 0.5–1.0 m²) before implementation at production scale.

Cleaning Considerations

The typical cleaning solution that offers repeatability and constancy in normalized water permeability (NWP) membranes fouled with *E. coli* cells is 0.5 M NaOH from room temperature to 40–45°C for 30–60 minutes.

For a better efficiency, thanks to its particular oxidizing action on cells and cell debris, a solution of NaOCl 300 ppm active chlorine at room temperature for 30–60 minutes may also be substituted for NaOH 0.5 M.

Scale-Up Considerations

By using a linearly scalable, flat sheet device, when the volume increases, membrane area increases linearly to perform the process in a defined period of time. Table 2 provides several examples of how to control the process impacts on the membrane area.

Conclusion

As shown in Table 3, operating parameters and membrane performance depend on a variety of factors including feed stream composition (*e.g.*, purity, and protein concentration, among other factors), membrane/device selection, and target final formulation. Success criteria imposed by the biopharmaceutical industry were achieved in most of the studies performed using linearly scalable flat sheet devices with open channel membrane.

TABLE 2. Example of scale-up.

Example of Process	Typical Process Batch Size (L)	Typical Process Time (h)	Area (m^2)
5 x concentration; 5 x diafiltration; final dry material up to 100–200 g/L; 15°C	100	3	3
	500		15
	1000		30
	2000		50

TABLE 3. Example of success criteria fulfillment.

Success Criteria	Acceptance Criteria	Comment
Yield	> 80	Usually yield around 86% after recovery with buffer
Non-Fouling Conditions	Possibility to maintain flux during concentration/diafiltration	Flux maintained between 20–25 LMH for 5 x concentration and 5 x diafiltation
Process Time (UF/DF)	< than 3 hours	6 m^2 = 3 x 2 m^2 (20% safety factor) allows processing 300 L in < 3 hours
Cleaning	Recovery of at least 80% of initial normalized permeability with water	0.5 M NaOH 40–45°C, 30–60 minutes usually allows recovery of > 90% initial NWP