Separating *E. coli*: sometimes smaller is faster

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The situation

Cross flow membrane microfiltration (MF) has become an established method for cell harvesting, cell washing, and lysate clarification. Open channel, hollow fiber membranes are considered by many investigators as the state-of-the-art technology for harvesting mammalian cells as well as processing fermentation cell broths.

Because MF membranes typically provide a higher initial permeation rate than ultrafiltration (UF) membranes, MF hollow fiber membrane separations cartridges are often perceived as the better choice.

However, the open pores of the MF membrane provide access sites for submicron-sized cellular debris in the feed stream, often causing pore plugging early on, with an associated decline in productivity.

The solution: GE Healthcare hollow fiber ultrafiltration cartridges

This cell debris is especially difficult with *E. coli* due to high levels of lipopolysaccharide (a "sticky" component that contributes greatly to membrane fouling). Though it is non-intuitive, some investigators have found that their needs are better served by moving to the smaller pore GE Healthcare 500,000 nominal molecular weight cutoff (NMWC) hollow fiber UF membrane.

While it is true that the more open the membrane, the higher the initial flux, for overall flux efficiency, customers should select the most dense membrane that allows significant passage of the desired molecule or largest contaminant.

Denser may be better in the long run. Compared to MF cartridges, the more dense 500,000 NMWC "skinned" UF membrane allows retained cellular material to remain in the bulk stream, where it does not block membrane pores, and where it provides improved flux stability over the course of the run. A graphical illustration of this relationship is shown in Figure 1.

E. coli example

Recently, one investigator working with *E. coli* cells utilized the GE Healthcare 500,000 NMWC UF membrane separations cartridge to concentrate the fermentation broth, then exchange the media with a storage buffer containing 10% glycerol.

A "snapshot" of the process is as follows:

- Growth media: Buffered vegetable protein hydrolysate
- Initial cell density: ~60 g wet cell wt/liter (OD 30)
- Initial volume: 16-18 liters
- Model number: UFP-500-C-6A (autoclavable)
- NMWC: 500,000
- Lumen diameter: 0.5 mm (20 mil)
- Membrane area: 0.48 m^2 (5.2 ft^2)
- Path length: 63.5 cm (25 in)
- Recirculation rate: 6 lpm (~11,000 sec^{-1} shear rate)
- Productivity (avg): ~35 lmh

Due to the relatively low cell densities involved, the investigator selected our 0.5 mm lumen diameter membrane. Normally the best choice for lumen diameter is 1 mm. Assuming that this channel diameter allows unimpeded passage of the cellular material, it can provide a "win-win" situation by maximizing membrane area and minimizing pump recirculation rates to achieve optimum cross flow velocity. Moreover, the unique ability to autoclave our ultrafiltration cartridges allowed the investigator to maintain an entirely aseptic process.

Successive runs prove the point

To evaluate the consistency of the flux performance of this membrane, four successive test runs were conducted. After each run, the cartridge was cleaned using 0.5 N NaOH at 50 °C for 30 minutes. The cartridge and tubing were autoclaved before each subsequent run. The consistent flux performance is clearly evident in Figure 2. This data also indicates the successful "clean and reuse" feature of hollow fiber membranes with fouling feed streams.

Scale-up is linear with GE Healthcare membrane cartridges

Extrapolating these results to our 4-inch diameter Steam-in-place 65MSM cartridge with its comparable path length, one could perform a similar concentration/diafiltration on a 200-liter batch in about the same time period.

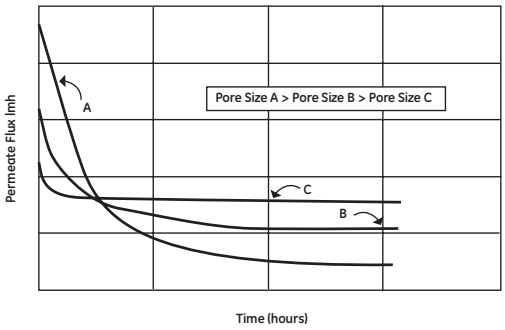

Figure 1. Flux curves by membrane pore size. Here pore size A, the largest, provides the greatest initial flux, but soon is blinded by particulate in the feedstream. An intermediate size, B, provides considerably more filtrate over time. The smallest size, C, while its initial flux is a fraction of A's, provides the greatest output over time.

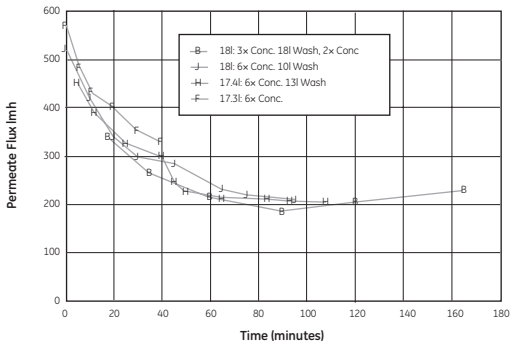

Figure 2. Concentration and media exchange of *E. coli* fermentation broth

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