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Tangential Flow Filtration for *E. coli* Harvest: Plasmid DNA Production Using the Sartoflow® 5000 SU System

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Abstract

Plasmid DNA produced in *Escherichia coli* serves as a critical starting material for gene therapy and nucleic acid-based applications. Harvesting the cells using hollow fiber tangential flow filtration (TFF) enables a closed, scalable, and low-shear concentration and washing step that preserves cell integrity and reduces premature lysis, host-cell DNA release, and endotoxin burden prior to downstream processing.

This application note demonstrates the use of the Sartoflow® 5000 SU system with hollow fiber TFF modules for harvesting *Escherichia coli* cells in plasmid DNA production. The process replaces traditional centrifugation with a closed, single-use TFF approach, reducing contamination risk and improving process efficiency.

The performance of the Sartoflow® 5000 SU TFF system was evaluated using a Sartorius 750 kDa single-use hollow fiber module in a cell harvest step of a plasmid DNA process. A total of 34.2 L of cell broth was harvested and subjected to concentration and diafiltration. The TFF approach delivered satisfactory yields, achieving complete recovery of wet cell weight and largely retaining the plasmid DNA in the cells.

Introduction

Industrial-scale plasmid DNA (pDNA) production typically involves *Escherichia coli* (*E. coli*) fermentation, followed by cell harvest, lysis, and downstream purification (Figure 1). The cell harvest step is critical because it directly influences both product yield and the impurity burden entering downstream processing. Harvesting technologies must efficiently concentrate and wash biomass while preserving cell integrity to prevent premature release of intracellular contaminants.

Conventional centrifugation is widely used for microbial cell harvest but presents several operational and process-related challenges. Open handling steps can increase contamination risk, while cleaning and sterilization require significant time and resources. In addition, the mechanical forces generated during centrifugation may cause partial cell lysis, resulting in the release of host-cell DNA, endotoxins, and other impurities that can complicate subsequent purification steps.

Tangential flow filtration (TFF) with hollow fiber membranes offers a closed, single-use alternative for microbial cell harvesting. By enabling gentle concentration and washing of *E. coli* cultures, hollow fiber TFF can help maintain cell integrity while reducing process complexity and contamination risks. The approach combines cell concentration and diafiltration in a single operation and can be readily scaled from process development to manufacturing.

The Sartoflow® 5000 SU TFF system has been developed to support high-performance ultrafiltration, diafiltration, and microfiltration applications across a wide range of batch sizes. The platform accommodates single-use hollow fiber modules and utilizes a four-piston membrane recirculation pump that delivers high flow rates under low-shear conditions, making it well suited for microbial cell harvesting applications. Integrated automation, precise monitoring of process parameters, and automated diafiltration capabilities support robust and reproducible operation, while the fully single-use flow path minimizes cleaning requirements and reduces the risk of cross-contamination. In combination with its low hold-up design and product recovery features, the system enables efficient biomass processing and high recovery yields (Figure 2).

This application note evaluates the use of the Sartoflow® 5000 SU system equipped with a 750 kDa single-use hollow fiber module for harvesting *E. coli* cells during pDNA production. A total of 34.2 L of fermentation broth from a Biostat® Microbial bioreactor was processed using concentration and diafiltration steps. The study demonstrates the capability of hollow fiber TFF to replace conventional centrifugation with a closed, scalable, and low-shear harvesting strategy while assessing biomass recovery, plasmid retention, and overall process performance.

Figure 1: Typical pDNA production workflow

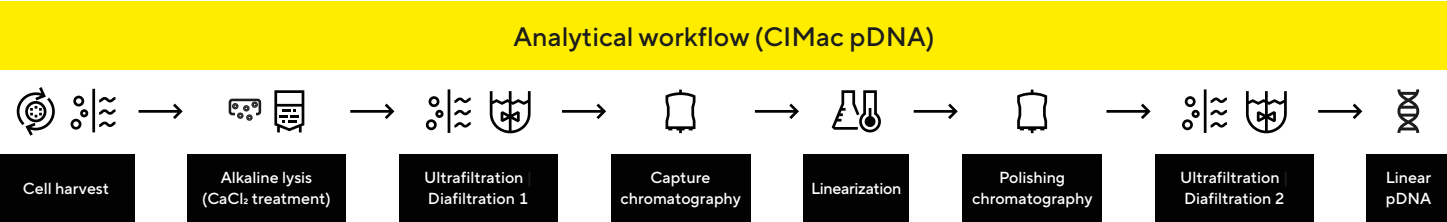
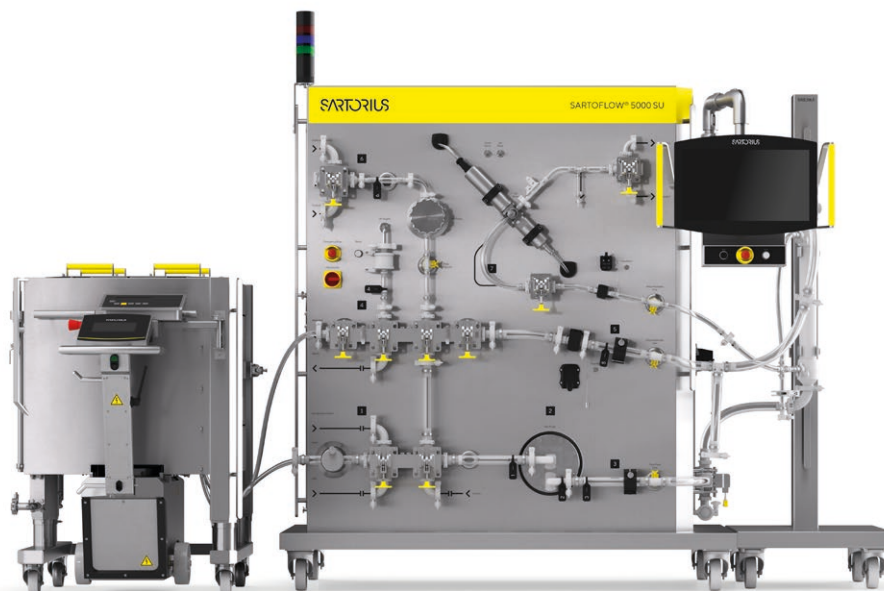


Figure 2: The Sartoflow® 5000 SU TFF system



Materials & Methods

A 34.2 L *E. coli* fermentation ($OD_{600} \approx 30$) with a wet cell weight of 101 g/L served as the feed. The broth was fed directly into the TFF system without centrifugation. The Sartoflow® 5000 SU system was equipped with a Sartorius single-use hollow fiber ultrafiltration module with a 12" length, 750 kDa molecular weight cut-off (MWCO), 1 mm inner diameter, and 0.63 m² surface area. This corresponded to a loading density of 54 L/m². The 750 kDa MWCO is suitable for retaining the bacteria while allowing soluble proteins and small molecules to pass.

The crossflow (feed flow) rate was initially set to 1,920 L/h, resulting in an estimated shear rate of 6,800 s⁻¹ in the fibers. After the start-up phase, the crossflow rate was set to 2,260 L/h, corresponding to a shear rate of approximately 8,000 s⁻¹. The retentate valve was completely opened to minimize transmembrane pressure (TMP). No permeate flow control was applied; therefore, the permeate valve was kept fully open throughout the entire process (2-fold concentration, diafiltration, and 2.5-fold final concentration). 3-fold diafiltration was performed using 25 mM Tris buffer (pH 8.0) to remove residual growth medium, an important step for eliminating endotoxins and media components that could interfere with lysis or downstream processing.

During final concentration, the bypass function of the Sartoflow® 5000 SU system was used to reach a final volume

of 7 L. In bypass operation, the bleeding function was used to homogenize the concentrated cells in the loop with the concentrate remaining in the tank after filtration of a defined volume.

Figures 3 and 4 illustrate bypass operation and tank-based recirculation, respectively. In bypass mode, the low hold-up volume in the loop leads to rapid cell accumulation, causing a pronounced increase in viscosity and limiting both pumpability and filtration efficiency.

As a result, the system periodically switches from bypass (Figure 3) to tank-based recirculation (Figure 4), bleeding concentrated retentate back into the recirculation tank, where mixing equalizes concentration between the tank and the loop. After homogenization, operation returns to concentration mode, and filtration continues under stable hydraulic conditions.

Wet cell weight and OD measurements were performed on the feed solution and monitored throughout the process. pDNA was isolated from *E. coli* using the QIAprep® Spin Miniprep Kit (QIAGEN), with OD₆₀₀-based sample preparation and centrifugation according to the manufacturer's protocol. The pDNA was eluted in kit buffer, quantified at 260 nm, and the concentration in the original cell broth was calculated as the mean of three measurements.

Figure 3: Bypass operation in the Sartoflow® 5000 SU system

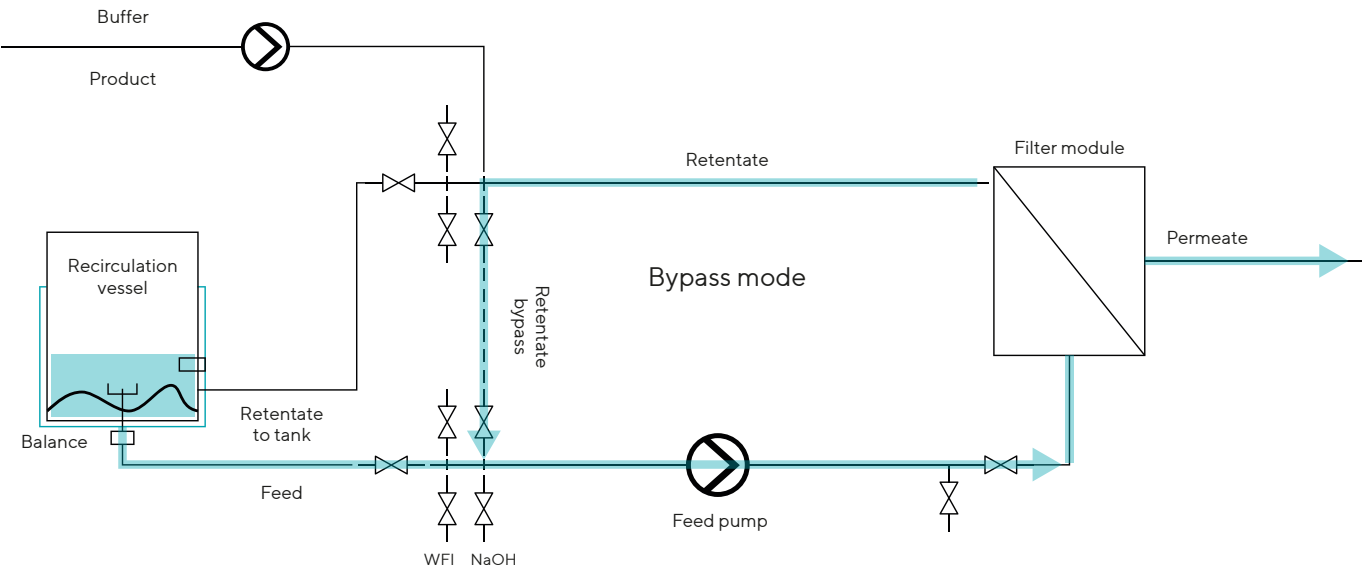
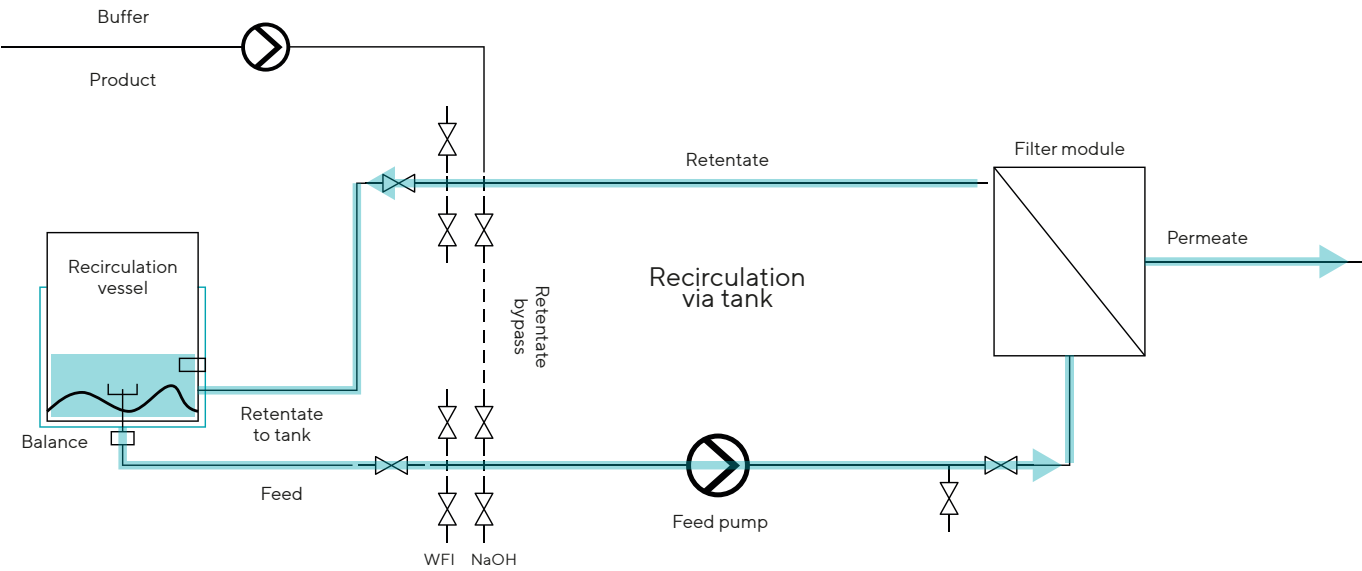


Figure 4: Tank-based recirculation for homogenization in the Sartoflow® 5000 SU system



Results

Process overview

The TFF harvest process was operated in a fully closed and aseptic manner and completed within approximately 7.5 hours. Once initiated, the process required minimal manual intervention. The extended process duration was mainly attributable to moderate flux rates and the implementation of multiple diafiltration volumes.

As shown in Figure 5, TFF was started with a gentle shear rate of $6,800\text{ s}^{-1}$. After 30 minutes, the flow rate was increased to 37.7 L/min ($2,260\text{ L/h}$), corresponding to a shear rate of approximately $8,000\text{ s}^{-1}$.

Bypass operation during final concentration

During final concentration using the Sartoflow® 5000 SU system's bypass function, a final volume of 7 L was achieved with a wet cell weight of 670 g/L . As shown in Figure 6, wet cell weight increased continuously during this phase because of volume reduction and concentration in the loop, indicated by the gray shaded areas.

The yellow shaded regions in Figure 6 denote bypass operation and highlight the transitions between concentration mode, where the suspension concentration is increased, and bypass mode, during which the cell suspension in both the tank and the loop is homogenized. Figure 6 shows that bypass operation can be successfully used to homogenize the suspension.

Figure 5: Process conditions during concentration, diafiltration, and final concentration in the Sartoflow® 5000 SU system

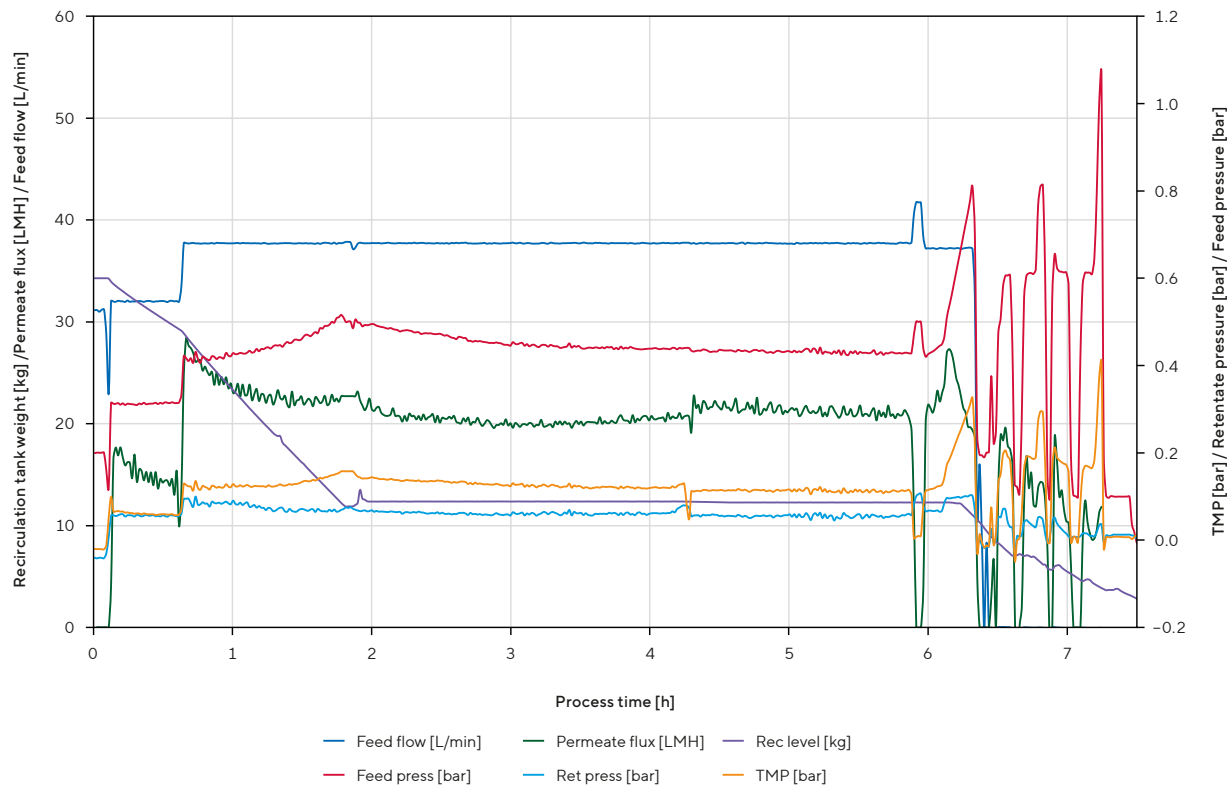
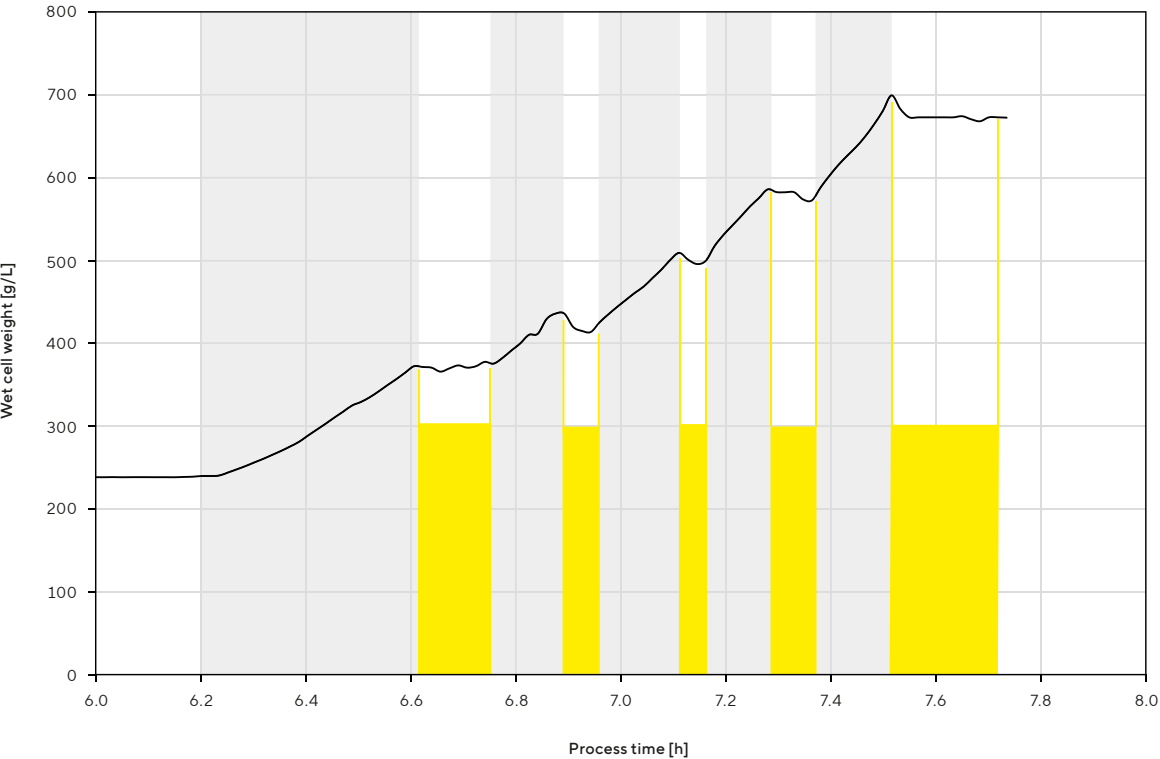


Figure 6: Process conditions during final concentration in the Sartoflow® 5000 SU system



Note. Active concentration is shown in gray, and homogenization in bypass operation is shown in yellow.

Analytical results

The final retentate (~ 7 L) contained the entire biomass from the initial 34.2 L *E. coli* fermentation, corresponding to an overall concentration factor of approximately 5-fold. Complete cell retention was achieved throughout the process, resulting in an overall biomass yield of ~ 100%. No bacterial breakthrough into the permeate was observed, as confirmed by permeate clarity and turbidity measurements.

Wet cell weight increased from 101 g/L in the feed to 670 g/L after final concentration (Table 1). Minor variations observed during intermediate steps are attributed to sampling variability and volume changes during diafiltration rather than biomass loss.

The majority of pDNA present in the feed was recovered. The relatively small deviation can be explained by biological variability.

Table 1: Analytical results for wet cell weight and pDNA

	Wet cell weight [g/L]	pDNA [mg/L]
Feed	101	213
Concentration	251	473
Diafiltration	213	358
Final concentration	670	1,047
Harvest 1 + 2	393	735

Following a 3-diavolume diafiltration, approximately 95% of residual cultivation medium components were removed from the *E. coli* suspension. The diafiltered biomass can then be subjected directly to alkaline lysis (NaOH | SDS), resulting in a cleaner lysate with significantly reduced carryover of media-derived impurities and an improved starting point for downstream processing.

Overall, the total recoverable pDNA yield after downstream processing was comparable to that obtained from conventional centrifugation-based cell harvest.

Discussion

Comparison with centrifugation

Table 2 compares the features of the two harvest methods: centrifugation and TFF using hollow fibers on the Sartoflow® 5000 SU system. A traditional disk-stack centrifuge can clarify approximately 34 L of *E. coli* broth in about 1 hour (including acceleration and deceleration), which is significantly faster than the 7–8 hours required for the TFF process. However, setup and cleanup times, as well as the overall process context, must be considered. Using the Sartoflow® 5000 SU TFF system, the harvest process was operated with minimal manual intervention; after initial setup, the system was fully automated. In contrast, centrifugation of multiple batches typically requires repeated loading and unloading cycles and increased operator involvement. A further advantage of TFF is its straightforward scalability, as capacity can be increased by adding membrane area while maintaining comparable process conditions.

In addition, centrifugation-based harvest often requires resuspension and washing of the cell pellet, representing an additional processing step. In the TFF process presented here, concentration and washing are integrated into a single operation. Analytical data showed that pDNA yields from TFF-harvested cells were equivalent to those obtained from centrifuge-harvested cells, with high supercoiled plasmid purity.

Another advantage of the TFF approach is the reduced level of impurities in the lysate. During TFF harvest, extracellular DNA, soluble proteins, and residual growth medium components are efficiently removed into the permeate, whereas these impurities may remain associated with the cell pellet after centrifugation.

Table 2: Harvest method comparison: TFF with hollow fibers vs. centrifugation using a 40 L *E. coli* broth example

	Harvest method	Harvest method
Criterion	Hollow fiber TFF (Sartoflow® 5000 SU system)	Centrifugation (disk-stack)
Throughput rate	~ 5 – 6 L/h (40 L in ~ 8 h)	~ 40 L/h (single run)
Batch mode	Continuous filtration of an entire batch	May require multiple runs, depending on working volume
Setup turnaround	~ 30 min to install the single-use kit; no cleaning-in-place needed	~ 1 – 2 h for cleaning-in-place and bowl sterilization
Sterility containment	Closed system comprising single-use bags and tubing; supports aseptic processing throughout the harvest	Open transfers using funnels or containers may be required unless a customized closed system is employed; higher contamination risk
Cell yield	~ 100% (no cell loss)	~ 98 – 99% with minor losses in the bowl and during transfers
Cell condition	Low shear distributed across the membrane; intact, minimally stressed cells	High g-force and shear upon impact; some cell breakage is likely
Impurity removal	Integrated diafiltration removes media and soluble impurities	Requires separate resuspension and wash step
Equipment footprint	Mobile skid; requires feed tank	Large floor-mounted centrifuge and feed tank
Scale-up	Scale by adding membrane area using multiple modules on the same skid, up to ~ 2,000 L	Scale by using a larger centrifuge or multiple parallel units; challenging beyond certain process volumes
Single-use vs. reuse	Single-use; consumable cost per batch and no cross-contamination risk	Reusable; fixed cost, but cleaning validation required
Maintenance	Minimal; preventative pump maintenance only	Frequent; decontamination, seal bearing wear, balancing, etc.
Operator skill	Recipe-driven; system monitoring with minimal intervention	Requires oversight during feed harvest, possibly with manual pellet handling

Process considerations

A limitation of the TFF harvest is the larger final recovery volume (~ 7 L) relative to centrifugation, which typically yields a compact pellet of ~ 1–2 L of wet solids. However, because the subsequent alkaline lysis step requires the addition of buffer volumes, this difference does not represent a practical disadvantage. Handling biomass as a homogeneous slurry can even facilitate mixing of lysis reagents compared to processing a dense cell pellet.

Overall, the TFF harvest achieved the same primary objective as centrifugation – efficient collection and washing of cells – with comparable yield and product quality, while offering advantages in terms of process integration, containment, and impurity removal.

Process parameters and optimization for TFF harvest

Implementing a hollow fiber TFF harvest successfully requires attention to certain key parameters:

Membrane selection (pore size | MWCO)

For cell harvest, the membrane pore size should be small enough to retain cells but large enough to permit high flux and passage of lysate impurities. In our case, 0.2–0.45 μm ($\approx$ 750 kDa MWCO) achieved a good balance, consistent with the literature on TFF-based cell harvest.

Crossflow (shear rate)

High crossflow helps prevent cell deposition on the fiber walls, maintaining flux. However, excessively high shear can damage cells or cause foaming. In a benchtop study conducted using a Sartoflow® Smart benchtop system, shear rates of approximately 6,800 s^{-1} at the beginning of filtration, and 8,000 s^{-1} during the later stages of the process, resulted in the best overall performance. The Sartoflow® 5000 SU pump can reach shear rates of 8,000–10,000 s^{-1} even with large modules, and its control system can ramp the speed as needed.

TMP

In microfiltration mode, optimal flux is usually obtained at low TMP ($\leq$ 0.2 bar). The goal is to operate in a pressure-controlled regime while avoiding conditions in which increasing TMP does not increase flux but instead promotes fouling. We observed that operating with the permeate line fully open maintained TMP at ~ 0.1–0.15 bar initially, which was optimal. Any attempt to “force” higher flux by increasing retentate backpressure simply compacted the filter cake.

Sartoflow® 5000 SU system automation can maintain a set TMP if desired; for example, it can hold a TMP of 0.15 bar by modulating membrane valves to ensure consistent performance. In summary, operation at the lowest TMP that provides reasonable flux is recommended, as the system typically reaches a stable operating equilibrium.

Flux and permeate control

Unlike ultrafiltration (where constant TMP operation is common), microfiltration cell harvest can benefit from operating at a fixed moderate flux and allowing TMP to increase gradually, or vice versa. We chose to operate initially at the maximum attainable flux (open permeate). Another approach is constant flux control, for example, setting the permeate valve to withdraw at 20 LMH and lowering the TMP to reduce fouling. The Sartoflow® 5000 SU system can implement flux control using an external permeate flow sensor or timed draw-off. In practice, the simpler open-permeate approach proved effective, as flux self-regulated with increasing concentration.

Volume reduction and diafiltration

Determining the target concentration factor is a critical decision. In this study, an approximately 6.5-fold concentration was achieved. The process was stopped at 7 L to maintain good flowability and avoid excessive TMP caused by increased feed pressure due to rising viscosity of the cell suspension. Further increases in concentration may be possible by activating permeate flow or TMP control to compensate for this effect.

After concentration, diafiltration was essential: by adding buffer and removing permeate, more than 95% of extracellular contaminants were flushed out. Typically, 3–5 diavolumes are sufficient for washing cells. We used three diavolumes and observed diminishing returns beyond the third (e.g., permeate OD was nearly water-clear). If extremely pure cells are required, up to five diavolumes may be used at the expense of additional processing time. Because diafiltration maintains volume, the cells remain at high concentration during washing. A slight flux decline was observed by the third wash due to fouling, but not enough to significantly prolong the process.

Foaming

E. coli broth can foam when pumped. Because of the design of the recirculation bag in combination with bypass operation, and because all relevant parameters are well controlled by the Biobrain® automation system, no antifoam agent was required.

Temperature

The process was conducted at room temperature (~ 20 – 25 °C). No active cooling was required, as the run duration was moderate. The Sartoflow® 5000 SU system also offers the option of using a temperature-control unit, which is fully integrated into the Biobrain® automation system.

Conclusion

In summary, TFF using hollow fiber modules on the Sartoflow® 5000 SU system represents a robust, scalable, and operationally efficient alternative to centrifugation for *E. coli* cell harvest in pDNA production. Critical process parameters requiring optimization include crossflow rate (balancing fouling and shear stress), TMP, and the extent of diafiltration, all of which can be precisely controlled within the system.

Despite longer processing times compared to centrifugation, TFF offers significant advantages, including high automation, reduced manual intervention, and elimination of cleaning-in-place requirements due to the single-use design. The closed system enables consistent aseptic operation, while integrated diafiltration improves biomass quality for downstream processing.

Operationally, the platform is low maintenance and straightforward to run, shifting emphasis toward process monitoring rather than equipment handling. Additionally, scalability is supported through the Sartorius hollow fiber portfolio, facilitating efficient small-scale process development.

Overall, TFF-based cell harvest simplifies process workflows and aligns well with modern GMP manufacturing strategies, particularly in flexible and single-use production environments.

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