



APPLICATION NOTE

Concentration of Myeloma Cells Using TFF

The objective of the study, summarized in this application note, was to concentrate myeloma cells using tangential flow filtration (TFF) prior to downstream processing. The following text outlines a case study performed at the Massachusetts Institute of Technology (MIT), demonstrating the effectiveness of using the **NovaSet-LS™** LHV tangential flow filtration device.

INTRODUCTION

In the early stages of process development, time and product are at a premium. TangenX **NovaSet-LS™** LHV cassettes provide a solution for evaluating process streams once a membrane has been selected and process development is underway. Data generated will influence decisions to be made regarding the system configuration and operating conditions for the final process. These elements must eventually coalesce into a scalable robust effective commercial manufacturing process.

Tangential Flow Filtration (TFF) is an effective downstream processing (DSP) method for the clarification and purification of biological solutions. Membrane clarification is a solid / liquid separation technique that is widely used in the biotechnology industry when isolating soluble components from a cell fermentation stream. Depending on the membrane porosity used, it can be classified as a microfiltration or ultrafiltration process. Microfiltration membranes have pore sizes typically between 0.1 μm and 10 μm . Ultrafiltration membranes have much smaller pore sizes and are classified by cutoff, which is based on their ability to retain the protein with a known molecular weight (i.e., 1kD to 1000kD). A good rule of thumb is to select the membrane cutoff three to six times smaller than the molecular weight of the species to be retained. TFF is widely used for concentration, desalting, buffer exchange, and fractionation of proteins. Until recently there were limited applications of TFF in cell harvest and cell lysate clarification. This case study describes the possible use of TFF for the concentration of a myeloma cell line prior to further downstream processing.

One of the primary advantages of using TFF is that it can be easily scaled up or scaled down by keeping the channel length and channel height constant. This becomes very important during bench-top trials where data will be later used to support the pilot and process scale. This is critical for current good manufacturing practices and manufacturing companies where

technology transfer and process development is very important. The NovaSet-LS LHV tangential flow filtration system is an ideal choice for such applications.

NOVASET-LS™ LHV Cassette and Systems For Early Stage Process Development



0.01m² & 0.02m²
NOVASET-LS PACKETS
All-in-one processing
convenience for
15 mL to 500 mL samples



- ❑ **Application Performance:**
NovaSet-LS 0.01m², 0.02m² & 0.1m² devices are designed for processing volumes from 10's to 1,000's of milliliters.
- ❑ **NovaSet-LS™ processing volume capability:**
Depends on MWCO, TMP and fouling, but as a guide, a 10KD membrane run at 2bar TMP typically handles 200LMH or 2LPH for the 0.01m², 4lph for the 0.02m² and 20lph for the 0.1m²
- ❑ Typically 3 - 100 X when used in conjunction with the low hold up volume (<10mL) **NovaSet-LS™** LHV Lab System.
- ❑ **NovaSet-LS™** cassettes are available in 0.01m², 0.02m² and 0.1m² surface area formats as well as three different channel configurations.
- ❑ **Reliable, Reproducible Cassette Performance:**
TangenX **NovaSet-LS™** cassettes represent the latest development in tangential flow filtration cassette design and performance. The TangenX line of cassettes has been designed to deliver optimal performance as well as demonstrate exceptional batch-to-batch reproducibility.

Researchers at academic institutions also benefit from the use of scaled down TFF technology. As the researcher is continuously working to develop novel methods and improve process efficiency, the ability to generate scalable data is very important. A case study performed at the Massachusetts Institute of Technology (MIT) demonstrates the efficiency of using TFF to concentrate myeloma cells prior to downstream processing.



BACKGROUND

Myeloma cells are a type of cancer cell and will therefore reproduce indefinitely. Myeloma cells are commonly fused with B-lymphocytes, the latter being isolated from a host organism. The resulting cells, known as hybridoma cells, produce desired antibodies and grow indefinitely. The exposure of the host to the target antigen results in the B-lymphocytes raising antibodies against the antigen. Thus myeloma cells are an important element of producing therapeutic proteins.

Bioreactors have long been used to develop and produce therapeutic and diagnostic agents serving many purposes, from combating infectious disease to facilitating analytical chemistry techniques. Critical to operation of bioreactors, which may vary in scale from 1 liter to 10,000 liters, is the ability to provide control for process variables including temperature, dissolved oxygen, pH, foam level control, and glucose/lactate concentrations. The bioreactor available for the project was a stirred-tank reactor for cell culture, designed for continuous operation as a perfusion system, i.e. fresh medium is added at the same rate as the spent medium is removed. In this case, myeloma cells were produced using a 10 Liter vessel while maintained in Dulbecco's modified eagle medium. Culture samples were tested for cell count and viability using a tripan blue assay.

CASE STUDY

This case study consisted of preparing Myeloma cells in a bioreactor followed by a concentration step using tangential flow filtration. Cell concentration and viability were monitored during the TFF concentration step to demonstrate the efficacy of the process. Figure 1 depicts a flow diagram typical of an up-stream cell harvest process.

In a typical process, the cells would be taken from the bioreactor, concentrated, and then diafiltered to remove extracellular proteins. The concentrated stream would then be passed through a homogenizer (or similar) for the purpose of lysing the cells prior to clarification. Once the cells are lysed, the intracellular proteins are released and the cell debris may be removed using a second TFF clarification step.

The primary focus of this case study was to perform the initial concentration of the cells produced and demonstrate the effectiveness of using TFF to concentrate the myeloma cells three to five times without negatively impacting the cell viability. In conjunction with this information, the performance of the membrane was evaluated while measuring operating pressures and flow rates. Additionally, the membrane's normalized water permeability (NWP) was measured

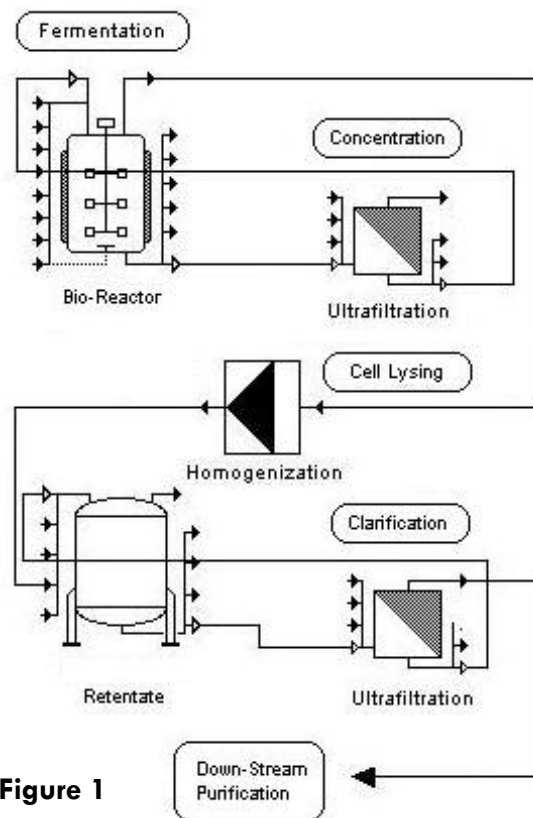


Figure 1

both prior to use and after the cleaning cycle in order to demonstrate the recovery of the membrane.

EQUIPMENT USED

The 0.02m² (200cm²) NovaSet-LS LHV tangential flow filtration system was chosen for this study, as it is ideally suited for studies where source material is in limited supply. Its low holdup design, clean fittings (no exposed threads), and integrated acrylic reservoir make it an excellent choice for many benchtop applications. An "S-channel" cassette was used as it was specifically designed for feed streams containing particulates such as cells or cell debris. The data generated when using **NovaSet-LS™** cassettes can be used to define operating parameters during scale-up. A 300kD **ProStream™** modified polyether sulfone membrane was selected for the concentration of the myeloma cells. The 300kD membrane is a good choice as it has excellent permeability and resists fouling due to its proprietary modifications. It is also has excellent chemical compatibility and is easily cleaned when using a basic cleaning solution such as sodium hydroxide.

The **NovaSet-LS™** LHV system was set up according to the operating instructions included with each system. Once the setup was complete the cassette was initially sanitized with 0.5M Sodium Hydroxide, flushed with deionized water, and the initial DI water permeability

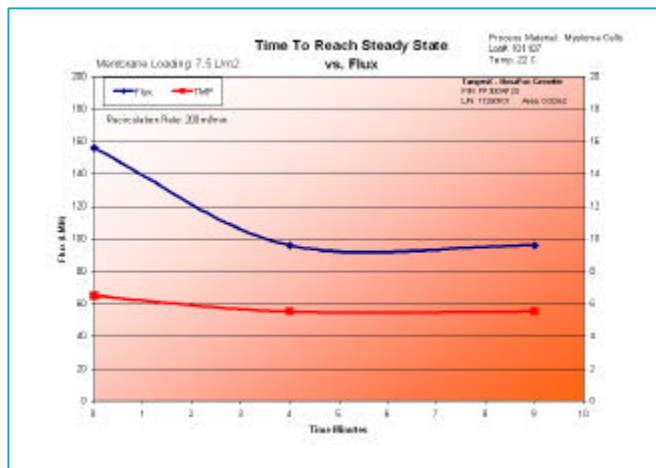


was measured. The system was then equilibrated with PBS buffer prior to use.

EXPERIMENTAL RESULTS

The filtration process began with collecting the cells from the bioreactor and measuring the cell viability and density of the process stream. The initial cell density was found to be 1.72×10^6 cells/mL with 96.4% viability. Once the stream was analyzed, 150mL of the cell culture was added to the graduated acrylic reservoir of the **NovaSet-LS™** LHV system. The pump was then turned on and slowly ramped up to 200mL/min and the system began operating in a recirculating mode with the filtrate directed back to the retentate reservoir.

Critical operating parameters such as the operating pressure, cross flow, and filtrate flow were measured and recorded at the initial start-up. The cross flow rate was set at 200mL/min and the trans-membrane pressure adjusted to approximately 60 psi. Data was then recorded every five minutes until the permeate flux had stabilized and a steady state was reached. The following graph shows the relationship between time and flux at a constant trans-membrane pressure

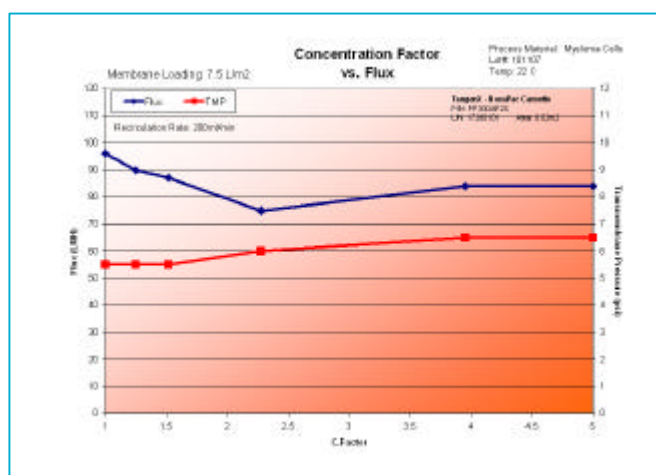


while the stream was in a recirculating mode.

The results of the initial recirculation experiment show the flux initially begins at 160LMH, but then falls over the next four to five minutes as the boundary layer establishes itself. Eventually the process flux stabilizes at approximately 100LMH after 5 minutes of recirculation. The trans-membrane pressure remained steady at 6psi throughout the recirculation process. This steady state shows the velocity at the membrane surface inhibits further fouling and facilitates adequate mass transfer.

Once a steady state was reached, the concentration process could begin. The target concentration factor

was a five-fold reduction by volume, reducing the feed stream from 150mL to 30mL. The filtrate was directed from the feed reservoir to a separate collection vessel and the myeloma cells were concentrated. Again critical operating parameters including volume, pressure, cross flow, and filtrate flow were measured and recorded as the concentration operation progressed. The cross flow rate was set at 200mL/min and the trans-membrane pressure ranged between 5 psi and 7 psi. Data was recorded until approximately 120mL of permeate was collected and the target concentration was reached. The following graph shows the relationship between concentration factor and flux. Additionally, the trans-membrane pressure is graphed alongside of the flux to demonstrate the trend of the permeate flux.



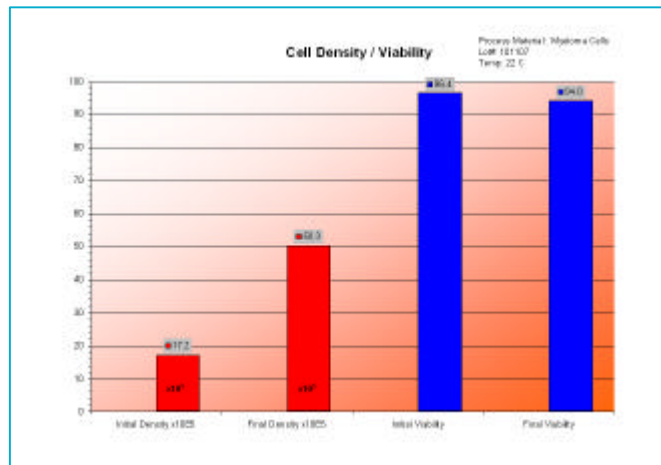
The permeate flux began at 100LMH and steadily decreased to 75 LMH as the cells were concentrated. Towards the second half of the concentration the flux began to gradually rise due to an increase in the transmembrane pressure from 5 psi to 7 psi. This rise in flux further demonstrated the membrane had not been significantly fouled and the filtration rate was still predominantly membrane controlled.

Once the target concentration was reached, the retentate valve was opened and the permeate valve was closed while the feed stream was gently recirculated for approximately five minutes to maximize cell recovery. The cell concentrate was then drained from the system and purged with 20mL of phosphate buffered saline solution. The final volume of the cell culture and filter wash was added together for a combined volume of 53mL, a three-fold volume reduction based on volume.

The concentrated cell culture was analyzed for cell density and viability following the TFF process. The graph below shows both the cell density before and after the concentration plotted in red. The initial density was found to be 1.72×10^6 cells/mL and the density

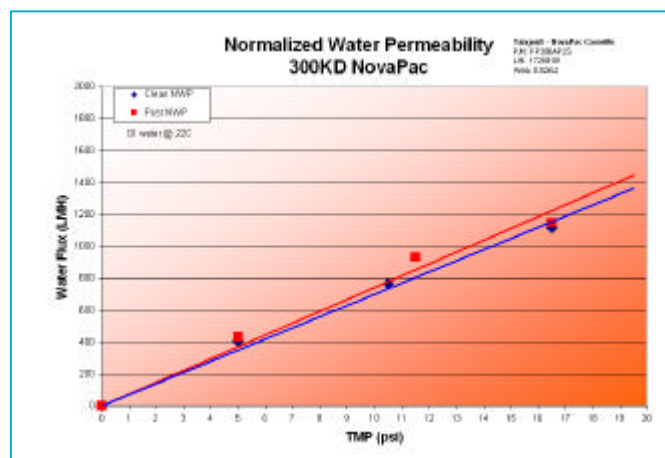


following the concentration was found to be 5.03×10^6 cells/mL; a three-fold increase in cell count. No cells were found in the permeate, demonstrating the 300kD membrane completely retained the cells. The mass balance of the cells from the TFF concentration step was 103.1%.



Additionally the concentrated cell culture was analyzed for viability and the results were plotted in blue in the graph above. The initial viability was found to be 96.4% and the viability following the concentration step was 94.0%. The total loss in viability was less than 2%, statistically equivalent. An ideal TFF process will permit a high percent recovery of a product with little or no loss in viability. The **NovaSet-LS™** LHV system has been optimized to minimize hold-up volume and support excellent product recovery. Additionally all valves and ports were designed to reduce un-wanted shear that may affect cell viability.

As with most tangential flow filtration membranes, TangenX **ProStream™** membranes are cleaned using a CIP procedure and reused. These membranes are manufactured using a durable modified polyether sulfone polymer and will afford a long service life. Once the myeloma cell concentration process was complete the membranes were cleaned using an 0.5M Sodium Hydroxide solution. Approximately 200mL of solution were recirculated through the TFF system for thirty minutes at 20°C. The purpose of this cleaning step was to remove residual contaminants from the system so the membrane could return to its original condition. The most effective way to determine a membrane's recovery is to compare the initial clean water permeability to the permeability following the cleaning cycle. Typically greater than 90% recovery is achievable and a membrane is considered "clean" at this point.



The graph above shows the clean water permeability for the 200cm² **NovaSet-LS™** cassette, both pre-use and post-use. The initial water permeability graphed in blue and post-use in red show 100% recovery, demonstrating the membrane is fully recovered following the Sodium Hydroxide cleaning.

This study demonstrated that the **NovaSet-LS™** LHV is able to successfully concentrate myeloma cells using TFF prior to downstream processing. One hundred percent of the cells were recovered with negligible loss in viability. The data generated using this system is directly scalable from 0.02m² to 60m² and may be used when planning future processes with this particular feed stream. As each application is unique, this information is intended as a reference only and should not be used to predict performance where the feed stream differs from the one outlined here.



RESOURCES

1. Biotechnology Recovery Module

Sponsored by:

Massachusetts Institute of Technology (MIT)
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2. Scaling up Monoclonal Antibody in Novel and Traditional Bioreactors Lab 13-3095

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DasGip mbH a Division of the Biotechnology Process Engineering Center (BPEC)
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