# *Implementation of a New Membrane and the Re-use in a Tangential Flow Filtration System*

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***Abstract*** *— Tangential Flow Filtration (TFF) is commonly used as the last steps in protein purification processes at Biopharmaceutical Plants for clarification and concentration of the target protein. Since the membranes are expensive, the industries should maximize its use in order to minimize associated costs. A common membrane is made Polyethersulfone. As specified from the manufacturer, it can be cleaned using Sodium Hypochlorite (NaOCl). Since TFF is the last step in the manufacturing process, a Biopharmaceutical Plant have the concern of using this cleaning agent since this solution can alter adversely the safety, identify, strength, purity and quality of the product. For this reason, it was chosen not to validate a cleaning strategy for Polyethersulfone TFF cassettes in a specific industry. Instead, new membranes are installed for the manufacturing process per production lot. A typical cost of membrane ranges between $5,000 and $7,000. For a process that requires eight membranes, the cost for a biotechnology plant is approximately $40,000 per lot. This proposed project consists in changing Polyethersulfone membranes to Regenerated cellulose, which can be easily cleaned with sodium hydroxide, commonly used in pharmaceutical industry. By demonstrating the effectiveness of membrane reuse via cleaning validation, a typical biotechnology industry can save more than $2 million in an annual basis.*

***Key Terms*** *— Biopharmaceutical, Cleaning, Membranes, Tangential Flow Filtration.*

## OBJECTIVES

One of the main objectives of this design project is to perform an economical evaluation and determine if the re-use of membranes is feasible, depending on the industry and process characteristics. In addition, DMAIC (Define, Measure, Analyze, Improve and Control) and Project Management tools will be used to define a plan for the evaluation and implementation of the new membranes and its re-use in a TFF Process. An additional objective is to demonstrate that the change of the filtration element from Polyethersulfone to Regenerated cellulose does not alter unfavorably the filtration capacity of the system.

## CONTRIBUTIONS

An effective implementation of the new membranes and the re-use after cleaning project can provide an industry with several significant improvements such as cost reduction. Cost reduction includes cost associated to material required per manufacturing lot (membranes) and disposition lot of the waste generated after completion of manufacturing process. In addition to these benefits, the cycle time associated to the membrane installation and removal will be eliminated in most part, since the membrane will only be installed and removed twice a year, depending on production plan for the year.

## LITERATURE REVIEW

In general, the costs associated to biotechnology products are higher than other dosage forms, such as solids and semi-solids. Based on this assumption, one of the main priorities of the biotechnology industry is to look forward for projects and process improvements that will

increase the plant's efficiency by reducing the cost associated to production batches.

Cost associated to membranes in a typical ultra-filtration system in a biotechnology plant can be more than $2 million, in an annual basis, if membranes are not re-used. Based on this, alternate ways to reduce or minimize this cost must be implemented. An important tool developed by Motorola and widely used by General Electric (GE) is Six Sigma. Basic concepts usually associated to this methodology will be used to implement new membranes used in a Tangential Flow Filtration system and validate the cleaning process for its re-use.

### Tangential Flow Filtration and Membranes

Separation and purification using membrane is mainly driven by a differential pressure, in which the main criterion for separation is the molecular weight of the components.

There are four main groups associated to filtration techniques, winch include Reverse Osmosis, Ultrafiltration, Nanfiltration and Microfiltration. In the case of the proposed project, the technique used is Ultrafiltration. The main objective of Ultrafiltration is to concentrate a biologic product (protein) by removing water and other low molecular weight solutes. The type of filtration technique mainly depends on the product to be filtrated, what is desired to eliminate and the molecular weight of all components, among other factors [1].

There are two main operations of membranes, which are known as Dead-End Filtration (Normal Flow Filtration, NFF) and Cross-Flow Filtration (Tangential Flow Filtration, TFF) [2]. In Normal Flow Filtration the fluid and pressure are parallel toward the membrane. When macromolecules are retained in the membrane's surface, a "cake" or layer can cause clogging of the membrane. The main disadvantage of this type of filtration is that the membrane or filtration elements can get easily clogged, decreasing the flux with time. In the other hand, for TFF (or Cross Flow Filtration) the fluid passes tangentially to the membrane, while a pressure is applied toward the membrane. The drag force of the fluid is constantly moving the retained macromolecules; therefore, clogging of the membranes is minimized. This provides a higher efficiency of the membranes. Figure no. 1 shows a schematic of the behavior for both dead-end filtration and cross flow filtration. Figure no. 2 provides a comparison between both operational modes.

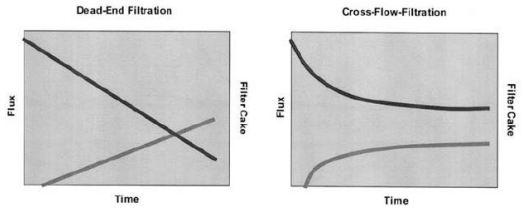


**Figure 1**
**Behavior of Dead-End Filtration and Cross Flow Filtration (Sartorius)**

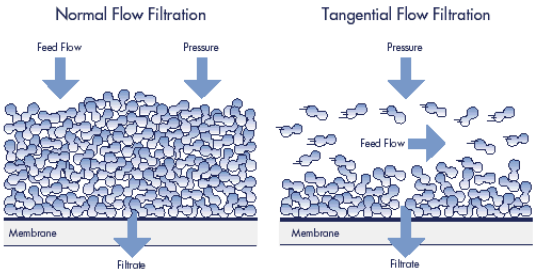


**Figure 2**
**Comparison between NFF and TFF [2]**

There are several important factors that must be considered in a membrane operation to guarantee the performance of the system and to improve filtration capacity. Some of these factors include type of the membrane, component to filtrate and configuration of the system. There are several types of material or polymers for the filtration elements. In addition, there are various types of membranes, including flat plate, spiral and hollow fiber. Refer to Figure no. 3.


**Figure 3**
**Types of Membranes (Millipore)**

Millipore provides general information on Process Economics. There are four major categories in which costs can be divided: overhead, labor, capital and materials. The industry should evaluate its process in order to determine how to minimize costs associated to materials. For example, costs associated to membrane re-use can increase since additional chemicals and water must be used during cleaning cycles. However, depending on the type, surface area and quantity of membranes required, the cost of the membrane is higher, as well as the cost per membrane [2].

**Cleaning**

Contamination in a purification step can be avoided by having good process hygiene, which includes the use of suitable cleaning and sanitization protocols. The impact of bioburden should be evaluated by each individual company and process, taking into consideration standards and regulations by the Food and Drug Administration (FDA) [3].

In terms of membrane cleaning, it is very important to find a method for cleaning that does not damage the system and membranes. Regarding chemical compatibility, it is very important to use a cleaning agent that is compatible with all materials, including gaskets, piping, pump, etc and that does not alter the integrity of subsequent lots. Data gathered from different studies showed the efficiency of cleaning using sodium hydroxide and sodium hypochlorite. Another important aspect is that cleaning validation demonstrates that there are no residual of previous lots that could create batch to batch contamination, and eventually produce bacterial growth. In addition, it is very important to demonstrate that all cleaning agent is removed or lowered to acceptable levels prior continue batch processing [3] [4] [5].

Another critical factor is cleaning water quality. That is, impurities found in the water can adversely affect the cleaning efficiency, affecting performance of the membranes. Among other factors or conditions to document during an execution of a cleaning protocol are: contact time, flow rate, pressure, temperature, concentration, among others [3] [4] [5].

An effective cleaning process should restore membrane performance. Moreover, cleaning agents must be sufficient to remove or degradate all fouling materials that can be found in the membrane's surface. Fouling and polarization is a common problem in filtration technology such as Reverse Osmosis (RO) and Ultrafiltration (UF) [6]. Buildup affects directly the membrane uses or lifetime and filtration capacity. That is, a buildup in the membrane surface eventually leads to a decrease in the permeate flow rate. Fouling can occur due to several factors, including pH, salt concentration and presence of oils in the product to be filtrate, as well as to the pore size of the membrane. Some types of fouling include:

- Microbiological Fouling- include the basic steps such as transport of the bacteria into the surface of the membrane, attachment and growth at the surface. This is also known as biofilm. The bacteria adhere to the surface of the membrane via hydrophobic interactions [6].
- Protein fouling- among others, a possible source of fouling is in low pH environments, in which the negative charges is reduced, therefore, causing aggregates formation, which can deposit on membrane's surface [6].

According to H. Lutz and B. Raghunath, understanding polarization and controlling its effects are essential to implement a good process [7]. In this case, this is of relevant importance, since currently membranes are not re-used; therefore, this is not a factor to consider. However, by implementing a new membrane and its re-use, this should be considered and evaluated to demonstrate the cleaning effectiveness.

Membranes can be cleaned using conventional Clean-In-Place (CIP) methods, as for vessels and pipes. However, with time, fouling can occur in the membrane's surface. In order to minimize or remove fouling, membranes can be flushed in a forward and reverse way. However, the effectiveness of this back flush mainly depends on

the capability of the membrane to sustain reverse tranmembrane pressure.

Millipore Corporation describes several elements that can be used to demonstrate cleaning effectiveness in a membrane [8]. Figure no. 4 was included to show several elements that should be considered and used as reference when determining cleaning effectiveness of a membrane system.

These elements are:

- Normal Water Permeability (NWP) - measures the clean water that passes through the membrane in given conditions of pressure and temperature. A membrane that is fouled usually will have NWP values lower than 50% [8].
- Total Organic Carbons (TOC) - TOC is measured in permeate and retentate flush to determine organic material in the membrane. Typical TOC values should be less than 1.0 parts per million (ppm) [8].
- Process reproducibility- a fouled membrane will reduce the process flux and yield. A sudden decrease in these values is an indication of fouling material in the membrane [8].

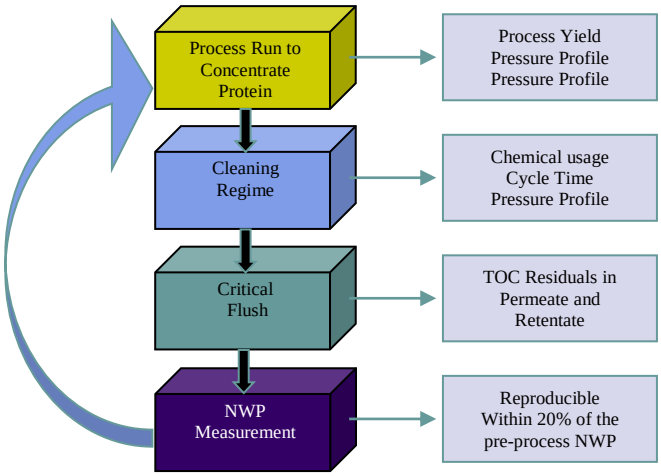


**Figure 4**
**Demonstrating Cleaning Effectiveness [8]**

In a case study presented by Millipore, two different membranes were used: polyethersulfone and regenerated cellulose. Polyethersulfone were cleaned using a combination of caustic and chlorine solution, while regenerated cellulose were cleaned using only a caustic solution. The duration of the cleaning cycle for both membranes was the same, as well as temperature and pressure conditions

Results obtained for the membranes showed that both polyethersulfone and regenerated cellulose membranes maintained process flux conditions and performance, which is an indication of the absence of fouling material. In addition, TOC results were less than 500 parts per billion (ppb) for both membranes. In terms of NWP, both membranes obtained satisfactory results [8].

This study demonstrated the effectiveness of cleaning of both membranes. Thereafter, membrane selection may be directly influence by the preference of the industry in terms of cleaning agents, or other factors such as process characteristics.

Lastly, membrane re-use should be validated. Data gathered during validation activities must be used to determine how many uses a membrane can have. The decision is based in reproducibility and based on data obtained from quality specifications. In addition, shelf life of the membrane shall be validated.

## METHODOLOGY

According to T. Pyzdek [9], the Six Sigma philosophy consists on observing an aspect on the process, determining an hypothesis, making predictions, testing by conducting experiments and repeating until there are no discrepancies.

Six Sigma uses the DMAIC methodology. Define- Measure- Analyze- Improve- Control is mostly used when a process, product or service can be improved to accomplish the project's purpose. Table 1 presents an overview of DMAIC.

**Table 1**
**DMAIC Overview [9]**

| | |
|---|---|
| **Define** | Define goal. Obtain goals from direct communication with customers, shareholders, etc. |
| **Measure** | Measure the existing process. Establish valid and reliable metrics to help monitor progress toward the goal(s) defined at the previous phase. |
| **Analyze** | Analyze the system to identify ways to |

| | |
|---|---|
| | improve the process. Statistical tools can be used to guide the analysis. |
| **Improve** | Improve the system. Use project management and other planning tools to implement the new approach. |
| **Control** | Controls the new system. Establish the system by modifying procedures, operating instructions, etc. Use statistical tools to monitor process. |

### The Define Phase

The first step in the DMAIC tool is Define. In this phase, the scope of the project will be well defined, taking into consideration what the customer wants. This phase will answer question such as [9]:

- Why is this project important?
- What business goals the project must achieve to be considered successful?
- What are the client's requirements on performance level?
- Who are the key players?

For this, three main steps should be completed in the Define Phase: develop business case, map the current process and listen to the voice of the customer

### The Measure Phase

The second phase of the DMAIC tool is Measure. The main goal of this phase is to build understanding of the existing process, its conditions and problems. This knowledge will help to narrow potential solutions, that will be investigated in the analyze step [9].

In this phase, data will be collected as a baseline for the project. It will help to measure the impact of changes in the process. The first step that needs to be completed in the Measure Phase is to determine if the implementation of a new membrane and its re-use through cleaning validation is economically feasible for a Biotechnology Industry. For this evaluation, aspects such as water and buffer usage, manpower (installation of membranes versus cleaning), validation cost, and automation process cost, among others should be considered [9].

After the economical evaluation is completed, it is intended to evaluate how the implementation of a new membrane and its re-use will impact the Ultrafiltration process. For this purpose, it is intended to create a process map, which will help to better understand current process flow. After the current process flow is completed, variables must be identified in order to determine possible process impact with the implementation of new membranes. These variables will be used to create a Prioritization Matrix to rank and weight parameters that need to be monitored during lab scale and engineering runs (Analyze Phase) [9].

Another tool that can be used is the FMEA (Failure Mode and Effect Analysis), which is mostly used to identify, estimate, prioritize and evaluate potential risk. A typical FMEA can be conducted in the following way [9]:

- Identify potential failure modes, that is, ways in which the process might fail.
- Identify potential effect of each failure (consequences) and rate its severity.
- Identify causes of the effects, and rate their probability of occurrence.
- Rate the ability to detect each failure mode.
- Multiply the three numbers together to determine the risk of each failure mode (RPN= Risk Priority Number)
- Identify ways to reduce or eliminate risk associated to high RPN's.

### The Analyze Phase

Since the proposed design project is to implement a new cleaning procedure, data must be gathered in order to properly determine if the new process is efficient and does not alter the current validated state in terms of processing conditions. For this reason, bench scale and Engineering runs must be executed [9].

Among the parameters and data that will be evaluated in the bench scale and Engineering runs are: process conditions with new membrane, run time, quantity of membranes required to maintain process yield, cleanability of the membranes, bacterial control, quantity of buffer required, and

efficiency of equipments, among others. The data obtained during these testing will be evaluated to determine [9]:

- If the proposed project is aligned with current practices and FDA requirements
- If the re-use of membranes has an impact in the production schedule and costs
- Any possible impact in the process, besides the cleaning of membranes
- Documentation impact and its costs (Change Control, Procedures, Validation records, etc)
- Environmental Impact, if any

Another tool that can be used in the Analyze phase is the Cause-and-Effect Diagram, or Fishbone. This type of diagram graphically displays potential causes for a problem. In this cause, the problem that will be evaluated is that membranes could not be cleaned for re-use. The categories that will be included in the evaluation are: material, method, media, machinery and manpower [9].

Since the cleaning of membranes can be a time constraint, all value added and non-value added activities must be evaluated to ensure that the implementation of this cleaning strategy does not alter in a significant way production schedules. Non-value added activities include waiting time, errors, preparation/set-up of the equipment, manual control (instead of automated process), additional testing, etc. All these activities must be evaluated in order to provide recommendations that at the end will develop into a cost reduction to the company.

### The Improve and Control Phases

The Improve and Control phases will be based on data obtained from testing performed during the Analyze Phase. Validation activities (Improve phase) and process monitoring (Control phase), are not considered under the scope of this design project. However, based on the results obtained during Engineering runs and bench scale testing, recommendations in terms of process economics, cleaning validation and process parameters, among others will be provided [9].

## RESULTS

### Financial Evaluation

As part of the evaluation of the proposed process, and based on data obtained in small scale testing, it was decided that twelve (12) Regenerated Cellulose membranes will replace the eight (8) Polyethersulfone membranes that are used in the TFF process. Based on the cost of approximately $5,000 per membrane, there will be an increase of about $20,000 per set of membranes. However, even though there is an increase in the basic cost of the membranes set, based on fact that the membranes will be re-used, this increase is insignificant in the long term. In this particular case, other costs associated with validation exercises will be neglected because all activities will be carried out with internal resources. Table 2 presents a summary of the financial benefit, assuming up to 30 uses of the membranes.

**Table 2**
**Financial Benefits**

| | Regenerated Cellulose | Polyethersulfone |
|---|---|---|
| **Lots in a year** | 60 | 60 |
| **Membrane Installations** | 2 | 60 |
| **Cost per set** | $60,000 | $40,000 |
| **Cost per year** | $120,000 | $2,400,000 |
| **Savings** | $2,280,000 | |

### Validation Strategy

As part of the validation initiatives, several activities were performed. First, Engineering runs were performed to confirm the performance of the membranes in the commercial scale environment. Basically, this means that three (3) manufacturing lots were run using the new Regenerated Cellulose membranes to confirm that all the parameters that were established during small scale testing meet the actual established criteria for the product. In addition, automation conditions as well as Standard Operating Procedure (SOP's) effectiveness were verified.

Once the Engineering runs were completed, process and cleaning validation exercises followed.

The purpose of the cleaning validation was to confirm that, once again, the results obtained from the small scale were adequate and cleaning effectiveness could be determined. In the same manner, process validation confirms that the process consistently delivers a product within the established acceptance criteria. It is important to establish that cleaning and process validation exercises are not associated to the extended re-use of the membranes, only up to three (3) re-uses, which were the runs performed during the validation process.

Once cleaning and process validation results are available and reports are approved, data supporting the new process must be submitted to the different regulatory agencies. It is important that this step is considered in the timeline because it could take up to 10 months to obtain approval in all the different regulatory jurisdictions. The product cannot be distributed until approval.

The final step was to perform the re-use validation. The purpose of this validation is to demonstrate that after "X" amount of uses, the membranes are effective and free of fouling that could affect the process performance. Since the process is already validated and the re-use of the membranes has been demonstrated up to three (3) uses, there was no need to collect additional data during these first three lots. To facilitate this validation process and avoid delays in the release of the affected batches, interim validation reports were submitted. Each validation report includes the results of 3-4 batches and was aligned with the release schedule from the logistics department.

Among the results needed to demonstrate the performance of the membranes are the NWP and Step Recovery. The results obtained for NWP were compared with the established criteria from small scale testing, engineering runs and process validation. Figure 5 includes the data obtained for the first 22 batches. All results obtained were within the established range of 70.0% - 130.0%. This data shows an ascending behavior, which is typical of this process and also observed in the small scale testing.

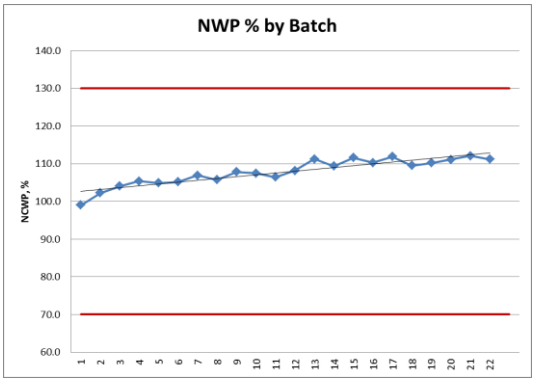


**Figure 5**
**Normalized Water Permeability Results**

Results obtained for Step Recovery not only meet the established action limits, but also exceeded current control limits. The values obtained for Step Recovery were above historical data, giving an additional benefit of approximately 2% increase. The first 115 data points shown in Figure 6 correspond to consecutive batches manufactured between 2012 and 2013. Based on this historical data, the average step recovery is 96.7%, with control limits of 93.8% - 99.7% (3 sigma). With the new process using Regenerated Cellulose, the new average for using the first 22 batches is 99.0%, with control limits of 97.0% - 101.0%. Using this data, control limits will be revised to be aligned with the new process.

The last two data points shown in Figure 6 are outside control limits. After investigation, it was found that these higher Step Recoveries are associated to a carryover of a human error in a previous process step. Given the fact that a categorical root cause was found for the event, these two values will be excluded from future evaluations.

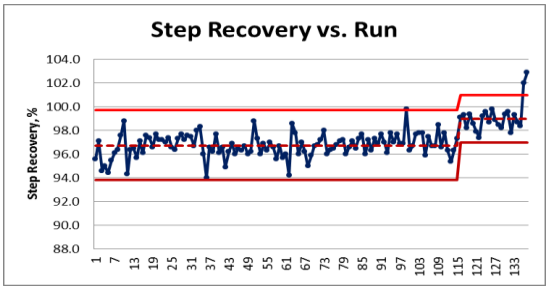


**Figure 6**
**Normalized Water Permeability Results**

## CONCLUSION

Results obtained from an effective validation strategy have demonstrated that the implementation of Regenerated Cellulose membranes has provided several benefits to a Biopharmaceutical industry. Among the benefit obtained are:

- Cost reduction of approximately $2,280.000 per year in membrane cost
- Increased 2% in process step recovery.
- Validated cleaning process with eliminates installation process per batch (less manual operations and man hours).

This reports includes data up to 22 consecutive batches, however, it is intended to validate up to 30 batches, maximizing the expected benefit.

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