THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF CHEMICAL ENGINEERING

FACTORS AFFECTING HOLLOW FIBER MEMBRANE VIRUS FILTER PERFORMANCE DUE TO PROTEIN FOULING

ADAM CATRAMBONE
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ABSTRACT

Therapeutic antibodies are used for the treatment of a wide variety of cancers and autoimmune diseases. This has created a need for new and improved technologies to support the production and purification of these complex biological molecules. This includes methods for viral clearance, which is required to ensure that there are no exogenous or adventitious viruses in the final formulated product. The objective of this thesis was to examine the effects of factors such as protein concentration, transmembrane pressure, and solution pH on membrane fouling using Planova™ BioEX hollow fiber virus filtration membranes. Experiments were performed with solutions of human immunoglobulin G (IgG), with limited data obtained with the Planova™ 20N membranes and with solutions of bovine serum albumin (BSA). The fouling of the BioEX membrane decreased slightly at high pressures and high protein concentrations, but was relatively independent of the solution pH. BSA showed very little fouling of the Planova™ hollow fiber membranes over a range of conditions. These results provide important insights into the factors controlling fouling during virus filtration as well as a foundation for further optimization of the performance characteristics of the Planova™ BioEX membrane.

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ACKNOWLEDGEMENTS

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1. Introduction

1.1 Antibodies in Vivo

The human immune system can be divided into two parts: the innate and the adaptive immune responses. While the innate immune response is inherent to every individual from birth, it is the adaptive immune response that evolves to target specific antigens, allowing for an organism to build resistance to threats it might face. Beyond resistance that arises purely naturally, the adaptive immune response is also the basis for vaccinations against viral and bacterial infections. The term antigen refers to any, typically foreign, substance that can initiate this adaptive immune response (Janeway et al., 1996). This recognition is accomplished through antibodies, glycoproteins produced by B cells possessing a molecular weight around 150 kDa (Lipman et al., 2005).

Human antibodies can be divided into five classes: IgM, IgD, IgE, IgA, and IgG, which is the most common type of antibody found in blood (Lipman et al., 2005). All antibodies are formed from four peptide chains: two longer molecules termed heavy chains and two shorter molecules termed light chains. Once together, they form a structure that resembles a “Y”. The antibody as a whole is structurally divided into two functional parts. The Fab domains, which recognize and bind a specific part of an antigen, an epitope, are located at the two prongs of the “Y” (Gao et al., 2018). The lower portion of the “Y” is the Fc domain, which is involved in the induction of the necessary immunological response (Lipman et al., 2005).

The adaptive immune system utilizes a complex mechanism of adaption and selection to allow the body to recognize such specific antigens. Antibodies are encoded by genes containing a series of transposable elements that are shuffled so that each B cell line within the body produces a different specific antibody. Antibodies that happen to recognize foreign antigens

return signals to their parent cells, causing these cells to proliferate. These cells also undergo random mutations, allowing a process akin to natural selection to generate antibodies with greater and greater affinity to the target antigen (Janeway et al, 1996).

1.2 The Medical Use of Antibodies

The history of antibody-based treatments in medicine stretches back over a century (Squaiella-Baptistão et al., 2018). Given that antibodies recognize specific foreign compounds or organisms within the body, there was early interest in identifying a way of transferring antibodies from one organism to another to allow temporary protection against disease. With the use of antivenin, or serum therapy, an organism is given successive doses of some natural toxin, such as the venom of a snake, to build up natural antibodies. The serum of this organism can then be extracted and introduced into another organism to give that organism a temporary immune response to the antigen (Pucca et al., 2019). The antibodies purified from serum are termed polyclonal, reflective of the fact that multiple B cell lines produce different antibodies that recognize the same antigen, possibly through different epitopes on that antigen (Lipman et al., 2005).

Monoclonal antibodies, however, are produced from a single, identical, B cell clone allowing a highly consistent product to be produced (Lipman et al., 2005). Today, monoclonal antibodies are used to treat a variety of diseases, including a range of cancers and autoimmune disorders (Singh et al., 2018). Antibodies can possess therapeutic effects in a variety of ways: they can bind an antigen so as to prevent it from interacting with a receptor or growth factor, they can trigger various immune responses in the patient, or they can direct an attached drug to a specific location or cell type (Chames et al., 2009).

1.3 The Industrial Production of Antibodies

Prescription therapeutic antibodies like Herceptin or Rituxan are "blockbuster drug[s]" with annual sales well in excess of $1 billion (Trefis Team, 2019). Most monoclonal antibodies are manufactured in Chinese hamster ovary (CHO) cells within suspension bioreactors, with the cell genetically engineered to produce the desired heavy and light chains. The antibodies are then purified using a combination of centrifugation, chromatography, and filtration steps to obtain the very high degrees of purity required for these injectable products (Kelley, 2009). The focus of this thesis is specifically on the removal of viruses, which is a critical step in the downstream purification process.

1.4 Virus Contamination in Bioprocessing

Since antibodies are produced within living mammalian cells, these cells can be infected with the same type of viruses that affect whole organisms. Viral contamination is of special concern in manufacturing since it cannot be detected by the same cultivation assays as living pathogens like bacteria and fungi. Viruses can be introduced into the production stream through the cell line itself, in contaminated media or buffers, or through process equipment. The virus contamination can not only harm the cell line within the bioreactor, but can also compromise the patient if they remain in the final formulation (Merten, 2002). As such, viral contamination can be incredibly costly for the manufacturing facility. For example, in 2009, a production plant owned by Genzyme Corporation was temporarily shut down due to contamination of a bioreactor by the virus Vesivirus 2117 (Bush, 2009). Although this virus was not harmful to humans, the losses associated with ceasing production and eliminating the viral contamination reached into

the hundreds of millions of dollars (Dimond, 2009). As such, preventing viral contamination is an absolutely essential component of monoclonal antibody production.

1.5 The Challenges of Filtration in Viral Clearance

Viral clearance can be accomplished through a number of methods, including low pH chemical inactivation, combinations of solvents and detergents, and both ion exchange and protein A affinity chromatography (Zhou, et al., 2008; Miesegaes, et al., 2010). Virus filtration is one of the more robust methods of virus clearance, providing a size-based removal of even very small parvovirus that can complement other inactivation and removal steps as part of the overall viral clearance strategy (Asahi Kasei Bioprocess, 2018). Several companies manufacture virus filtration membranes, including MilliporeSigma, Pall, Sartorius, and Asahi Kasei (Insight Partners, 2018). While the first three of these companies make flat sheet virus removal membranes, Asashi Kasei manufactures several hollow fiber membrane filters. The focus of this thesis was on the performance of Asahi Kasei's Planova™ BioEX membrane.

Hollow fiber membranes like the BioEX, are, as their name suggests, constructed of an array of hollow tubular fibers. The fluid to be filtered is fed into the lumen, or interior space, of the hollow fiber membranes. The fluid then passes through the membrane, which forms the wall of the fiber and has pores that are narrow enough to retain even very small viruses (Asahi Kasei Bioprocess, 2018). One of the major challenges in the operation of these virus filters is the buildup of material on and within the hollow fiber membrane, a process known as fouling. Since the viruses are present at incredibly low levels in production, if at all, the dominant foulant in virus filtration is the protein product, often in the form of aggregates or product variants, i.e., mis-folded forms of the protein (Syedain, et al., 2006).

The overall objective of this thesis was to evaluate some of the key factors influencing the fouling characteristics of the Planova™ BioEX virus filtration membrane, including the solution pH and the operating pressure.

2. Materials and Methods

2.1 Solution Preparation

A pH 7.2 solution of phosphate buffer was the primary buffer used in the experimental studies. The phosphate buffer was prepared by dissolving 4.083 g of KH_2PO_4 (Sigma-Aldrich) and 4.26 g of Na_2HPO_4 (Sigma-Aldrich) in one liter of deionized distilled water obtained from a Direct-Q® 3 UV Water Purification System (MilliporeSigma, Billerica, MA). The pH of the solution was then adjusted by the addition of 1 M NaOH as needed. Experiments were also performed using a phosphate buffer at pH 8.0, prepared by dissolving 0.381 g of KH_2PO_4 and 8.072 g of Na_2HPO_4 in 300 mL of deionized distilled water. A pH 8.5 carbonate buffer was prepared using 8.023 g of $NaHCO_3$ (Sigma-Aldrich) and 0.477 g of Na_2CO_3 (Sigma-Aldrich) in 1 L of deionized distilled water.

Human serum immunoglobulin G (IgG) (Sigma Aldrich, Milwaukee, WI) was used in most of the experiments. Protein solutions were prepared by dissolving a pre-weighed quantity of IgG in the desired buffer. Protein concentrations were measured with a NanoDrop Microvolume Spectrophotometer based on the recorded absorbance at 254 nm. Limited experiments were performed using bovine serum albumin (BSA) (Sigma Aldrich, Milwaukee, WI). All protein solutions were filtered through 0.22 μm VWR syringe filters (VWR, Radnor, PA) to remove any large aggregations or un-dissolved components prior to use.

2.2 Hollow Fiber Membranes

Two different hollow fiber membranes were used. The Planova™ BioEX membrane cartridge (Asahi Kasei, Tokyo, Japan) consisted of 12 fibers made from a hydrophilized polyvinylidene fluoride (PVDF), providing a total surface area of 0.001 m^2. The Planova™ 20N

(Asahi Kasei, Ltd, Tokyo, Japan) cartridge possessed the same effective surface area, though its fibers were made from a regenerated cellulose; the 20N is widely used for virus filtration of plasma-derived products (Asahi Kasei Bioprocess, 2018).

2.3 Filtration Apparatus

Filtration experiments were performed using the set-up shown in Figure 1. The driving pressure within the feed vessel was provided with compressed nitrogen, with the specific pressure controlled manually via adjustment of the pressure regulator. The exit point for the permeate consisted of a short length of tubing open to air; the permeate was allowed to drip into a collection vessel set atop an electronic scale. Filtrate mass was recorded directly and converted to volume by taking the density of each filtrate as being roughly equal to that of water at the same temperature. The gauge pressure within the feed vessel was assumed to be equal to the transmembrane pressure since the pressure in the permeate line was approximately atmospheric pressure.

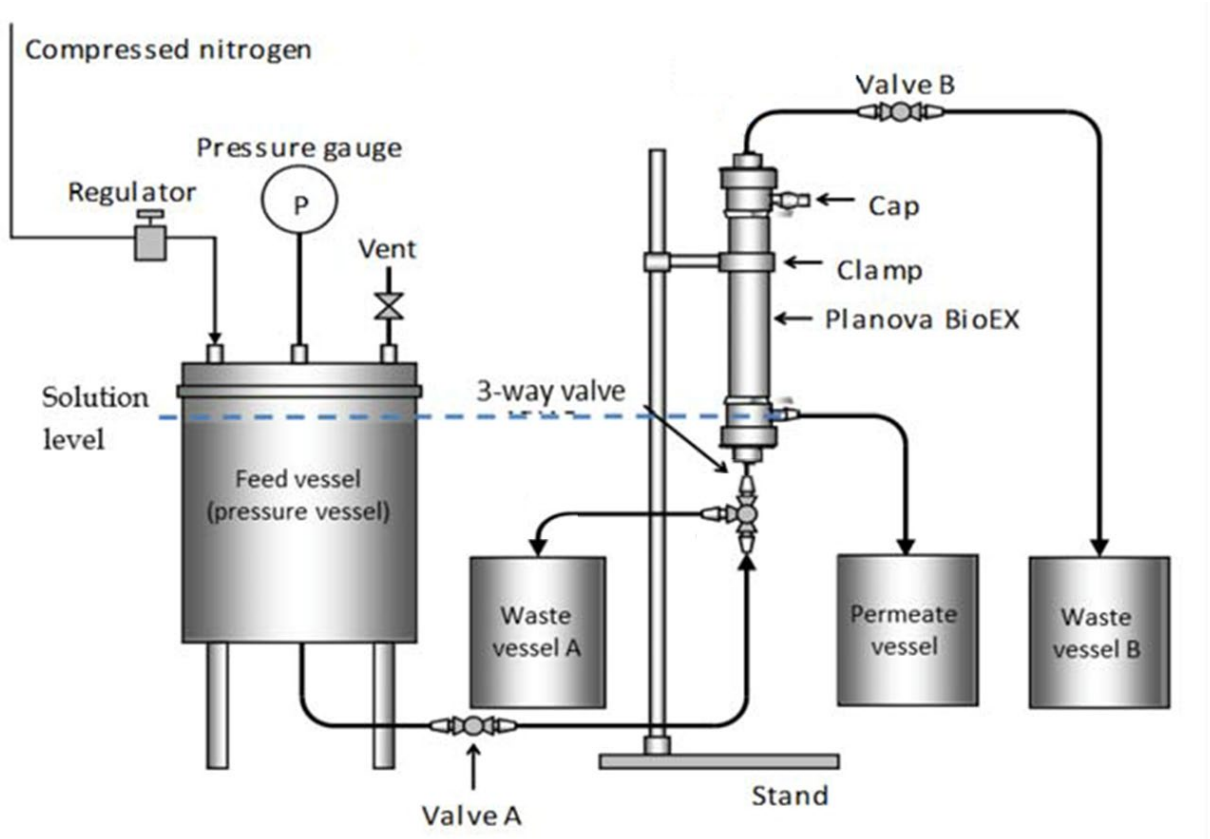

Figure 1. The equipment used in the filtration experiments. Note that the exit point for the permeate was maintained at roughly the same height as the level of the solution within the feed vessel. Modified from Filtration Procedure: Planova™ BioEX Filtration (2015).

Prior to each trial, the filters were flushed thoroughly with buffer using the following procedure. Keeping Valve A closed, the pressure was brought to 2-3 psig. The three-way valve was opened so as to direct the feed to waste vessel A, then Valve A was opened to remove air from the feed tubing. Once there were no visible air bubbles in the tubing, Valve A was closed, the three-way valve was changed to direct feed into the filter, and Valve B was opened. Once the feed had passed beyond Valve B, Valve B and Valve A were both closed. The feed vessel was then pressurized to the desired pressure for the trial, and Valve A was re-opened.

Prior to each protein filtration, experimental data for the membrane permeability were obtained with buffer as the feed. Filtrate flux data were collected over ten minutes to evaluate the initial buffer flux (J_o) and the membrane permeability, $L_p = J_o/\Delta P$ where ΔP is the transmembrane pressure difference.

3. Results and Discussion

3.1. The Effects of Driving Pressure on Protein Fouling

Typical experimental data for the filtrate flux as a function of time at three different transmembrane pressures are shown in Figure 2. The data were obtained with solutions of 1 g/L IgG in a pH 7.2 phosphate buffer at transmembrane pressures of 10, 30, and 45 psi (69, 210, and 310 kPa). The data are plotted as the normalized flux, defined as the flux with the protein solution divided by the buffer flux evaluated immediately prior to the protein filtration, as a function of the volumetric throughput, which is equal to the cumulative collected filtrate volume divided by the effective membrane area of the hollow fiber module. The runs in Figure 2 correspond to total filtration times of more than 5 hours. The scatter in the data reflects the dropwise collection of the filtrate. The filtrate flux initially declines nearly linearly with the volumetric throughput, with the flux then declining more slowly approaching a value of zero as the volumetric throughput continued to increase.

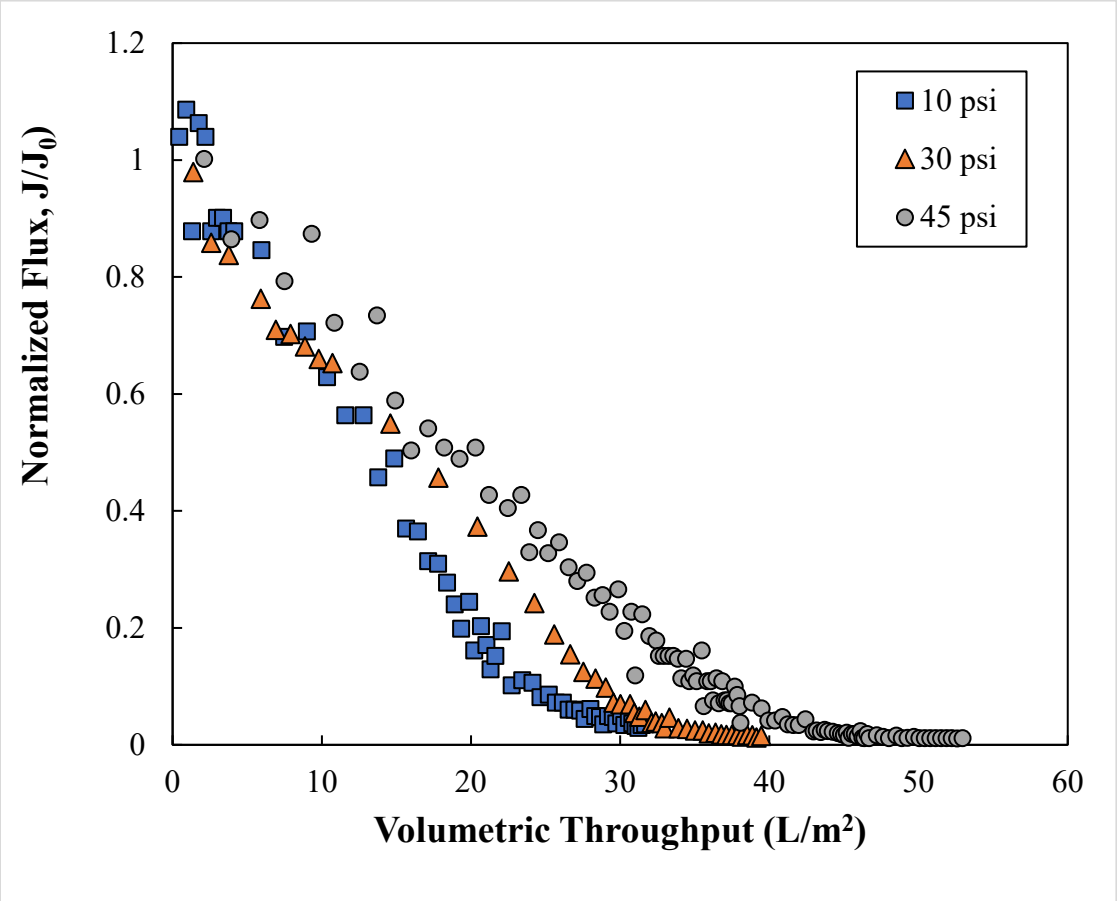

Figure 2. Normalized flux through the BioEX membrane as a function of the total volumetric throughput during filtration of 1 g/L IgG solutions at pH 7.2 at various transmembrane pressures.

As seen in Figure 2, the rate of flux decline was greatest at the lowest transmembrane pressure, with the rate of fouling decreasing with increasing pressure. This effect can be seen even more clearly in Figure 3 in which the data have been re-plotted as the logarithm of the filtrate flux. The data over the first 10 – 15 L/m^2 look fairly similar, but the flux then begins to decrease more rapidly, beginning first at the lowest transmembrane pressure; this transition point for the flux did not occur until nearly 25 L/m^2 for the run at 45 psi. The net result is that the normalized flux decreased by a factor of 10 after only 23 L/m^2 at 10 psi but not until 37 L/m^2 for the run at 45 psi.

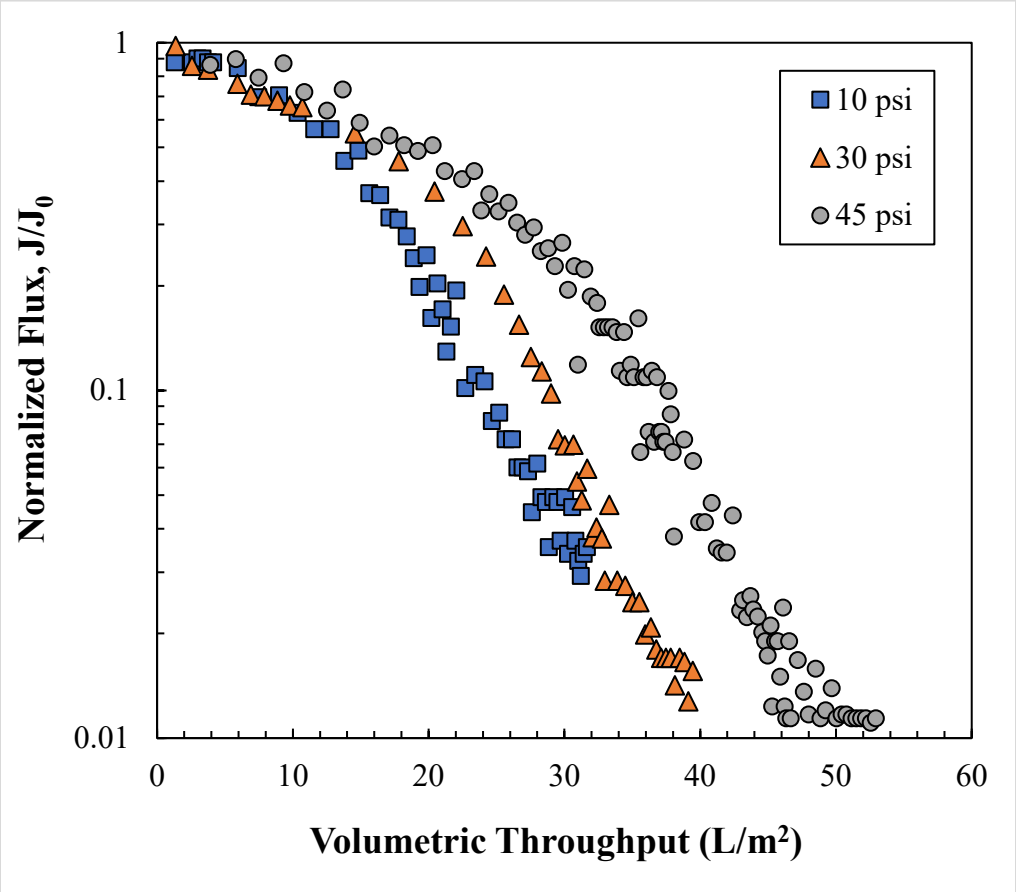

Figure 3. Filtrate flux data from Figure 2 re-plotted on a logarithmic scale.

One possible explanation for the pressure effects seen in Figures 2 and 3 was that the membrane itself might compress or deform at the higher pressures. In order to explore whether there was any significant material deformation of the membrane over this range of pressures, a pressure excursion experiment was performed using the pH 7.2 phosphate buffer with results shown in Figure 4. The filter was initially pressurized to 10 psi, the filtrate flux was measured over a period of 10 minutes, the driving pressure was then increased by 5 psi and the entire procedure repeated until attaining a pressure of 45 psi. Upon completion of the pressure excursion, the entire experiment was repeated with the same filter cartridge, beginning at 45 psi

and decreasing the pressure in 5 psi increments every 10 minutes. The filtrate flux data with ascending and descending pressures were nearly identical, with less than a 5% difference in the flux values. In addition, both sets of data were highly linear with R-squared values greater than 0.998 for ascending pressures and 0.997 for descending pressure, as determined by simple linear regression. These data strongly suggest that there is no mechanical deformation of the BioEX membrane at pressures up to at least 45 psi.

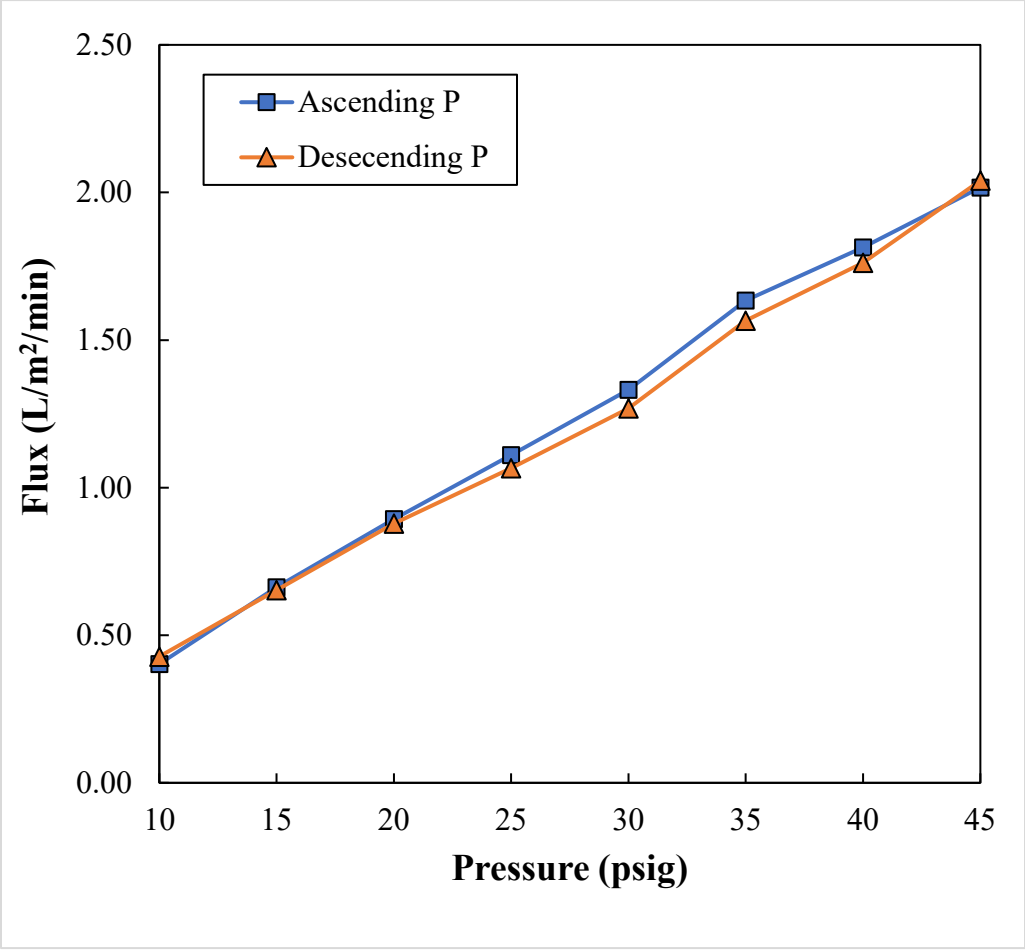

Figure 4. Filtrate flux through the BioEX membrane using pH 7.2 phosphate buffer for both ascending and descending pressures.

In order to help determine whether the flux decline seen in Figures 2 and 3 was reversible or irreversible, a membrane was fouled to approximately a 50% flux decline at a transmembrane pressure of 10 psi, after which the IgG feed was replaced with a protein-free buffer. The feed was immediately re-pressurized to 10 psi and the buffer flux measured as a function of time. The data for two separate repeat experiments using this procedure are shown in Figure 5. The filtrate flux during the buffer filtration was essentially constant over 20 L/m^2, corresponding to more than 100 min of filtration, with no measurable increase in the flux, suggesting the fouling was largely irreversible during flushing of the membrane over the range of conditions examined in Figure 5.

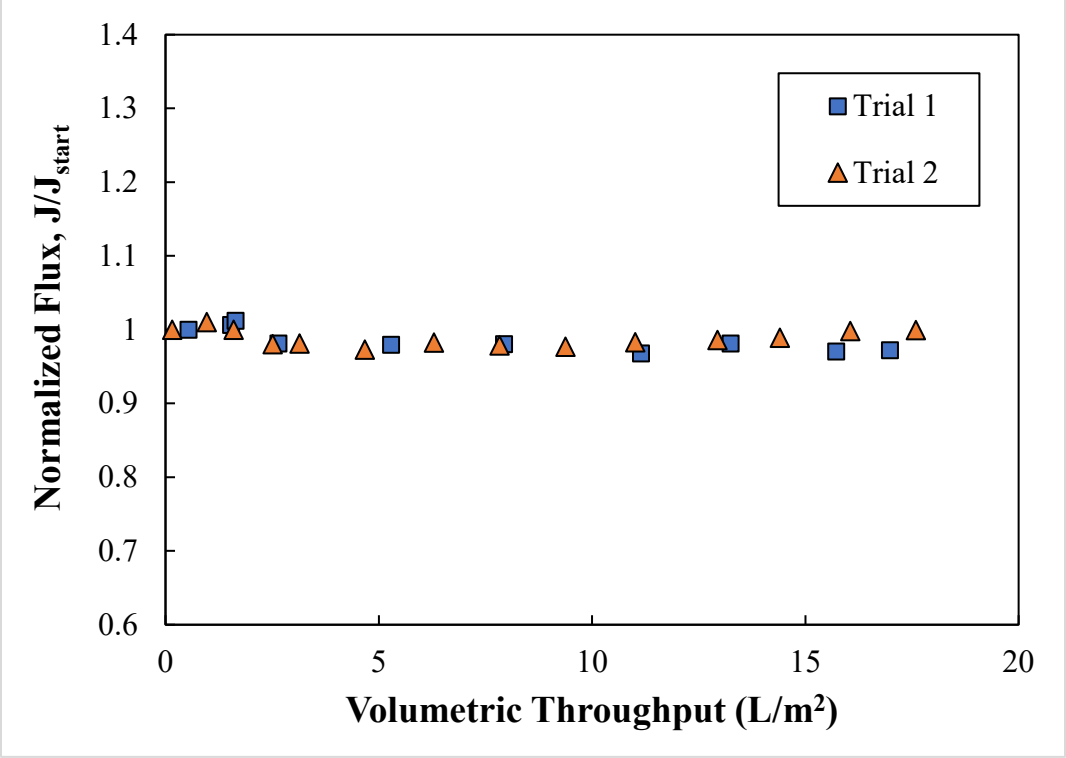

Figure 5. Change in flux during filtration of a pH 7.2 buffer solution through a BioEX membrane previously fouled to 50% flux decline by 1 g/L IgG at 10 psig. Data are normalized using the flux at the start of the buffer filtration, J_{start}.

To determine whether the higher pressure might have any effect on the fouling itself, a separate experiment was performed by again fouling the membrane with IgG at a pressure of 10 psig to a flux decline of 50%, but when the feed was replaced with buffer, the system was re-pressurized to 45 psig. As would be expected, the increase in pressure resulted in an immediate increase in flux by a factor of approximately five, slightly above a factor of 4.5 that might have been predicted from the ratio of pressures. More importantly, however, the data in Figure 6 show a slight increase in flux, of approximately 5%, while flushing the filter with 30 L/m^2 of buffer at the higher pressure. This behavior was reproducible, although the magnitude of the flux increase was somewhat less pronounced in Trial 2.

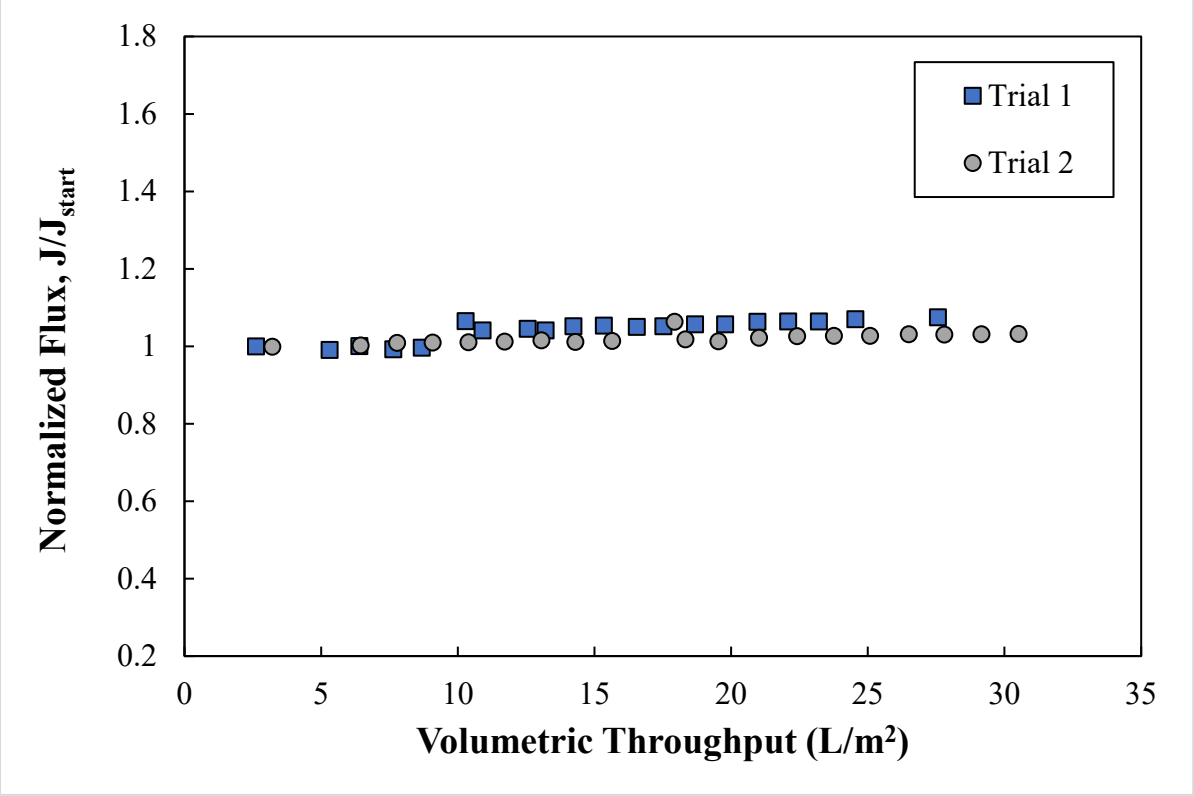

Figure 6. Filtrate flux during filtration of a pH 7.2 buffer at 45 psig through a BioEX membrane previously fouled to 50% flux decline by IgG at 10 psig. Data are normalized using the flux at the start of the buffer filtration, J_{start}.

3.2 Secondary Filtration of IgG Solutions

Further investigation was undertaken to determine the mechanism by which the protein solution fouls the hollow fiber membrane. First, a 1g/L solution of IgG in a pH 7.2 buffer was filtered through a Planova BioEX membrane at a driving pressure of 45 psig. The filtrate from that run was collected and pooled for use as the feed for another filtration run at the same transmembrane pressure. The concentration of IgG in the collected permeate from this initial filtration was 0.70 g/L, as determined by a NanoDrop Microvolume Spectrophotometer. For comparison, a fresh solution of 0.7 g/L IgG in a pH 7.2 buffer was prepared and also run at a pressure of 45 psig in a separate trial, with results shown in Figure 7. There is a significant flux decline for both IgG solutions, although there was slightly less flux decline observed for the IgG that had been prefiltered through a BioEX membrane. This difference is quite small for the first 30 L/m^2, but the data diverge more clearly at longer filtration times. The reduced fouling for the IgG that had been prefiltered through the BioEX membrane may reflect the removal of some large aggregates or other foulants during the initial filtration run.

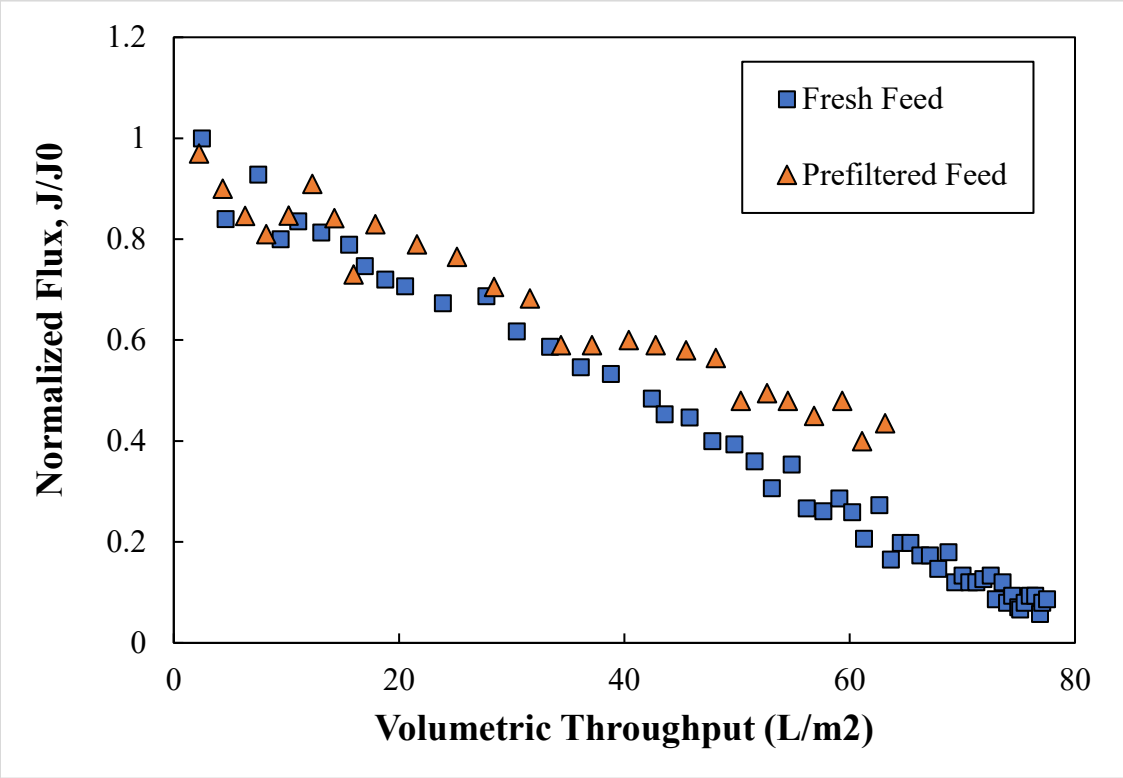

Figure 7. Flux profile for a fresh and pre-filtered 0.7 g/L IgG solution through separate Planova™ BioEX cartridges at 45 psig.

3.3 Varying IgG Concentration

The effect of the concentration of IgG on the flux profile was examined in a series of experiments using IgG solutions with 0.25 g/L, 1 g/L, and 4 g/L IgG concentrations in a pH 7.2 buffer. Results for filtration at 30.0 psig are shown in Figure 8. As would be expected, the flux decline occurs more rapidly relative to volumetric throughput for the runs at higher IgG concentrations, as the same volume of liquid is carrying more foulant.

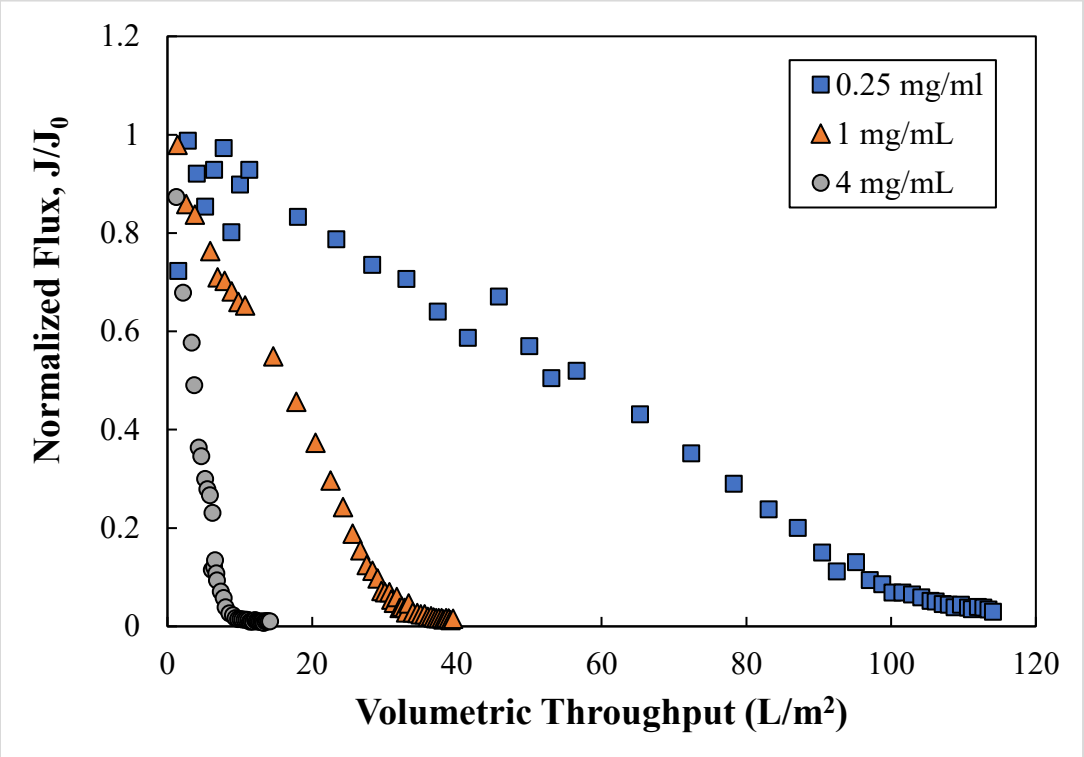

Figure 8. Normalized flux through the BioEX membrane as a function of the total volumetric throughput for filtration of 0.5, 1.0, and 4.0 g/L solutions of IgG at pH 7.2 at a 30 psig pressure.

The data in Figure 8 have been replotted in Figure 9 in terms of mass throughput, defined as the cumulative mass of IgG that has passed through the filter divided by the membrane area. When doing so, the characteristic curving of the flux decline profile is still preserved. Portraying the data in such a way causes the data for the three IgG concentrations to align more closely, particularly during the initial stage of the filtration until mass throughput of IgG of approximately 15 g/m^2. When plotted logarithmically as in Figure 10, it becomes clearer how the runs diverge at higher mass throughput values. The most rapid flux decline is seen for the most dilute solution (0.25 g/L), although there is no obvious trend in the data between the three concentrations. As such, over the range of concentrations tested here, fouling appears directly proportional to the concentration of foulant.

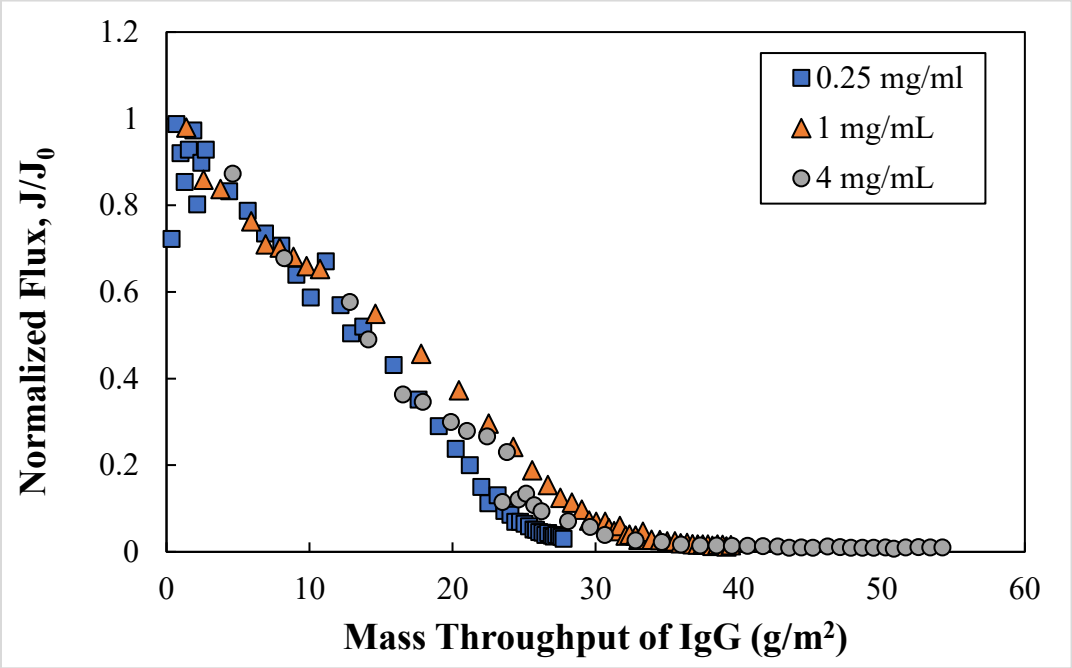

Figure 9. Normalized flux through the BioEX membrane as a function of the cumulative mass of IgG filtered through the membrane at three different IgG concentrations at pH 7.2 and 30 psig.

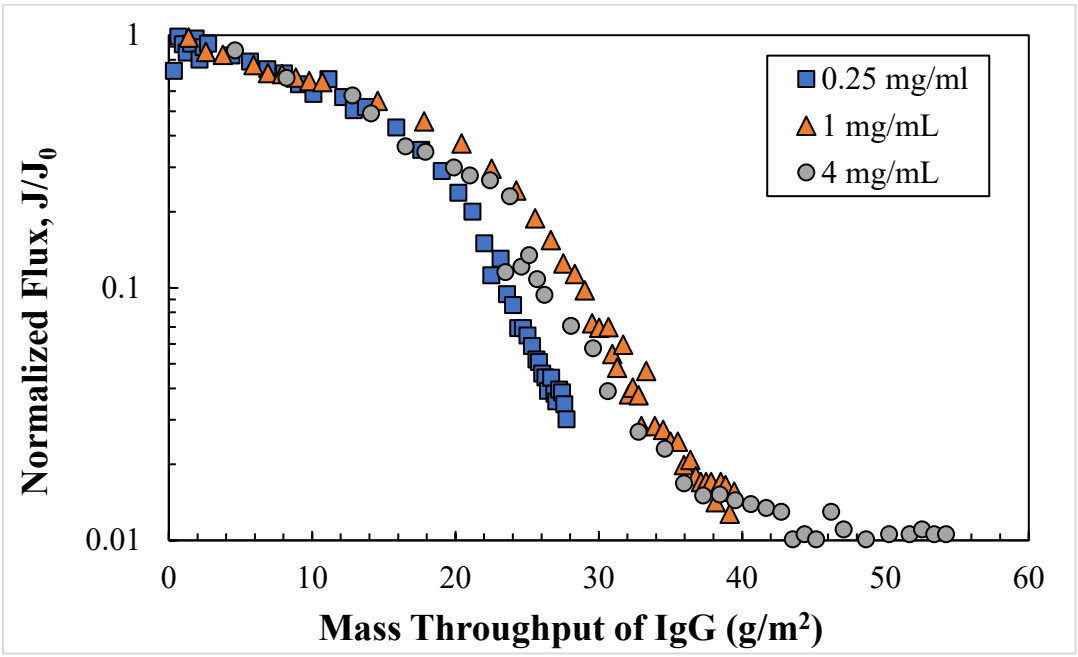

Figure 10. Filtrate flux data from Figure 9 re-plotted on a logarithmic scale.

3.4 Effect of Buffer pH

Another factor that can potentially influence fouling and flux decline is the pH of solution. Human IgG possesses an isoelectric point of between pH 6.6 and 7.2, meaning that it would exist in primarily a neutral state when dissolved in a pH 7.2 buffer (Agrisera, 2019). To test whether a partially charged form of the molecule would exhibit different fouling behavior, trials were performed with IgG dissolved in buffers at pH 8.0 and 8.5. At these higher pH values, some of the amino acid residues would become deprotonated, lending the protein an overall net negative charge. To perform these trials, 1 g/L IgG were dissolved in the appropriate buffer at driven by a pressure of 30.0 psig as shown in Figure 11. The runs correspond to total filtration times of approximately an hour. While there is slight variability between the trials, there are no obvious trends in the data, particularly at volumetric throughput greater than 20 L/m^2. These results suggest that the pH of the solution has no significant effect on flux decline in these experiments.

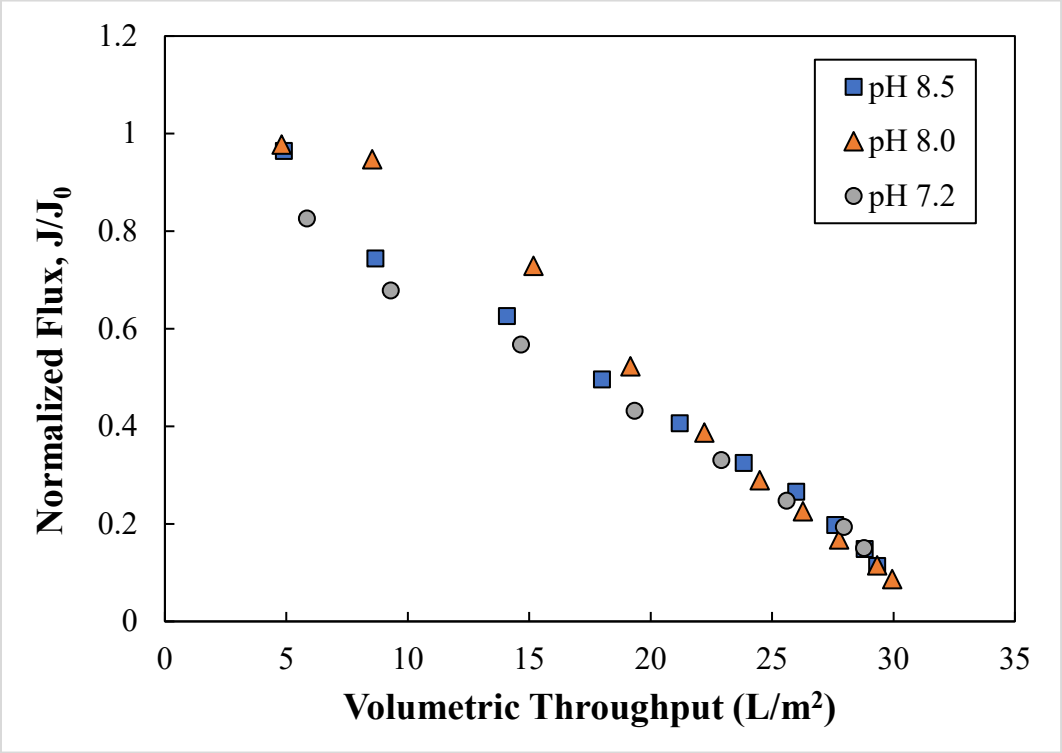

Figure 11. Effects of solution pH on the flux of 1 g/L IgG solutions at 30 psig through the Planova™ BioEX membranes.

3.5 BSA fouling

Although the primary interest of this work was in the fouling behavior of IgG as a model for current monoclonal antibody products, limited experiments were performed using bovine serum albumin, BSA, as a possible foulant. Experimental data for the filtration of 4 g/L solutions of BSA in a pH 7.2 phosphate buffer at 10, 30, and 45 psig are shown in Figure 12. These runs correspond to total filtration times of more than 120 minutes. No flux decline was seen with the BSA solutions over the range of these experiments, with the normalized flux after 80 L/m^2 varying between 1.0 and 1.2. The small increase in flux during the initial phase of the filtration is unexpected. This behavior may be due to either wetting of the pores or small variations in the pressure driving force during the start-up of the filtration process.

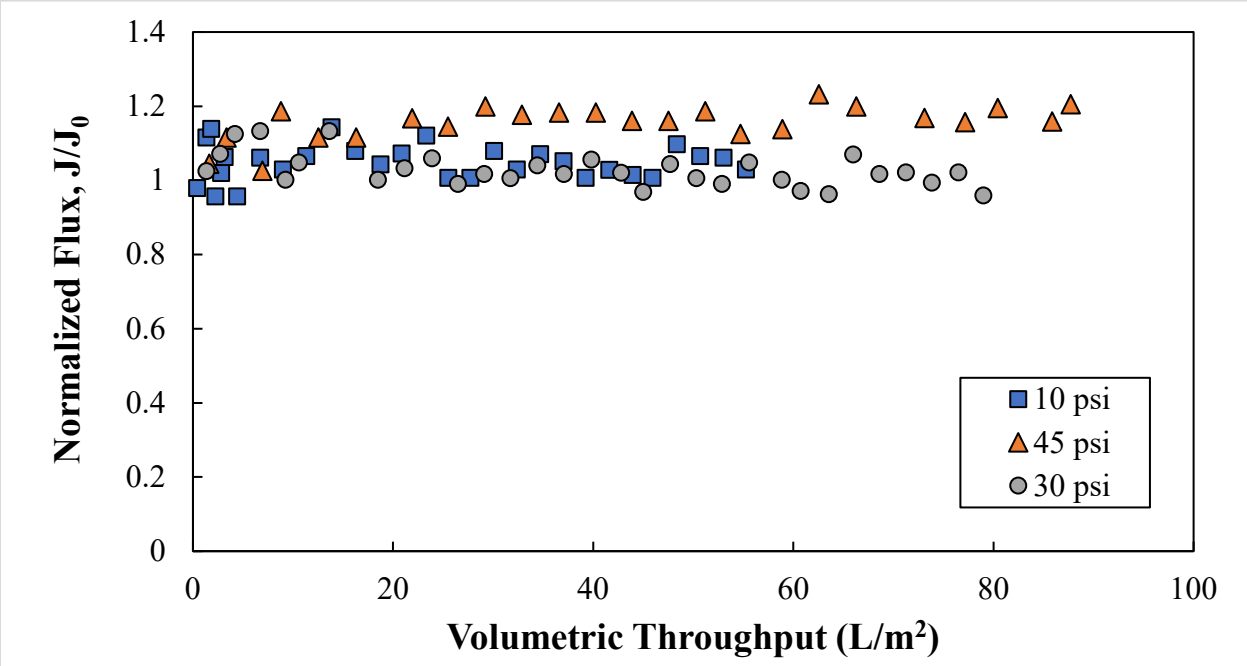

Figure 12. Flux of 4 g/L solutions BSA in pH 7.2 buffer through the BioEX membrane at varying pressures.

Additional insights into the fouling behavior of the different proteins were obtained using a sequential filtration experiment. A membrane cartridge was initially fouled with a 1 g/L solution of IgG until approximately a 50% decline in flux was achieved. At this point, the feed was replaced with a 4 g/L solution of BSA, with the system rapidly re-pressurized to 30 psig. Results during the IgG and BSA filtration are shown in Figure 13. There was no measurable fouling during the BSA filtration over approximately 30 L/m^2 of cumulative filtrate volume, suggesting that the IgG does not constrict the pores sufficiently to allow BSA to foul the membrane.

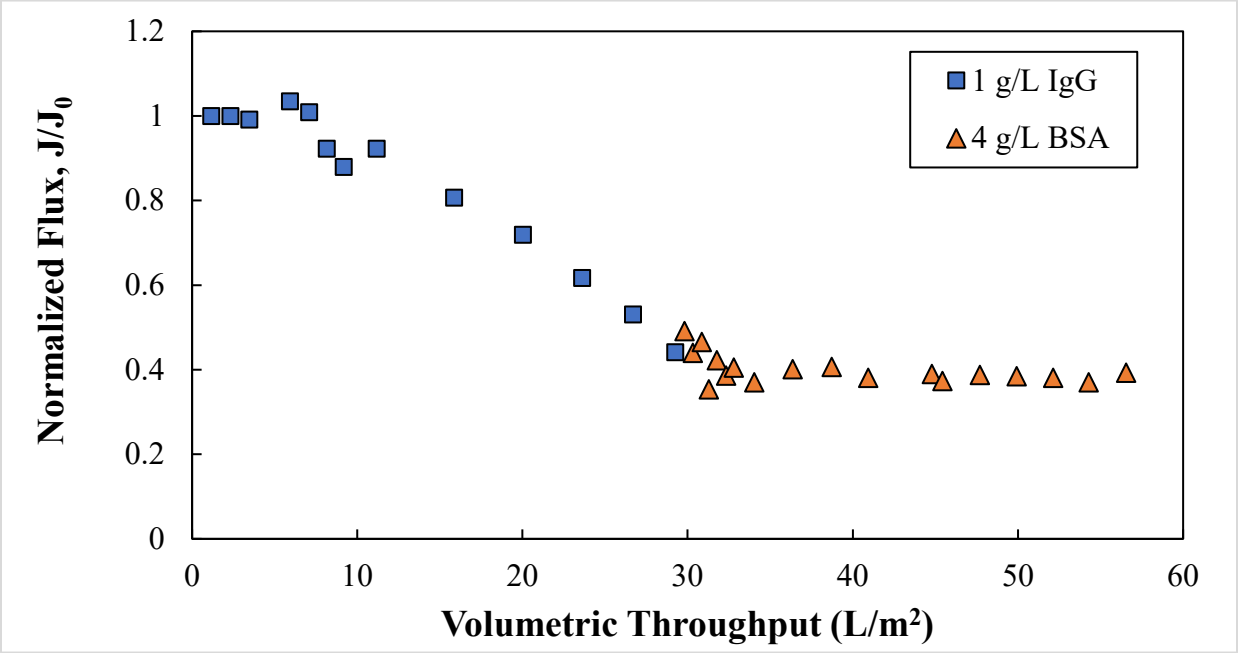

Figure 13. Sequential fouling of a Planova™ BioEX membrane using first 1 g/L IgG followed by 4 g/L BSA at a pressure of 30 psig.

3.6 Fouling of the Planova™ 20N Membrane

The Planova BioEX membrane is the current "state-of-the-art" virus filter manufactured by Asahi Kasei (Asahi Kasei Bioprocess, 2018). However, there is still considerable interest in the performance of Asahi's older hollow fiber virus filter, the Planova 20N. Limited experiments were performed with the 20N membranes using 1 g/L solutions of IgG in a pH 7.2 phosphate buffer, but at transmembrane pressures of 5, 10, and 14 psig (34, 69, and 97 kPa). The Planova 20N membrane possesses a smaller range of pressure over which it is intended to be operated; its maximum operating transmembrane pressure is only 15 psi as specified by the manufacturer (Strauss, et al., 2017). Typical data are shown in Figure 14 for runs of more than 3 hours total filtration time. In contrast to the results with the BioEX membrane, the flux decay profiles were essentially independent from pressure over the entire experiment. Only small differences were

seen at volumetric throughput beyond 50 L/m^2, where the highest flux decay was seen in the 14 psig run. These data clearly indicate that the transmembrane pressure plays a much smaller role in determining the fouling behavior of the Planova 20N compared to that observed with the BioEX.

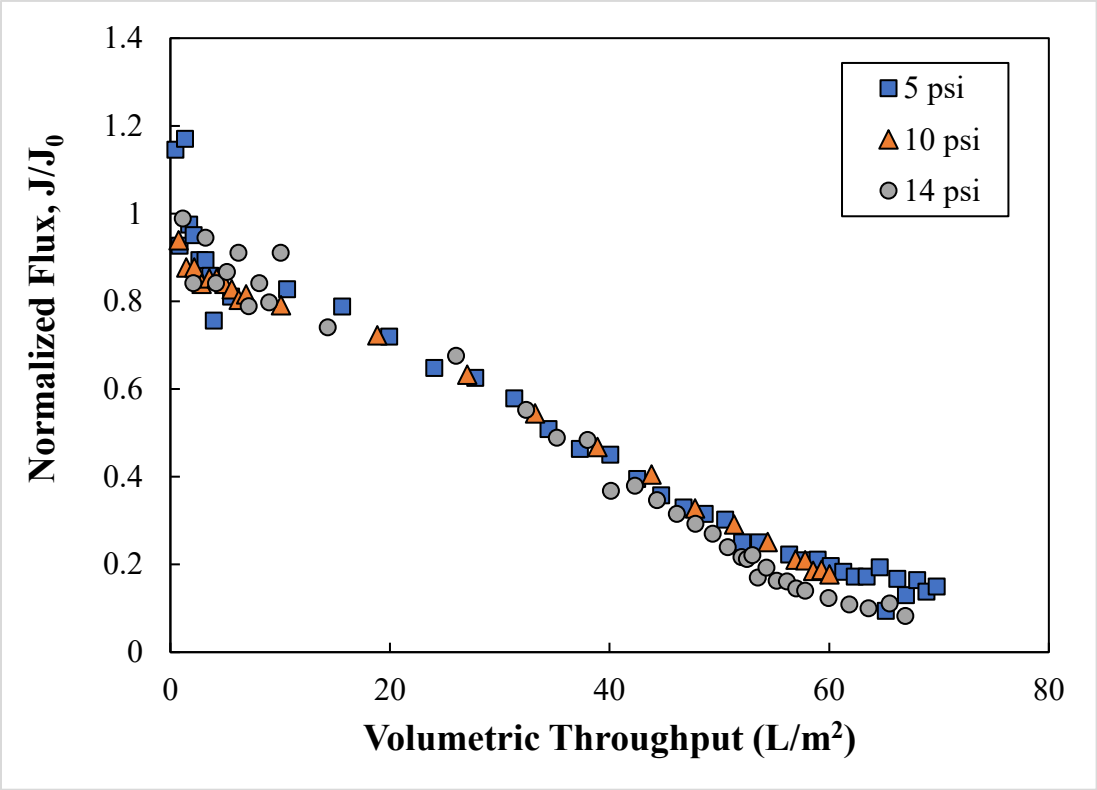

Figure 14. Normalized flux through the Planova 20N membrane as a function of the total volumetric throughput during filtration of 1 g/L IgG solutions at pH 7.2 at various driving pressures.

In order to further explore the fouling behavior of the Planova 20N membrane, a sequential filtration experiment was performed with the permeate obtained during filtration of a 1 g/L solution of IgG in a pH 7.2 buffer at 15 psig used as the feed for a subsequent filtration experiment under otherwise identical conditions. The concentration of IgG in the pooled permeate from the initial filtration, as measured by the NanoDrop Microvolume

Spectrophotometer, was 0.67 g/L, slightly lower than but comparable to the permeate concentration recorded in the BioEX run. A parallel experiment was conducted by running a fresh solution of 0.67 g/L IgG in a pH 7.2 buffer through another 20N membrane. In contrast to the results obtained with the BioEX membrane (Figure 7), the pre-filtered IgG solution shows nearly identical flux behavior as the fresh solution when filtered again through the Planova 20N membrane.

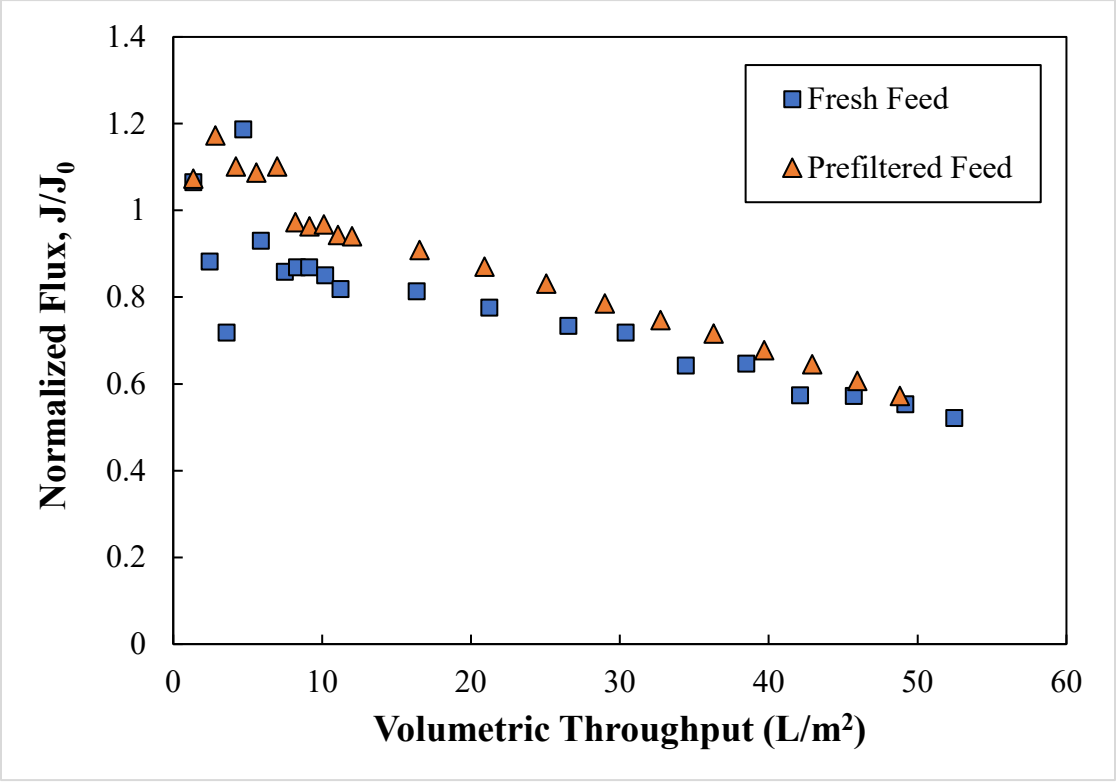

Figure 15. Flux profile for a fresh and prefiltered 0.67 g/L IgG solution through separate Planova™ 20N cartridges at 15 psig.

As with the BioEX membrane, a lack of association between pH and fouling behavior over the range examined was observed in 20N filters, as shown in Figure 16. To run those trials a similar procedure was followed: 1g/L of IgG was dissolved in the requisite buffer and was then driven through the system with a pressure of 10 psig, to reflect the lower pressure tolerances of the 20N filter. Over the runs of over 3 hours, the recorded fluxes did not diverge.

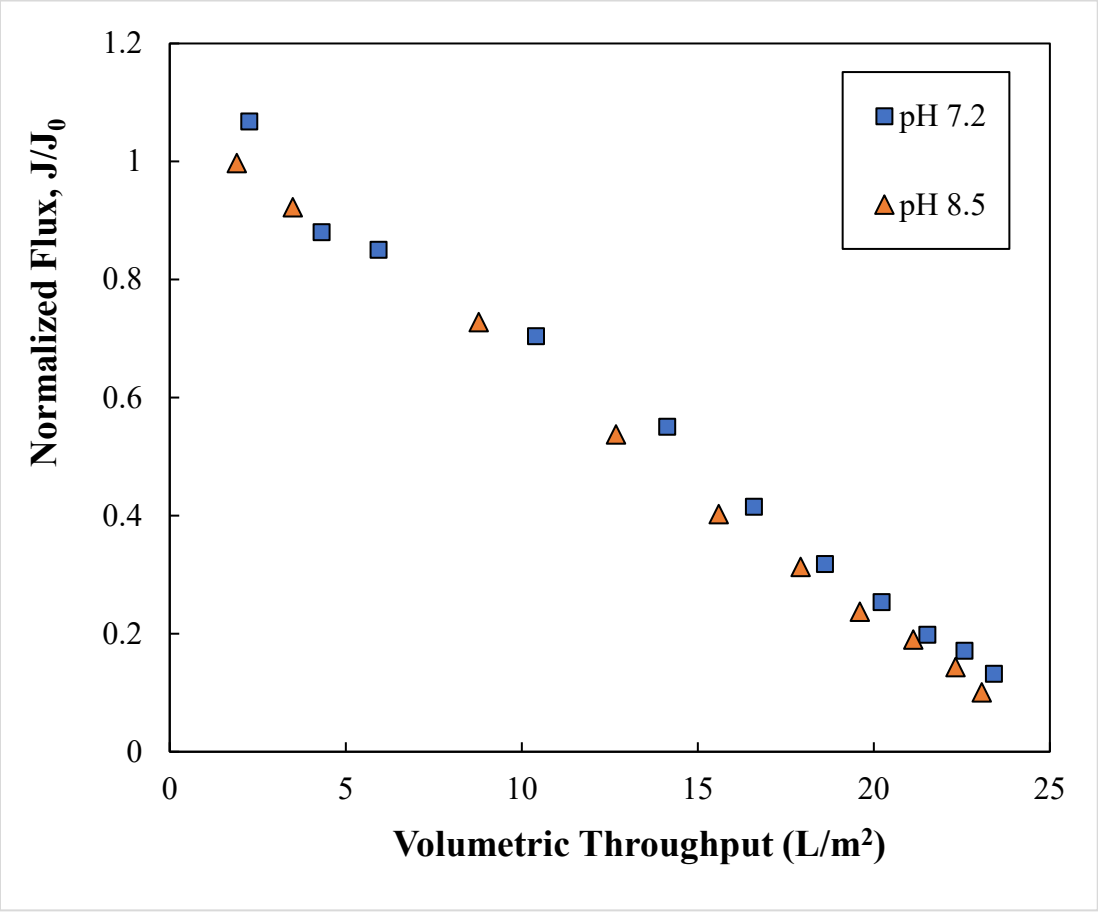

Figure 16. Effects of varying buffer pH on the flux profile of 1 g/L IgG driven at 10.0 psig in a Planova™ 20N cartridge.

Limited data were also obtained for the filtration of BSA through the Planova 20N membrane. Given the lack of fouling seen at low BSA concentrations, experiments were performed using both 4 and 40 g/L solutions of BSA dissolved in pH 7.2 buffer, with results at a pressure of 5 psig shown in Figure 17. The data with the 40 g/L BSA did show a small, under 10%, flux decline over the first 10 L/m^2, but the flux then remained almost constant over the rest of the filtration, dropping only slightly. Thus, BSA fouling of the Planova 20N filter is negligible even at concentrations as high as 40 g/L in the conditions of these experiments. As such, while BSA can interact with and foul the 20N membrane to some degree, it would require unrealistically high concentrations to be noticeable at these experimental time scales.

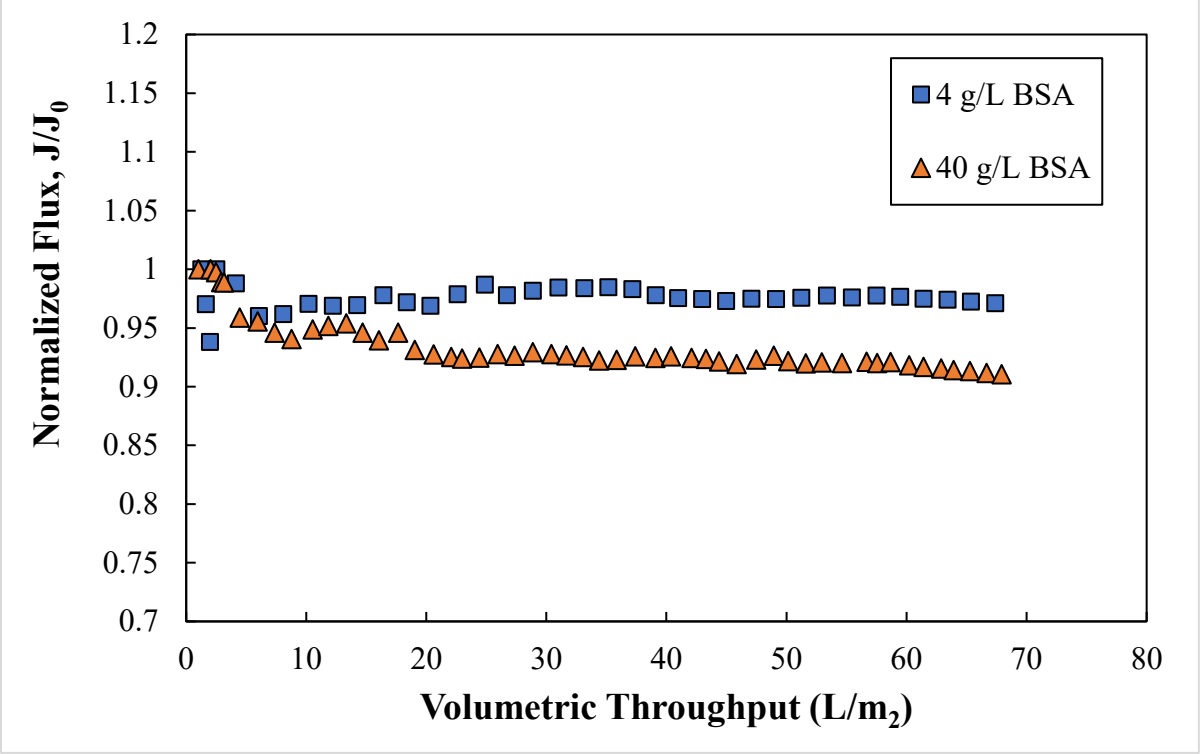

Figure 17. Comparison of the flux profiles of 4 g/L and 40 g/L BSA solutions in pH 7.2 buffer through the Planova 20N membrane at 5 psig.

4. Conclusions

The objective of this thesis was to obtain a better understanding of the fouling behavior of the Planova™ BioEX hollow fiber virus filtration membranes, focusing on serum immunoglobulin G (IgG) as a model protein. Experimental data obtained using 1 g/L solutions of IgG show more than an order of magnitude decline in filtrate flux after less than 30 L/m^2 filtration when at a pressure of 30 psi, which would severely limit the applicability of these filters for processing an IgG feed of this type. This fouling was directly due to some aspect of the protein solution; data obtained during filtration of unaltered buffer showed no flux decline over the course of the run. However, the fouling cannot be simply due to the presence of large aggregates of IgG already present in solution; pre-filtration of the IgG solution through the BioEX membrane had a relatively small effect on the subsequent fouling behavior within a fresh BioEX membrane.

The rate of flux decay for the BioEX filters was still less pronounced when operating at higher pressures, however. The capacity of the filter, if defined as the total volumetric throughput corresponding to a flux decline of 90% from the initial value, increased by more than 50% as the pressure was increased from 10 to 45 psig. This increase in capacity was not due to any changes in the structure of the membrane, which shows nearly constant permeability over this pressure range. Instead, the changes in flux associated with operation at high pressures could reflect the removal of previously deposited IgG from within the porous filter or some reorganization of the deposited protein to yield slightly less resistance to flow.

Several other factors were explored to determine their effects on IgG's fouling behavior within the BioEX membrane. The degree of fouling was essentially independent of the concentration of the IgG in solution as long as the data were plotted as a function of the total IgG

mass throughput rather than the volumetric throughput. Similarly, there was no obvious effect of buffer pH on the fouling behavior.

Similar trends were observed with the Planova 20N membrane, which is one of the most widely used virus filters in the plasma processing industry. However, the flux decline through the 20N was essentially independent of the transmembrane pressure over a range from 5 to 14 psig. Additionally, the capacity of the membrane was much greater with the Planova 20N than with the BioEX. It is unclear to what extent these differences are related to the different surface chemistry or pore structures of the different membranes.

In an attempt to expand the characterization of the BioEX membrane further, beyond simply IgG as the foulant, bovine serum albumin (BSA) was also tested for its fouling properties. In contrast to the results obtained with IgG, the Planova BioEX and 20N membranes showed almost no fouling when challenged with solutions of BSA. This was true, to an extent, even up to BSA concentrations of 40 g/L. Indeed, the rate of fouling was low enough to require lengths of time that would have made it impractical to study with the current experimental set-up. This is contrary that what might be expected from trials run with the Viresolve Pro membrane, where BSA yielded fouling on scales similar to that of IgG (Zydney & Fallahianbijan, 2018). BSA is somewhat smaller than IgG, with a molecular weight of 67 kDa compared to that of 150 kDa, although it is unclear if this difference in size is the cause of the very different fouling behavior (Rizvi, 2010). These data show strongly how differences between the behavior of membranes with similar applications might vary unpredictably. As such, broad, wide-ranging investigations into their properties are crucial.

This thesis provides a foundation for future research to provide more detailed insights into the fouling characteristics of these important virus filtration membranes. Although the

experimental data reported in this thesis showed little effect of buffer pH on IgG fouling, these studies were limited to neutral to basic buffers at relatively high ionic strength. Further research might involve the study the behavior of IgG in acidic buffers, in which the protein would have a significant positive charge, and the effects of varying ionic strength on the fouling behavior. Another avenue of investigation would be to determine the source behind BSA's resistance to fouling within Planova™ hollow fiber membrane filters This would likely require the examination of the fouling behavior of other proteins besides IgG and BSA. These studies should also include more extensive characterization of the properties of the proteins using dynamic light scattering, to determine the protein's effective size, and analytical hydrophobic interaction chromatography. Additionally, other experiments could be performed to determine how the specific characteristics of the membranes themselves influence fouling, by examining other virus filters having similar pore size but different surface chemistries or pore morphologies. Whatever the case, it is clear that there is still much to be understood about protein fouling within the Planova™ BioEX membrane.

Appendix A

Verification of Elements of the Experimental Method for Pausing Filtration

Several of the trials performed in this thesis required the filtration to be paused for a period of time during the course of the filtration experiment, such as to exchange the feed solution for another. However, it was important to confirm that this pause in the filtration process would not cause unusual artifacts in the fouling behavior of the filter. In order to examine this more closely, a 1 g/L solution of IgG in pH 7.2 buffer was filtered through a BioEX membrane at a constant pressure of 45 psig (Figure 18) or 10 psig (Figure 19). When the total average flux reached approximately a 50% decline, corresponding to between 15 and 20 L/m^2 of filtration, Valve A was closed for 15 minutes. The filtration was then re-started by opening Valve A, with the pressure maintained at the same value used in the first portion of the filtration experiment. The data in Figures 19 and 20 show what appears to be a smooth continuous decline in flux, with the data before and after the pause showing no differences in either value or rate of flux decline. Simply pausing and re-starting the filtration did not have any observable effect on the flux behavior. Thus, pauses were used in experiment design without concerns of overly influencing the data.

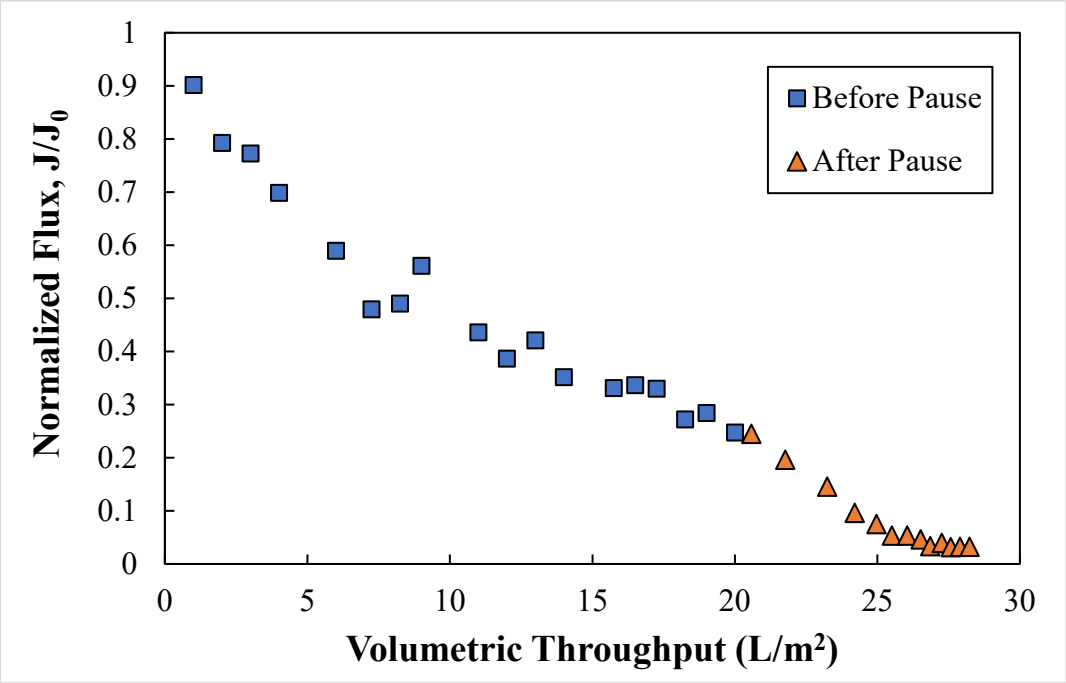

Figure 18. Effects of a 15-min pause on the flux profile during filtration of 1 g/L IgG in pH 7.2 buffer at 45 psig through a BioEX membrane.

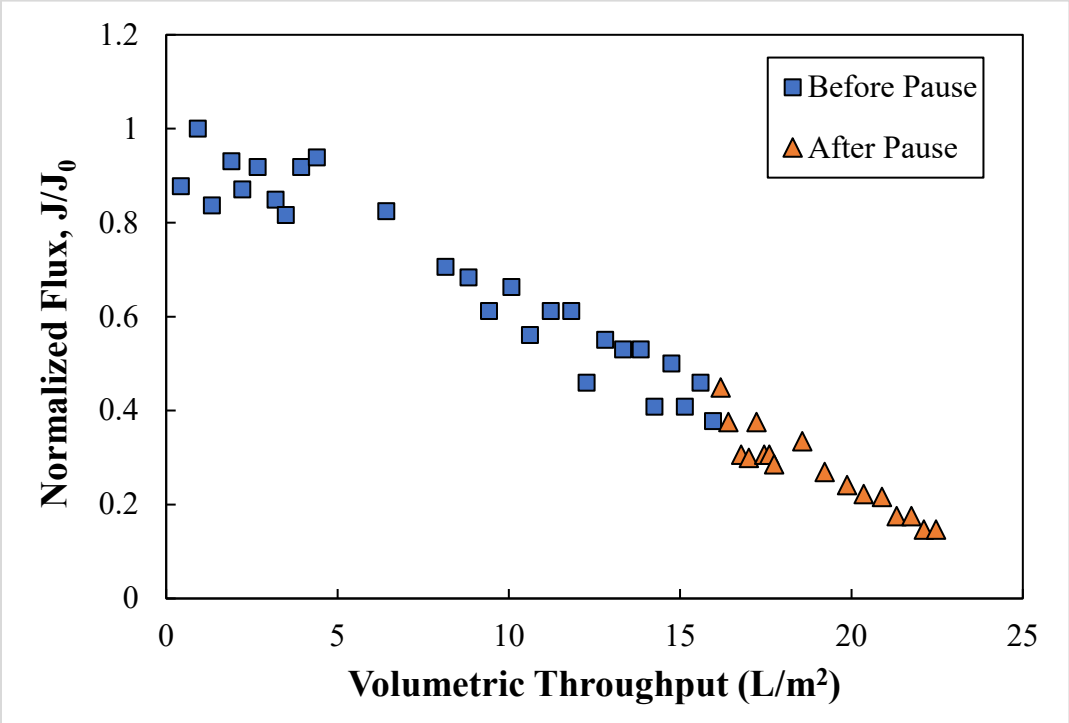

Figure 19. Effects of a 15-min pause on the flux profile during filtration of 1 g/L IgG in pH 7.2 buffer at 10 psig through a BioEX membrane.

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