flexibility has led to a growing interest in the potential of continuous bioprocessing.

[0006] U.S. Patent No. 10,183,108 to the Pall Corporation describes a diafiltration system designed for continuous buffer exchange. While these stages provide continuous buffer exchange, the system requires larger buffer volumes to achieve the same level of buffer exchange as a corresponding batch system, and the multihead pumping system is more complex.

[0007] Rucker-Pezzini et al. describe a three-stage single pass diafiltration system (see Rucker-Pezzini et al., "Single pass diafiltration integrated into a fully continuous mAb purification process." *Biotechnology and Bioengineering.* 2018; 115:1949-1957).

[0008] Nambiar et al. describe using single pass tangential flow filtration (SPTFF) stages for continuous diafiltration (see Nambiar et al., "Countercurrent staged diafiltration for formulation of high value proteins." *Biotechnology and Bioengineering.* 2018; 115:139-144). The individual stages were arranged in a countercurrent configuration to reduce buffer requirements.

[0009] There remains a need for improved diafiltration systems and methods which reduce the impact on the biologic product, reduce the consumption of diafiltration buffer and reduce the size of the necessary equipment.

SUMMARY

[0010] Disclosed herein are systems and methods for the efficient diafiltration of biologic products. The systems and methods can support continuous or batch processes as well as reusable or single use processing.

[0011] The systems disclosed herein offer distinct advantages over traditional tangential flow filtration (TFF) systems used for the diafiltration of biologic products. In particular, diafiltration carried out with the systems according to the examples operate such that the biologic product experiences only one pump pass compared to about 40 to about 80 pump passes in tradition TFF systems. In this way, potential impact on the biologic product is reduced.

[0012] Another advantage of the systems disclosed herein is a substantial reduction in the use of diafiltration buffer. For example, the use of diafiltration buffer can be reduced by at least about 60% compared to traditional TFF systems, achieving 8 diavolumes of diafiltration with 3

diavolumes of diafiltration buffer using a 3-stage system.

[0013] A further advantage of the systems disclosed herein is that the equipment size may be substantially reduced compared to traditional TFF systems. In some examples, the feed flow rate of the system is about 40 to about 80 fold lower than the feed flow rate of a traditional TFF system. In certain examples, the system is more easily produced with a single use product contacting flow path compared to a traditional TFF system. The size of a traditional TFF system might preclude the use of single use materials of construction.

[0014] The systems and methods described herein accomplish buffer exchange, or diafiltration, at a constant retentate concentration. The TFF cassettes used in the systems and methods each include a diafiltration channel, a flat first filtration membrane, a retentate channel, a flat second filtration membrane, and a permeate/diafiltration buffer collection channel, configured such that (i) the flat first filtration membrane delimits the diafiltration channel and the retentate channel from one another, (ii) the flat second filtration membrane delimits the retentate channel and the permeate/diafiltration buffer collection channel from one another, (iii) the diafiltration channel is fluidly connected to at least one inlet for the diafiltration medium, (iv) the retentate channel is fluidly connected to at least one inlet for the feed fluid and to at least one outlet for the retentate, and (v) the permeate/diafiltration buffer collection channel is fluidly connected to at least one outlet for the permeate/diafiltration buffer. The systems and methods described herein provide a stepwise diafiltration of a feed medium that comprises a molecule of interest (e.g., a protein or other biologic molecule). The feed medium is diafiltered against a diafiltration buffer across an appropriate membrane, with the diafiltration taking place several times (e.g., two, three, four or more times) in adjacent diafiltration cassettes. This stepwise diafiltration is characterized by a countercurrent flow of the diafiltration buffer against the feed medium. The permeate (and diafiltration buffer, if any remains) leaving the system from the first diafiltration cassette (e.g., the left-most cassette shown in Fig. 1) comprises washed-out impurities, mixture of ions etc., while the retentate leaving the system after the final diafiltration cassette (e.g., the right-most cassette shown in Fig. 1) comprises the molecule of interest exchanged into the diafiltration buffer.

[0015] A system for single-pass, countercurrent diafiltration of a fluid feed is disclosed, the system comprising: two, three, four or more filtration stages; conduits to facilitate fluid communication between manifolds in the filtration stages; a conduit and at least

one feed pump to provide a fluid feed to the first filtration unit; a conduit and at least one buffer pump to provide diafiltration buffer to the final filtration stage; a conduit and at least one buffer pump per filtration stage to provide permeate and diafiltration buffer from a subsequent to a prior filtration stage; and a conduit and a filtrate pump to remove permeate (and diafiltration buffer, if any remains) from the system;

[0016] wherein each filtration stage comprises:

[0017] (i) a manifold segment and (ii) one or more TFF cassettes as described herein; wherein the manifold segment comprises: a first manifold (i.e., inlet) for receiving and carrying the fluid feed or retentate into the filtration stage, a second manifold (i.e., outlet) for receiving and carrying retentate out of the filtration stage, a third manifold (i.e., inlet) for receiving and carrying permeate and diafiltration buffer through the filtration stage, and a fourth manifold (i.e., outlet) for receiving and carrying permeate and diafiltration buffer out of the filtration stage; and wherein the manifold segment is fluidly connected to the one or more TFF cassettes as described herein;

[0018] wherein the filtration stages are fluidly connected through the manifold segments to provide a flow path between filtration stages, by coupling of the second manifold in a manifold segment to the first manifold of a manifold segment in a subsequent filtration stage, such that the retentate of one stage serves as the feed for the next filtration stage, and by coupling of the fourth manifold in a manifold segment to the third manifold of a manifold segment in the prior filtration stage;

[0019] wherein the first filtration stage in the system receives the fluid feed from a conduit connected to a feed pump; and wherein the second manifold on the final (i.e., last) filtration stage is a retentate outlet that allows the retentate to leave the system and does not connect to a prior filtration stage.

[0019a] In one aspect, the present disclosure provides a system for single-pass, countercurrent diafiltration of a fluid feed comprising: two or more filtration stages; conduits to facilitate fluid communication between manifolds in the filtration stages; a conduit and at least one feed pump to provide a fluid feed to the first filtration stage; a conduit and at least one buffer

pump to provide diafiltration buffer to the final filtration stage; a conduit and at least one buffer pump per filtration stage to provide permeate and diafiltration buffer from a subsequent to a prior filtration stage; and a conduit and a filtrate pump to remove permeate (and diafiltration buffer, if any remains) from the system;

wherein each filtration stage comprises:

(i) a manifold segment and (ii) one or more tangential flow filtration ("TFF") cassettes; wherein the manifold segment comprises: a first manifold (i.e., inlet) for receiving and carrying the fluid feed or retentate into the filtration stage, a second manifold (i.e., outlet) for receiving and carrying retentate out of the filtration stage, a third manifold (i.e., inlet) for receiving and carrying permeate and diafiltration buffer through the filtration stage, and a fourth manifold (i.e., outlet) for receiving and carrying permeate and diafiltration buffer out of the filtration stage; and wherein the manifold segment is fluidly connected to the one or more TFF cassettes;

wherein the filtration stages are fluidly connected through the manifold segments to provide a flow path between filtration stages, by coupling of the second manifold in a manifold segment to the first manifold of a manifold segment in a subsequent filtration stage, such that the retentate of one stage serves as the feed for the next filtration stage, and by coupling of the fourth manifold in a manifold segment to the third manifold of a manifold segment in the prior filtration stage;

wherein the first filtration stage in the system receives the fluid feed from a conduit connected to a feed pump; and wherein the second manifold on the final filtration stage is a retentate outlet that allows the retentate to leave the system and does not connect to a prior filtration stage; and

wherein the TFF cassettes comprise a diafiltration channel, a flat first filtration membrane, a retentate channel, a flat second filtration membrane, and a permeate/diafiltration buffer collection channel, configured such that (i) the flat first filtration membrane delimits the diafiltration channel and the retentate channel from one another, (ii) the flat second filtration membrane delimits the retentate channel and the permeate/diafiltration buffer collection channel from one another, (iii) the diafiltration channel is fluidly connected to at least one inlet for the diafiltration medium, (iv) the retentate channel is fluidly connected to at least one inlet for the

feed fluid and to at least one outlet for the retentate, and (v) the permeate/diafiltration buffer collection channel is fluidly connected to at least one outlet for the permeate/diafiltration buffer.

[0020] In certain examples, the system comprises two filtration stages. In certain examples, the system comprises three filtration stages.

[0021] A method of filtering a fluid feed using the system described herein is provided.

[0021a] In another aspect, the present disclosure provides a method for filtering a fluid feed, the method comprising: (i) passing a fluid feed through a single-pass, countercurrent diafiltration system as described herein, and (ii) recovering permeate and retentate from the system in separate containers.

[0022] A method of manufacturing a biologic product of interest is provided, the method comprising the steps of:

[0023] (I) cultivating a eukaryotic cell expressing the biologic product of interest in cell culture;

[0024] (II) harvesting the biologic product of interest from the cell culture in the form of a fluid feed comprising (a) the biologic product of interest and (b) one or more impurities and/or buffer components;

[0025] (III) purifying the fluid feed comprising the biologic product of interest and the one or more impurities and/or buffer components to isolate the biologic product of interest from the fluid feed; and

[0026] (IV) optionally formulating the biologic product of interest into a pharmaceutically acceptable formulation suitable for administration; and

[0027] wherein the method further comprises: (a) passing the fluid feed through a single-pass, countercurrent diafiltration system, and (b) recovering permeate (and diafiltration buffer, if any remains) and retentate from the system in separate containers; and

[0028] wherein the system is a system according to the examples described herein.

[0028a] In another aspect, the present disclosure provides a method of manufacturing a biologic product of interest comprising the steps of:

(I) cultivating a eukaryotic cell expressing the biologic product of interest in cell culture;

(II) harvesting the biologic product of interest from the cell culture in the form of a fluid feed comprising (a) the biologic product of interest and (b) one or more impurities and/or buffer components;

(III) purifying the fluid feed comprising the biologic product of interest and the one or more impurities and/or buffer components to isolate the biologic product of interest from the fluid feed; and

(IV) optionally formulating the biologic product of interest into a pharmaceutically acceptable formulation suitable for administration; and

wherein the method further comprises:

(a) passing the fluid feed through a single-pass, countercurrent diafiltration system, and (b) recovering permeate (and diafiltration buffer, if any remains) and retentate from the system in separate containers;

wherein the single-pass, countercurrent diafiltration system comprises: two or more filtration stages; conduits to facilitate fluid communication between manifolds in the filtration stages; a conduit and at least one feed pump to provide the fluid feed to the first filtration stage; a conduit and at least one buffer pump to provide diafiltration buffer to the final filtration stage; a conduit and at least one buffer pump per filtration stage to provide permeate and diafiltration buffer from a subsequent to a prior filtration stage; and a conduit and a filtrate pump to remove permeate (and diafiltration buffer, if any remains) from the system;

wherein each filtration stage comprises:

(i) a manifold segment and (ii) one or more tangential flow filtration ("TFF") cassettes; wherein the manifold segment comprises: a first manifold (i.e., inlet) for receiving and carrying the fluid feed or retentate into the filtration stage, a second manifold (i.e., outlet) for receiving and carrying retentate out of the filtration stage, a third manifold (i.e., inlet) for receiving and carrying permeate and diafiltration buffer through the filtration stage, and a fourth manifold (i.e., outlet) for receiving and carrying permeate and diafiltration buffer out of the filtration stage; and wherein the manifold segment is fluidly connected to the one or more TFF cassettes;

wherein the filtration stages are fluidly connected through the manifold segments to provide a flow path between filtration stages, by coupling of the second manifold in a manifold segment to the first manifold of a manifold segment in a subsequent filtration stage, such that the retentate of one stage serves as the feed for the next filtration stage, and by coupling of the fourth manifold in a manifold segment to the third manifold of a manifold segment in the prior filtration stage;

wherein the first filtration stage in the system receives the fluid feed from a conduit

connected to a feed pump; and wherein the second manifold on the final (i.e., last) filtration stage is a retentate outlet that allows the retentate to leave the system and does not connect to a prior filtration stage; and

wherein the TFF cassettes comprise a diafiltration channel, a flat first filtration membrane, a retentate channel, a flat second filtration membrane, and a permeate/diafiltration buffer collection channel, configured such that (i) the flat first filtration membrane delimits the diafiltration channel and the retentate channel from one another, (ii) the flat second filtration membrane delimits the retentate channel and the permeate/diafiltration buffer collection channel from one another, (iii) the diafiltration channel is fluidly connected to at least one inlet for the diafiltration medium, (iv) the retentate channel is fluidly connected to at least one inlet for the feed fluid and to at least one outlet for the retentate, and (v) the permeate/diafiltration buffer collection channel is fluidly connected to at least one outlet for the permeate/diafiltration buffer.

[0029] Additional advantages of the subject technology will become readily apparent to those skilled in this art from the following drawings and the detailed description. The drawings and description are to be regarded as illustrative in nature, and not as restrictive.

BRIEF DESCRIPTION OF FIGURES

[0030] **Figure 1** is a diagram illustrating an exemplary single pass countercurrent diafiltration system.

[0031] **Figure 2** is a graph of the diavolumes (buffer flow rate / feed flow rate) versus tracer clearance for a system with one TFF cassette according to the embodiments.

[0032] **Figure 3** is a graph of the diafiltration efficiency of a 2-stage single pass countercurrent diafiltration system.

[0033] **Figure 4** is a graph of the natural log clearance of glucose versus diavolumes of diafiltration buffer for a 3 stage single pass counter current diafiltration system. The horizontal