and low viabilities often associated with highly productive perfusion processes further accelerate membrane fouling. Additionally, as membranes plug with cell debris they also begin to effectively function as ultrafiltration devices, retaining the high molecular weight product proteins within the bioreactor in a not easily predictable or reproducible manner. This is a disadvantage because in a continuous perfusion bioreactor system it is advantageous to have the product of interest be continuously removed from the bioreactor in the cell-free harvest and delivered to the downstream operation in a consistent manner. Other problems with cell retention devices known in the art include overheating, low separation efficiencies and long residence times in the device.

[0006] As a result, conventional continuous perfusion cell culture systems usually have working volumes below 2,000 L, and if operated under conditions where productivity is highest (e.g. high viable cell densities and high perfusion rates), require frequent change out of the membrane-based cell retention device due to plugging and product retention. Additionally, cell retention devices capable of handling very large volumes of cell-free culture harvest can be quite complex (e.g. many moving parts), expensive, damaging to cells through excessive shear forces, and prone to failure. As the cell retention devices are typically external to the bioreactor, effective cleaning and sterilization can also be challenging at the large scale. Moreover, batch and fed-batch remain the most common type of mammalian cell culture, regardless of scale.

[0007] It is known that a perfusion N-1 (seed) bioreactor using a membrane based cell retention device linked to an about 5-fold to about 20-fold larger continuous-flow stirred-tank reactor (CSTR, or chemostat) can enhance productivity (see, e.g., U.S. Patent Publication No. 2019/0031997 to Boehringer Ingelheim International GmbH).

[0008] There remains a need for an alternative cell culture method or system that overcomes the limitations associated with the current conventional perfusion culture systems, particularly for large-scale bioprocessing.

SUMMARY ~~OF THE INVENTION~~

[0009] Disclosed herein are bioprocessing systems and methods and more particularly, systems and methods for producing a biologic molecule of interest (e.g., an antibody) which

include at least one hydrocyclone serving as a cell retention device.

[0010] ~~In a first aspect, disclosed~~Disclosed herein is a system for continuous production of at least one biologic molecule of interest, which system comprises: (i) at least one culture bioreactor (for example, a N-1 bioreactor or a N-1 perfusion bioreactor); (ii) at least one hydrocyclone; and (iii) at least one production bioreactor (for example, a N bioreactor or a continuously stirred tank reactor (CSTR) production bioreactor); wherein the culture bioreactor and the production bioreactor are linked by the hydrocyclone that serves as a cell retention device for the culture bioreactor, wherein the hydrocyclone produces an underflow stream containing concentrated cell culture and a partially cell-free overflow stream, and wherein the underflow stream containing concentrated cell culture is returned to the culture bioreactor and the partially cell-free overflow stream is directed to the production bioreactor.

[0010a] In one aspect, disclosed herein is a system for continuous production of at least one biologic molecule of interest, the system comprising: (i) at least one culture bioreactor; (ii) at least one hydrocyclone; and (iii) at least one production bioreactor; wherein the culture bioreactor and the production bioreactor are linked by the hydrocyclone that serves as a cell retention device for the culture bioreactor, wherein the hydrocyclone produces an underflow stream containing concentrated mammalian cell culture and a partially cell-free overflow stream, and wherein the underflow stream containing concentrated mammalian cell culture is returned to the culture bioreactor and the partially cell-free overflow stream is directed to the production bioreactor.

[0011] In one ~~embodiment~~example, the system can operate in a highly productive steady state for more than six weeks, more than eight weeks, more than three months, more than six months or more than a year. A “highly productive” steady state is considered to be a calculated steady-state volumetric productivity of at least about 0.80 g/L/day, or about 0.84 g/L/day.

[0012] In a particular ~~embodiment~~example, the system can operate in a highly productive steady state indefinitely by restarting the culture bioreactor with cells of a lower generational number.

[0013] ~~In a second aspect, disclosed~~Disclosed herein is a system for continuous production of at least one biological molecule of interest, which system comprises: (i) at least one continuous (e.g., perfusion) culture bioreactor (for example, an N-1 bioreactor or N-1 perfusion bioreactor); (ii) at least one hydrocyclone; (iii) at least one production bioreactor (for example, an N bioreactor or a continuously stirred tank reactor (CSTR) production bioreactor); and (iv) at least one downstream component (e.g, capture or purification components); wherein the culture bioreactor and the production bioreactor are linked by the hydrocyclone that serves as a cell retention device for the culture bioreactor, wherein the hydrocyclone produces an underflow stream containing concentrated cell culture and a partially cell-free overflow stream, and wherein the underflow stream containing concentrated cell culture is returned to the culture bioreactor and the partially cell-free overflow stream is directed to the production bioreactor.

[0013a] In another aspect, disclosed herein is a system for continuous production of at least one biological molecule of interest, the system comprising: (i) at least one culture bioreactor; (ii) at least one hydrocyclone; (iii) at least one production bioreactor; and (iv) at least one downstream component; wherein the culture bioreactor and the production bioreactor are linked by the hydrocyclone that serves as a cell retention device for the culture bioreactor, wherein the hydrocyclone produces an underflow stream containing concentrated mammalian cell culture and a partially cell-free overflow stream, and wherein the underflow stream containing concentrated mammalian cell culture is returned to the culture bioreactor and the partially cell-free overflow stream is directed to the production bioreactor.

[0014] ~~In a third aspect, disclosed~~Disclosed herein is a method for continuously producing a biologic molecule of interest (e.g., an antibody), comprising (i) culturing a plurality of host cells capable of producing a biologic molecule of interest (e.g. an antibody) in at least one culture bioreactor (for example, an N-1 bioreactor or N-1 perfusion bioreactor); (ii) inoculating one or more production bioreactors (for example, an N bioreactor or a continuously stirred tank reactor (CSTR) production bioreactor) with cells obtained from step (i); and (iii) culturing the cells in the at least one production bioreactor under conditions that allow production of the biologic molecule of interest (e.g., an antibody), wherein the culture bioreactor and the production bioreactor are linked by a hydrocyclone which serves as a cell retention device for the culture bioreactor, which culture bioreactor receives the underflow from the hydrocyclone while the production bioreactor receives the overflow, thereby inoculating the production bioreactor.

[0014a] In another aspect, disclosed herein is a method for producing a biologic molecule of interest, the method comprising: (i) culturing a plurality of host cells capable of producing a biologic molecule of interest in at least one culture bioreactor; (ii) inoculating at least one production bioreactor with cells obtained from step (i); and (iii) culturing the cells in the production bioreactor; wherein the culture bioreactor and the production bioreactor are linked by at least one hydrocyclone that serves as a cell retention device for the at least one culture bioreactor; and wherein the host cells comprise mammalian cells.

[0014b] In another aspect, disclosed herein is a method for producing a biologic molecule of interest, the method comprising: (i) culturing a plurality of host cells in at least one culture bioreactor; (ii) inoculating at least one production bioreactor with cells obtained from step (i); and (iii) culturing the cells in the production bioreactor under conditions that allow production of the biologic molecule of interest; wherein the culture bioreactor and the production bioreactor are linked by at least one hydrocyclone that serves as a cell retention device for the culture bioreactor, wherein the host cells comprise mammalian cells, wherein the hydrocyclone produces an underflow stream containing concentrated mammalian cell culture and a partially cell-free overflow stream, and wherein the underflow stream containing concentrated mammalian cell culture is returned to the culture bioreactor and the partially cell-free overflow stream is directed to the production bioreactor.

[0015] In one ~~embodiment~~example, the method further comprises (v) harvesting a portion of the cultured host cells from the production bioreactor. In certain ~~embodiments~~examples, the step of harvesting a portion of the cultured host cells from the production bioreactor occurs continuously. In certain ~~embodiments~~examples, the step of harvesting a portion of the cultured host cells from the production bioreactor occurs periodically, for example one time per day, two times per day, three times per day, or one time per two days. Optionally, one or more additional steps can be conducted to further process the biologic molecule of interest. In one ~~embodiment~~example, the one or more steps are selected from clarifying, capturing, purifying, polishing, formulating, packaging steps or a combination thereof. These one or more additional steps may be continuous or non-continuous in nature, thereby providing a hybrid or continuous end-to-end process.

[0016] Compared with systems and/or methods which utilize a membrane-containing host cell retention device rather than a hydrocyclone to provide the link between the culture bioreactor and the production bioreactor, the systems and/or methods disclosed herein offer one or more improved properties, particularly when applied to large-scale systems. For example, in systems that exceed 1000 L in working volume of the production bioreactor, the systems and/or methods described herein, which utilize a hydrocyclone to provide the link between the culture bioreactor and the production bioreactor large, may provide certain improvements including, increased total separation efficiency, decreased media usage and/or increased production of at least one biologic

molecule of interest. In certain ~~embodiments~~examples, the improved properties are present at high viable cell densities (VCD) and or high perfusion rates (PF). In certain ~~embodiments~~examples where the systems are of very large scale, systems which utilize a membrane-containing host cell retention device rather than a hydrocyclone to provide the link between the culture bioreactor and the production bioreactor, may be hindered by insufficient membrane area, which would result in lower overall productivities when compared to a hydrocyclone linked bioreactor system.

[0017] 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

[0018] **Figure 1** is a diagram of the fluid dynamics in an exemplary hydrocyclone. Culture enters the hydrocyclone swirl chamber through two tangential inlets at the top of the device. A centrifugal field creates a primary vortex which pushes the cells towards the wall of the swirl chamber while the conical taper guides the cells downward until they exit in the underflow stream at the bottom of the device. Simultaneously, partially cell free material reverses direction and enters into a secondary vortex which travels upward and exits through the overflow stream at the top of the device.

[0019] **Figure 2** is a schematic illustrating an exemplary bioreactor configuration. In this configuration, the N-1 perfusion bioreactor using a hydrocyclone as the cell retention device continuously supplies the partially cell free overflow stream to a second continuous-flow stirred tank reactor (CSTR) operating as the N stage production bioreactor. The N-1 perfusion bioreactor was maintained at constant volume and was fed with perfusion medium. A 600 series Watson Marlow peristaltic pump supplies N-1 culture into the hydrocyclone at 3 L/min to reach an operating pressure of 2.4 bar. The underflow stream containing concentrated cell culture was recirculated back to the N-1 bioreactor while the partially cell-free overflow stream was added to the production CSTR with a second peristaltic pump (500 series Watson Marlow) at a flow rate of 370 mL/min. Concentrated nutrient feeds were also added directly to the production CSTR. The production CSTR did not use a cell retention device and whole cell harvest was removed from the bioreactor to maintain the production CSTR at constant volume.