Cell retention systems are difficult to operate and design for use at the large scale (>1,000 L). For example, nearly any cell retention device that uses a membrane (as do many that are currently in use at large scale) will eventually plug with cell debris. This plugging is more likely to occur with low cell viability cultures as the cells are more fragile, and the particulates that are formed are often similar in size to the pores of the membrane (0.2-5 micron). 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.

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 (ultrafiltration). Additionally, cell retention devices capable of handling very large volumes of cell-free culture harvest can be quite complex (e.g. many moving parts) and 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. These factors can result in high cost of operation, significant loss of productivity and inefficiencies in production of a protein of interest. Therefore, there still remains a need for an alternative cell culture method or system that overcomes the limitations associated with the current conventional perfusion culture systems.

SUMMARY OF THE SUBJECT TECHNOLOGY

The subject technology is illustrated, for example, according to various aspects and embodiments listed below.

The subject technology relates to a method of producing a protein of interest, including: (a) culturing cells comprising a gene that encodes the protein of interest in a culture bioreactor (N-1 bioreactor); (b) inoculating a production bioreactor (N bioreactor) with cells

obtained from step (a); and (c) culturing the cells in the production bioreactor under conditions that allow production of the protein of interest.

In one aspect, the subject technology relates to a method of producing a protein of interest, comprising: (a) culturing cells comprising a gene that encodes the protein of interest in a culture bioreactor (N-1 bioreactor); (b) inoculating a production bioreactor (N bioreactor) with cells obtained from step (a); and (c) culturing the cells in the production bioreactor under conditions that allow production of the protein of interest, wherein the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor, wherein the cell transfer is by cell bleed in semi-continuous mode comprising the cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween.

In one or more examples relating directly or indirectly to the above, the method further comprises step (d) harvesting the protein of interest from the production bioreactor; the culture bioreactor is a continuous perfusion culture bioreactor and the production bioreactor is a continuously stirred tank reactor (CSTR) production bioreactor; the production bioreactor has no cell retention device; volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:20; volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:5; volume ratio of the culture bioreactor to the production bioreactor is about 1:5; the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor; the cell transfer is by cell bleed in continuous or semi-continuous modes; the cell transfer is in semi-continuous mode including the cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween; step (a) optionally alternates between a first and second culture bioreactors to allow for renewal and continuous production of culture cells for use in step (b); the second culture bioreactor is a continuous perfusion culture bioreactor; the production bioreactor operates continuously for a period of greater than 3 weeks; the production bioreactor operates continuously for a period of greater than 4 weeks; the production bioreactor operates continuously for a period of greater than 5 weeks; the production bioreactor operates continuously for a period of greater than 6 weeks; harvesting step (d) is continuous; the cells are CHO cells, HEK-293 cells, VERO cells, NSO cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells or COS cells; the production bioreactor has a volumetric productivity of at least 0.6 grams per liter per day for a

period of at least 14 days; the production bioreactor has a volumetric productivity of at least 0.6 grams per liter per day for a period of at least 20 days; the production bioreactor has a volumetric productivity of at least 0.6 grams per liter per day for a period of at least 30 days; the production bioreactor has a product residence time of about 1 to about 10 days; the production bioreactor has a dilution rate of about 1 to about 0.1 volume per day; the production bioreactor is fed with a diluent solution; the diluent solution is water or saline.

The subject technology also relates to a linked culture process, including: (a) culturing cells comprising a gene that encodes the protein of interest in a culture bioreactor (N-1 bioreactor); (b) inoculating a production bioreactor (N bioreactor) with cells obtained from step (a); and (c) culturing the cells in the production bioreactor under conditions that allow production of the protein of interest.

In another aspect, the subject technology relates to a linked culture process, comprising: (a) culturing cells comprising a gene that encodes the protein of interest in a culture bioreactor (N-1 bioreactor); (b) inoculating a production bioreactor (N bioreactor) with cells obtained from step (a); and (c) culturing the cells in the production bioreactor under conditions that allow production of the protein of interest, wherein the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor, wherein the cell transfer is by cell bleed in semi-continuous mode comprising the cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween.

In one or more examples relating directly or indirectly to the above, the linked culture process further includes step (d) harvesting the protein of interest from the production bioreactor; the culture bioreactor is a continuous perfusion culture bioreactor and the continuous production bioreactor is a continuously stirred tank reactor (CSTR) production bioreactor; the production bioreactor has no cell retention device; volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:20; volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:5; volume ratio of the culture bioreactor to the production bioreactor is about 1:5; the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor; the cell transfer is by cell bleed in continuous or semi-continuous modes; the cell transfer is in semi-continuous mode comprising the cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween; step (a) optionally alternates between a first and second culture bioreactors to allow for renewal and continuous production of culture cells for use in step (b); the second culture bioreactor is a continuous perfusion culture bioreactor; the production bioreactor operates continuously for a period of greater than 3 weeks; the production bioreactor operates continuously for a period of greater than 4 weeks; the production bioreactor operates continuously for a period of greater than 5 weeks; the production bioreactor operates

continuously for a period of greater than 6 weeks; harvesting step (d) is continuous; the cells are CHO cells, HEK-293 cells, VERO cells, NSO cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells or COS cells; the production bioreactor has a volumetric productivity of at least 0.6 grams per liter per day for a period of at least 14 days; the production bioreactor has a volumetric productivity of at least 0.6 grams per liter per day for a period of at least 20 days; the production bioreactor has a volumetric productivity of at least 0.6 grams per liter per day for a period of at least 30 days; the production bioreactor has a product residence time of about 1 to about 10 days; the production bioreactor has a dilution rate of about 1 to about 0.1 volume per day; the production bioreactor is fed with a diluent solution; the diluent solution is water or saline.

The subject technology also relates to a linked culture system, including: a culture bioreactor (N-1 bioreactor) for culturing cells comprising a gene that encodes the protein of interest; and a production bioreactor (N bioreactor) which is linked to the culture bioreactor and receives cells as inoculum from the culture bioreactor, wherein the production bioreactor operates under conditions that allow production of the protein of interest.

In another aspect, the subject technology relates to linked culture system, comprising: a culture bioreactor (N-1 bioreactor) for culturing cells comprising a gene that encodes the protein of interest; and a production bioreactor (N bioreactor) which is linked to the culture bioreactor and receives cells as inoculum from the culture bioreactor, wherein the production bioreactor operates under conditions that allow production of the protein of interest, wherein the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor, wherein the cell transfer is by cell bleed in semi-continuous mode comprising the cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween.

In one or more examples relating directly or indirectly to the above, the system is linked to a downstream purification system for harvesting the protein of interest from the production bioreactor; the culture bioreactor is a continuous perfusion culture bioreactor and the production bioreactor is a continuously stirred tank reactor (CSTR) production bioreactor; the production bioreactor has no cell retention device; volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:20; volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:5; volume ratio of the culture bioreactor to the production bioreactor is about 1:5; the inoculation is by transferring cells from the culture bioreactor to the production bioreactor; the cell transfer is by cell bleed in continuous or semi-continuous modes; the cell transfer is in semi-continuous mode comprising the cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween; the system includes a second culture bioreactor that operates in parallel with a first culture bioreactor and alternates in producing inoculum for transfer to the production bioreactor; the second culture bioreactor is a continuous perfusion culture bioreactor; the production bioreactor operates continuously for a period of greater than 3 weeks; the production bioreactor operates continuously for a period of greater than 4 weeks; the production bioreactor operates continuously for a period of greater than 5 weeks; the production bioreactor operates

continuously for a period of greater than 6 weeks; the harvesting is continuous; the cells are CHO cells, HEK-293 cells, VERO cells, NSO cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells or COS cells; the production bioreactor has a volumetric productivity of at least 0.6 grams per liter per day for a period of at least 14 days; the production bioreactor has a volumetric productivity of at least 0.6 grams per liter per day for a period of at least 20 days; the production bioreactor has a volumetric productivity of at least 0.6 grams per liter per day for a period of at least 30 days; the production bioreactor has a product residence time of about 1 to about 10 days; the production bioreactor has a dilution rate of about 1 to about 0.1 volume per day; the production bioreactor is fed with a diluent solution; the diluent solution is water or saline.

The subject technology also relates to a protein of interest produced by a method including: (a) culturing cells comprising a gene that encodes the protein of interest in a culture bioreactor (N-1 bioreactor); (b) inoculating a production bioreactor (N bioreactor) with cells obtained from step (a); and (c) culturing the cells in the production bioreactor under conditions that allow production of the protein of interest. In one or more examples relating directly or indirectly to the above, the method further comprises step (d) harvesting the protein of interest from the production bioreactor; the culture bioreactor is a continuous perfusion culture bioreactor and the continuous production bioreactor is a continuously stirred tank reactor (CSTR) production bioreactor; the production bioreactor has no cell retention device; volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:20; volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:5; volume ratio of the culture bioreactor to the production bioreactor is about 1:5; the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor; the cell transfer is by cell bleed in continuous or semi-continuous modes; step (a) optionally alternates between a first and second culture bioreactors to allow for renewal and continuous production of culture cells for use in step (b); the second culture bioreactor is a continuous perfusion culture bioreactor; the production bioreactor operates continuously for a period of greater than 3 weeks; the production bioreactor operates continuously for a period of greater than 4 weeks; the production bioreactor operates continuously for a period of greater than 5 weeks; the production bioreactor operates continuously for a period of greater than 6 weeks; harvesting step (d) is continuous; the protein of interest is an antibody or a fusion protein; the cells are CHO cells, HEK-293 cells, VERO cells, NSO cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells or COS cells; the production bioreactor is fed with a diluent solution; the diluent solution is water or saline.

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 THE DRAWINGS

CLAIMS

What is claimed is:

1. A method of producing a protein of interest, comprising:
   (a) culturing cells comprising a gene that encodes the protein of interest in a culture bioreactor (N-1 bioreactor);
   (b) inoculating a production bioreactor (N bioreactor) with cells obtained from step (a); and
   (c) culturing the cells in the production bioreactor under conditions that allow production of the protein of interest, wherein the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor, wherein the cell transfer is by cell bleed in semi-continuous mode comprising the cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween.
2. The method according to claim 1, wherein the method further comprises step (d) harvesting the protein of interest from the production bioreactor.
3. The method according to any of the preceding claims, wherein the culture bioreactor is a continuous perfusion culture bioreactor and the production bioreactor is a continuously stirred tank reactor (CSTR) production bioreactor and/or
   the production bioreactor has no cell retention device.
4. The method according to any of the preceding claims, wherein volume ratio of the culture bioreactor to the production bioreactor is 1:1 to 1:20 or 1:1 to 1:5 or 1:5.
5. The method according to any of the preceding claims, wherein step (a) optionally alternates between a first and second culture bioreactors to allow for renewal and continuous production of culture cells for use in step (b).

6. The method according to claim 5, wherein the second culture bioreactor is a continuous perfusion culture bioreactor.
7. The method according to any of the preceding claims, wherein the production bioreactor operates continuously for a period of greater than 3 weeks or for a period of greater than 4 weeks or for a period of greater than 5 weeks or for a period of greater than 6 weeks.
8. The method according to any of claims 2 to 7, wherein harvesting step (d) is continuous.
9. The method according to any of the preceding claims, wherein the cells are CHO cells, HEK-293 cells, VERO cells, NSO cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells or COS cells.
10. The method according to any of the preceding claims, wherein the production bioreactor has a product residence time of 1 to 10 days.
11. The method according to any of the preceding claims, wherein the production bioreactor has a dilution rate of 1 to 0.1 volume per day.
12. A linked culture process, comprising:

    (a) culturing cells comprising a gene that encodes the protein of interest in a culture bioreactor (N-1 bioreactor);

    (b) inoculating a production bioreactor (N bioreactor) with cells obtained from step (a); and

    (c) culturing the cells in the production bioreactor under conditions that allow production of the protein of interest, wherein the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor, wherein the cell transfer is by cell bleed in semi-continuous mode comprising the cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween.

13. A linked culture system, comprising:

    a culture bioreactor (N-1 bioreactor) for culturing cells comprising a gene that encodes the protein of interest; and

    a production bioreactor (N bioreactor) which is linked to the culture bioreactor and receives cells as inoculum from the culture bioreactor, wherein the production bioreactor operates under conditions that allow production of the protein of interest, wherein the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor, wherein the cell transfer is by cell bleed in semi-continuous mode comprising the cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween.

14. The linked culture system according to claim 13, wherein the system is linked to a downstream purification system for harvesting the protein of interest from the production bioreactor.