[0005] For the foregoing reasons, a need persists in the art for biomolecule (*e.g.*, biologic) purification processes involving a harvest recovery step that efficiently and cost-effectively results in high yield separations of biologics from other cell culture components arising from turbid perfusion cultures with packed cell volumes (PCV) of at least 16% at the time of harvest.

Summary

[0005a] In one aspect, the disclosure provides a method for separating a recombinant protein produced in a perfusion culture from at least one other perfusion culture component comprising: (a) harvesting a pool or eluate stream from a perfusion culture comprising a recombinant protein and at least one other perfusion culture component, the perfusion culture having a packed cell volume (PCV) of at least about 16% at harvest; (b) introducing the harvest pool or eluate stream into at least one continuous solids discharge disc stack centrifuge; (b) operating the continuous solids discharge disc stack centrifuge, thereby separating fluid components into a centrate and a high-density composition; (c) collecting the centrate; (d) adding a flocculant to the centrate at a temperature of 8°C to12°C; and (e) subjecting the centrate to a filtration step.

[0005b] In another aspect, the disclosure provides a method for producing an isolated, purified recombinant protein from a perfusion culture initiated in a single use bioreactor comprising the steps of (a) inoculating a bioreactor with cells engineered to recombinantly express a protein of interest; (b.) culturing the cells until a perfusion culture has a packed cell volume of at least 16%; (c.) reducing the temperature of the culture to between 8°C and 12°C and collecting the perfusion culture from the bioreactor as a harvest pool or eluate stream; (d.) introducing the harvest pool or eluate stream into at least one continuous solids discharge disc stack centrifuge, wherein the harvest pool or eluate stream is between 4°C and 12°C; (e.) collecting a centrate from the centrifuge; (f.) adding a flocculant to the centrate at a temperature of 8°C and 12°C; (g.) passing the flocculated centrate through a depth filter at a at a flux rate of 90 to 150 LMH and at a pressure of 10 psi or less, wherein the flocculated centrate is between 4°C and 12°C; (h.) subjecting the filtered centrate to one or more chromatography, filtration, and or UFDF unit operations; and; (i.) obtaining an isolated, purified, recombinant protein.

[0006] The disclosure provides a method for separating a recombinant protein produced in a perfusion culture from at least one other perfusion culture component comprising: (a) harvesting a pool or eluate stream from a perfusion culture comprising the recombinant protein and at least one other perfusion culture component, the perfusion culture having a packed cell volume (PCV) of at least about 16% at harvest; (b) introducing the harvest pool or eluate stream into at least one continuous solids discharge disc stack centrifuge; (b) operating the continuous solids discharge disc stack centrifuge, thereby separating fluid components into a centrate and a high-

density composition; (c) collecting the centrate; (d) adding a flocculant to the centrate at a temperature of 8°C to12°C; and (e) subjecting the centrate to a filtration step.

[0007] In some examples, the perfusion culture of any of the disclosed methods is harvested from single-use bioreactor, such as a single-use 500L bioreactor or a single-use bioreactor that is 2,000L or more.

[0008] In some examples, the prefusion culture harvest pool or eluate stream of any of the disclosed methods had a turbidity of at least 180 NTU at harvest. In some examples, the prefusion culture harvest pool or eluate stream of any of the disclosed methods had a viable cell density of at least 2 x 10^7 viable cells/ml at harvest or a viable cell density of at least 3 x 10^7 viable cells/ml at harvest or a viable cell density of at least 5x10^7 viable cells/ml at harvest.

[0009] In some examples, the prefusion culture of any of the disclosed methods had a PCV of at least about 20% at harvest, or at least about 24% at harvest, or at least about 26% at harvest, or at least about 30% at harvest.

[0010] In some examples, the temperature of the perfusion culture at harvest in any of the disclosed methods is about 8°C to about 12°C. For example, the temperature of the perfusion culture at harvest in any of the disclosed methods is about 10°C.

[0011] In some examples, a continuous harvest eluate stream from the perfusion culture of any of the disclosed methods is introduced into the continuous solids discharge disc stack centrifuge.

[0012] In other examples, a discontinuous batch of perfusion harvest pool of any of the disclosed methods is introduced into the continuous solids discharge disc stack centrifuge.

[0013] In some examples, the turbidity of the of the centrate of any of the disclosed methods is greater than about 160 NTU, such as a turbidity greater than about 180 NTU, or greater than about 200 NTU, or greater than about 250 NTU, or greater than about 300 NTU, or greater than about 500 NTU, or greater than about 600 NTU, or greater than about 750 NTU.

[0014] In some examples, the flocculant is added to the centrate of any of the disclosed methods at a temperature of about 10°C. In an exemplary example, the flocculant is poly(diallyldimethylammonium chloride) (pDADMAC) or its monomer diallyl dimethyl ammonium chloride (DADMAC), and pDADMAC or DADMAC is added to at least 0.04% (w/w), or is added to 0.04-0.15% (w/w), or is added to 0.05% (w/w).

[0015] In any of the disclosed methods, the recombinant protein is a eukaryotic protein, such as a mammalian protein. In some examples, the mammalian protein is an antigen-binding protein.

[0016] In any of the disclosed methods, the recombinant protein is a mammalian antigen-binding protein and the antigen-binding protein is a monoclonal antibody, a bispecific antibody, a multispecific antibody, a bispecific T-cell engager molecule (BiTE®).

[0017] In other examples, the protein of any of the disclosed methods is a granulocyte colony-stimulating factor, an erythropoiesis stimulating agent, a HER receptor, a cell adhesion molecule, a growth factor, an osteoinductive factor, insulin, a coagulation protein, a colony stimulating factor, a blood group antigen; a growth hormone, a growth hormone receptor, a T-cell receptor; a neurotrophic factor, a neurotrophin, a relaxin, an interferon, an interleukin, a viral antigen, a lipoprotein, an integrin, a rheumatoid factor, an immunotoxin, a surface-membrane protein, a transport protein, a homing receptor, an addressin, a regulatory protein, or an immunoadhesin.

[0018] In some examples, the filtration step in any of the disclosed methods includes a depth filtration. Exemplary depth filters that may be used in any of the disclosed methods include MILLISTAK+® C0HC filter, a MILLISTAK+® C0SP filter, a SARTOCLEAR® DL60 filter, or a SARTOCLEAR® DL75 filter. In one example, the depth filter is MILLISTAK+® C0HC filter or a MILLISTAK+® C0SP filter and wherein the centrate passes through the depth filter at a flux rate of 150 LMH or less and at a pressure of 10 psi or less. In some examples, the pressure is 2 psi or less. In some examples, the flux rate is 90 to 150 LMH.

[0019] The disclosure also provides for any of the disclosed methods which further comprise at least one additional chromatography step. Exemplary additional chromatography steps

include ion exchange chromatography, hydrophobic interaction chromatography, or multimodal chromatography.

[0020] In addition, the disclosure provides for any of the disclosed methods which further comprise at least one or more viral filtration step, viral inaction step, and/or UFDF step.

[0021] The disclosure provides for a target protein made by any of the methods disclosed herein.

[0022] In another example, the disclosure provided provide a method for maintaining low differential pressure during depth filtration of a load feed derived from a perfusion culture, the method comprising the steps: obtaining a flocculated centrate derived from a perfusion culture having packed cell volume (PCV) of at least 16% at the time of harvest; passing the centrate through a depth filter at a flux rate of 150 LMH or less and a filter pressure differential pressure of 10psi or less; and recovering the eluate.

[0023] The disclosure also provides for methods of producing an isolated, purified recombinant protein from a perfusion culture comprising the steps of (a) initiating a perfusion culture in a single use bioreactor; (b.) inoculating the bioreactor with cells engineered to recombinantly express a protein of interest; (c.) culturing the cells until the perfusion culture has a packed cell volume of at least 16%; (d.) reducing the temperature of the culture to between 8°C and 12°C and collecting the perfusion culture from the bioreactor as a harvest pool or eluate stream; (e.) introducing the harvest pool or eluate stream into at least one continuous solids discharge disc stack centrifuge, wherein the harvest pool or eluate stream is between 4°C and 12°C; (f.) collecting a centrate from the centrifuge; (g.) adding a flocculant to the centrate at a temperature of 8°C and 12°C; (h.) passing the flocculated centrate through a depth filter at a at a flux rate of 90 to 150 LMH and at a pressure of 10 psi or less, wherein the flocculated centrate is between 4°C and 12°C; (i.) subjecting the filtered centrate to one or more chromatography, filtration, and or UFDF unit operations; and (j.) obtaining an isolated, purified, recombinant protein.

[0024] In another example, the disclosure provides for pharmaceutical compositions comprising the isolated, purified, recombinant protein produced with any of the methods disclosed herein.

Brief Description of the Drawings

[0025] **Figure 1. Effect of flocculation on small-scale normalized depth filter loading level.** Figure 1 shows normalized small-scale depth filtration performance using untreated and flocculated material from large-scale single use continuous solids discharge disc stack

Claims

What is claimed is:

1. A method for separating a recombinant protein produced in a perfusion culture from at least one other perfusion culture component comprising:

(a) harvesting a pool or eluate stream from a perfusion culture comprising a recombinant protein and at least one other perfusion culture component, the perfusion culture having a packed cell volume (PCV) of at least about 16% at harvest;

(b) introducing the harvest pool or eluate stream into at least one continuous solids discharge disc stack centrifuge;

(c) operating the continuous solids discharge disc stack centrifuge, thereby separating fluid components into a centrate and a high-density composition;

(d) collecting the centrate;

(e) adding a flocculant to the centrate; and

(f) subjecting the centrate to a filtration step.

2. The method of claim 1, wherein the continuous solids discharge disc stack centrifuge is a single-use continuous solids discharge disc stack centrifuge.

3. The method of any one of claims 1-2, wherein the perfusion culture is harvested from single-use bioreactor.

4. The method of claim 3, wherein the single-use bioreactor is at least 500L.

5. The method of any one of claims 1-4, wherein the prefusion culture harvest pool or eluate stream had a turbidity of at least 180 NTU at harvest.

6. The method of any one of claims 1-5, wherein the perfusion culture harvest pool or eluate stream had a viable cell density of at least 2×10^7 viable cells/ml at harvest.

7. The method of claim 6, wherein the perfusion culture harvest pool or eluate stream had a viable cell density of at least 3×10^7 viable cells/ml at harvest.

8. The method of claim 6 wherein the perfusion culture harvest pool or eluate stream had a viable cell density of at least $5x10^7$ viable cells/ml at harvest.

9. The method of any one of claims 1-8, wherein the perfusion culture harvest pool or eluate stream had a packed cell volume of at least about 18% at harvest.

10. The method of claim 9, wherein the perfusion culture had a packed cell volume of at least about 20% at harvest.

11. The method of claim 9, wherein the perfusion culture had a packed cell volume of at least about 24% at harvest.

12. The method of claim 9, wherein the perfusion culture had a packed cell volume of at least about 26% at harvest.

13. The method of any one of claims 1-12, wherein a continuous harvest eluate stream from the perfusion culture is introduced into the continuous solids discharge disc stack centrifuge.

14. The method of any one of claims 1-13, wherein a discontinuous batch of perfusion harvest pool is introduced into the continuous solids discharge disc stack centrifuge.

15. The method of any one of claims 1-14, wherein the turbidity of the of the centrate is greater than about 160 NTU.

16. The method of any one of claims 1-15, wherein the flocculant is poly(diallyldimethylammonium chloride) (pDADMAC).

17. The method of claim 16, wherein poly(diallyldimethylammonium chloride) (pDADMAC) as flocculant is added to at least 0.04% (w/w).

18. The method of claim 16, wherein poly(diallyldimethylammonium chloride) (pDADMAC) as flocculant is added to 0.04-0.15% (w/w).

19. The method of any one of claims 1-18, wherein the recombinant protein is a eukaryotic protein.

20. The method of claim 19, wherein the eukaryotic protein is a mammalian protein.

21. The method of claim 20, wherein the mammalian protein is an antigen-binding protein.

22. The method of claim 21, wherein the antigen-binding protein is a monoclonal antibody, a bispecific antibody, a multispecific antibody, a bispecific T-cell engager molecule (BiTE®).

23. The method of any one of claims 1-22, wherein the filtration includes a depth filter.

24. The method of claim 23, wherein the centrate passes through the depth filter at a flux rate of 150 LMH or less and at a pressure of 10 psi or less.

25. The method of claim 24, wherein the pressure is 2 psi or less.

26. The method of claim 24, wherein the flux rate is 90 to 150 LMH.

27. A method for maintaining low differential pressure during depth filtration of a load feed derived from a perfusion culture, the method comprising the steps:

(a) obtaining a flocculated centrate derived from a perfusion culture having packed cell volume (PCV) of at least 16% at the time of harvest;

(b) passing the centrate through a depth filter at a flux rate of 150 LMH or less and a pressure of 10psi or less; and

(c) recovering the eluate.

28. A method for producing an isolated, purified recombinant protein from a perfusion culture initiated in a single use bioreactor comprising the steps of

a. inoculating a bioreactor with cells engineered to recombinantly express a protein of interest;

b. culturing the cells until a perfusion culture has a packed cell volume of at least 16%;

c. reducing the temperature of the culture to between 8°C and 12°C and collecting the perfusion culture from the bioreactor as a harvest pool or eluate stream;

d. introducing the harvest pool or eluate stream into at least one continuous solids discharge disc stack centrifuge, wherein the harvest pool or eluate stream is between 4°C and 12°C;

e. collecting a centrate from the centrifuge;

f. adding a flocculant to the centrate at a temperature of 8°C and 12°C;

g. passing the flocculated centrate through a depth filter at a at a flux rate of 90 to 150 LMH and at a pressure of 10 psi or less, wherein the flocculated centrate is between 4°C and 12°C;

h. subjecting the filtered centrate to one or more chromatography, filtration, and or UFDF unit operations; and

i. obtaining an isolated, purified, recombinant protein.