typically has reached its greatest output. While this may result in a single high titer harvest, it is at the expense of a non-productive turnaround time to initiate the next run and the lag time to once again achieve peak production. In a continuous harvest systems, where product containing permeate is collected from the cell culture on a continuous basis throughout the production phase, the cell culture duration is extended, but at the expense of lower product titers and higher volumes of waste cell culture fluid to be dealt with during the harvest and purification stages.

Cell culture and harvest process development is ultimately is an exercise in process optimization, trading variables such as processing speed for product titer and product quality. The challenges include, for example, maintaining cell viability, achieving a workable product titer, and balancing the output from the upstream process with what the harvest and downstream processes can handle.

New process methods that provide even incremental improvements in recombinant protein production and recovery are valuable, given the expense of large scale cell culture processes and the growing demand for greater quantities of and lower costs for biological products. Improvements to cell culture processes that can lead to greater product recovery, thereby reducing the costs associated with manufacturing protein therapeutics are needed. The invention fulfills these needs by providing such methods and materials for extending cell culture duration while increasing protein recovery.

SUMMARY

In one aspect, the present invention refers to a method for an extended periodic harvest comprising establishing a cell culture by inoculating a bioreactor with mammalian cells expressing a recombinant protein, maintaining the cell culture by perfusing fresh cell culture medium into the bioreactor, passing the cell culture through a filter and collecting a permeate, wherein a null permeate is collected using a hollow fiber filter having a pore size or molecular weight cut off that retains the recombinant protein in the bioreactor wherein when the null permeate is

collected the host cells and the expressed recombinant proteins remain in the retentate in the bioreactor and the permeate is substantially free of or has significantly less of either, wherein a harvest permeate is collected using a hollow fiber filter having a pore size or molecular weight cut off that does not retain the recombinant protein in the bioreactor wherein when the harvest permeate is collected the host cells are retained in the bioreactor and the expressed recombinant proteins pass into the permeate, the fresh cell culture medium is formulated with or supplemented to achieve at least 5g/L of a non-ionic block copolymer; wherein the null permeate is initially collected until a first predetermined parameter is reached, at which time the harvest permeate is collected for a predetermined time, the harvest permeate comprising the recombinant protein, this is followed by alternately collecting the null permeate until a second predetermined parameter is reached, then collecting the harvest permeate for a predetermined time, wherein the alternating collection of null permeate and harvest permeate continues until the cell culture is terminated. Also described herein is a method for an extended periodic harvest comprising establishing a cell culture by inoculating a bioreactor with mammalian cells expressing a recombinant protein, maintaining the cell culture by perfusing fresh cell culture medium into the bioreactor, passing the cell culture through a filter and collecting a permeate, wherein a null permeate is initially collected until a first predetermined parameter is reached, at which time a harvest permeate is collected for a predetermined time, this is followed by alternately collecting a null permeate until a second predetermined parameter is reached, then collecting a harvest permeate for a predetermined time, wherein the alternating collection of null permeate and harvest permeate continues until the cell culture is terminated.

In one example the predetermined parameter is selected from time, viable cell density, packed cell volume or titer.

In one example the first predetermined parameter is at least 12 hours to 25 days following the establishment of the cell culture. In one example the first predetermined parameter is at least 24 to 72 hours following the establishment of the cell culture. In one example the first predetermined parameter is at least 4 days following the

establishment of the cell culture. In one example the first predetermined parameter is at least 5 or more days following the establishment of the cell culture. In one example the first predetermined parameter is at least 25 days following the establishment of the cell culture. In one example the first predetermined parameter is at least 5 to 25 days following the establishment of the cell culture. In one example the first predetermined parameter is at least 10 to 12 days following the establishment of the cell culture.

In one example the second predetermined parameter is at least 12 to 72 hours following the collection of the harvest permeate. In one example the second predetermined parameter is at least 24 to 72 hours following the collection of the harvest permeate. In one example the second predetermined parameter is at least 24 to 48 hours following the collection of the harvest permeate.

In one example in the predetermined time is at least 12 to 72 hours. In one example the predetermined time is at least 24 to 72 hours. In one example the predetermined time is at least 24 to 48 hours.

In one example the null permeate is initially collected for at least 24 hours to 25 days, at which time a harvest permeate is collected for 12 to 72 hours, followed by alternately collecting a null permeate for at least 24 hours to 25 days, then collecting a harvest permeate for 12 to 72 hours.

In one example when the null permeate is collected, the filter is a hollow fiber filter having a pore size or molecular weight cut off (MWCO) that retains the recombinant protein in the bioreactor. In a related example the molecular weight cutoff is 300 kDa or less. In a related example the hollow fiber filter is an ultrafilter.

In one example when the harvest permeate is collected, the filter is a hollow fiber filter having a pore size or molecular weight cut off (MWCO) that does not retain the recombinant protein in the bioreactor. In a related example the molecular weight cutoff is at least 500 kDa. In a related example the hollow fiber filter is a microfilter.

In one example the filter is a single unit filter system. In a related example rein the single unit filter system comprises at least one hollow fiber filter component having a pore size or molecular weight cut off (MWCO) that retains the recombinant protein in the bioreactor and at least one hollow fiber filter component having a pore size or molecular weight cut off (MWCO) that does not retain the recombinant protein in the bioreactor. In a related example the molecular weight cutoff of at least one hollow fiber filter component that retains the recombinant protein in the bioreactor is 300 kDa or less. In a related example the molecular weight cutoff of at least one hollow fiber filter component that does not retain the recombinant protein in the bioreactor is at least 500 kDa. In a related example at least one hollow fiber filter component that retains the recombinant protein in the bioreactor is an ultrafilter and at least one hollow fiber filter component that does not retain the recombinant protein in the bioreactor is a microfilter. In a related example the single unit filter system is contained within a housing. In a related example the single unit filter system further comprises a spacer between at least two of the hollow fiber filter components.

In one example when the null permeate is collected it is drawn from at least one hollow fiber filter component having a pore size or molecular weight cut off (MWCO) that retains the recombinant protein in the bioreactor.

In one example when the harvest permeate is collected, it is drawn from at least one hollow fiber filter component having a pore size or molecular weight cut off (MWCO) that does not retain the recombinant protein in the bioreactor.

In one example when the permeate is collected from a filter that is a hollow fiber filter having a pore size or molecular weight cut off that does not retain the recombinant protein in the bioreactor, the fresh cell culture medium is formulated with or supplemented to achieve at least 5g/L of a non-ionic block copolymer. In a related example the non-ionic block copolymer is a polyoxypropylene-polyoxyethylene block copolymer. In a related example the non-ionic block copolymer is poloxamer 188.

In one example the above method further comprising taking samples during the cell culture processes, evaluating the samples to quantitatively and/or qualitatively monitor

characteristics of the recombinant protein and/or the cell culture process. In a related example the samples are quantitatively and/or qualitatively monitored using process analytical techniques.

In one example the perfusing is continuous perfusion. In one example the rate of perfusion is constant. In one example the perfusing is performed at a rate of less than or equal to 1.0 working volume per day. In one example the perfusing is accomplished by a peristaltic pump, a double diaphragm pump, a low shear pump or alternating tangential flow. In a related example the perfusing is accomplished by alternating tangential flow.

In one example the method above further comprises subjecting the cell culture to a temperature shift wherein the cells are cultured a) at first temperature for a first period of time and b) at second temperature for a second period of time. In a related example the temperature shift occurs at the transition between the growth phase and production phase. In a related example the temperature shift occurs during the production phase. In a related example the temperature shift is in response to a predetermined parameter. In a related example the temperature shift is in response to a predetermined parameter wherein achieving

the predetermined parameter is determined using a capacitance based biomass probe.

In one example the cell culture is established by inoculating the bioreactor with at least 0.1 x 10^6 viable cells/mL. In a related example the inoculum was grown by means of a perfusion process using alternating tangential flow filtration.

In one example prior to entering the bioreactor, the cell culture medium is treated using nanofiltration, high temperature short time (HTST), or UV in combination with filtration.

In one example the bioreactor is a production bioreactor. In a related example the bioreactor has a capacity of at least 500L. In a related example the bioreactor has a capacity of at least 500L to 2000L. In a related example the bioreactor has a capacity of at least 1000L to 2000L.

In one example the cell culture medium is a serum-free cell culture medium. In one example the cell culture medium is a serum-free chemically defined cell culture medium. In one example the cell culture medium is a perfusion cell culture medium.

In one example the mammalian cells are Chinese Hamster Ovary (CHO) cells. In one example the recombinant protein is selected from the group consisting of a human antibody, a humanized antibody, a chimeric antibody, a recombinant fusion protein, or a cytokine.

In one example the recombinant protein is purified from the harvest permeate by one or more of flocculation, precipitation, centrifugation, depth filtration, affinity chromatography, size exclusion chromatography, ion exchange chromatography, mixed mode anion exchange chromatography, hydrophobic interaction chromatography or hydroxyapatite chromatography In one example the method above further comprises taking samples during the

purification process, evaluating the samples to quantitatively and/or qualitatively monitor characteristics of the recombinant protein and the purification process.

In one example the recombinant protein is formulated into a pharmaceutically acceptable formulation. In one example is provided a recombinant protein produced by the above method.

Also described herein is a method for harvesting a recombinant protein comprising establishing a cell culture by inoculating a bioreactor with mammalian cells expressing a recombinant protein, maintaining the cell culture by perfusing the cell culture with fresh cell culture medium formulated or supplemented to achieve a concentration of at least 5 g/L of a non-ionic block copolymer and passing the cell culture through a hollow fiber filter having a pore size or molecular weight cut off (MWCO) that does not retain the recombinant protein in the bioreactor and collecting a permeate containing the recombinant protein.

In one example the molecular weight cutoff is at least 500 kDa. In one example the hollow fiber filter is a microfilter.

The disclosure also provides a method for harvesting a recombinant protein comprising establishing a cell culture by inoculating a bioreactor with mammalian cells expressing a recombinant protein, maintaining the cell culture by perfusing the cell culture with fresh cell culture media formulated or supplemented to achieve a concentration of at least 1 g/L of a non-ionic block copolymer and passing the cell culture through a hollow fiber filter having a pore size or molecular weight cut off (MWCO) that retains the recombinant protein in the bioreactor, and collecting a permeate; once a predetermined parameter is reached perfusing the cell culture with fresh cell culture medium formulated or supplemented to achieve a concentration of at least 5 g/L of a non-ionic block copolymer and passing the cell culture through a hollow fiber filter having a pore size or molecular weight cut off (MWCO) that does not retain the recombinant protein in the bioreactor and collecting a permeate containing the recombinant protein.

In one example the molecular weight cutoff of the hollow fiber filter having a pore size or molecular weight cut off that retains the recombinant protein in the bioreactor is 300 kDa or less. In one example the hollow fiber filter having a pore size or molecular weight cut off that retains the recombinant protein in the bioreactor is an ultrafilter.

In one example the molecular weight cutoff of the hollow fiber filter having a pore size or molecular weight cut off (MWCO) that does not retain the recombinant protein in the bioreactor is at least 500 kDa. In one example the hollow fiber filter having a pore size or molecular weight cut off (MWCO) that does not retain the recombinant protein in the bioreactor is a microfilter.

In one example the a hollow fiber filter having a pore size or molecular weight cut off that retains the recombinant protein in the bioreactor and the hollow fiber filter having a pore size or molecular weight cut off that does not retain the recombinant protein in the bioreactor are components of a single unit filter system.

In one example the non-ionic block copolymer is a polyoxypropylene-polyoxyethylene block copolymer. In one example the non-ionic block copolymer is poloxamer 188.

In one example the method above further comprises taking samples during the cell culture processes, evaluating the samples to quantitatively and/or qualitatively monitor characteristics of the recombinant protein and/or the cell culture process. In one example the samples to quantitatively and/or qualitatively monitored using process analytical techniques.

In one example the perfusing is continuous perfusion. In one example the rate of perfusion is constant. In one example the perfusing is performed at a rate of less than or equal to 1.0 working volume per day. In one example the perfusing is accomplished by a peristaltic pump, a double diaphragm pump, a low shear pump or alternating tangential flow. In one example the perfusing is accomplished by alternating tangential flow.

In one example the method above further comprises subjecting the cell culture to a temperature shift wherein the cells are cultured a) at first temperature for a first period of time and b) at second temperature for a second period of time. In a related example n the temperature shift occurs at the transition between the growth phase and production phase. In a related example the temperature shift occurs during the production phase. In a related example the temperature shift is in response to a predetermined parameter. In a related example the temperature shift is in response to a predetermined parameter wherein reaching the predetermined parameter is determined using a capacitance based biomass probe.

In one example the cell culture is established by inoculating the bioreactor with at least 0.1 x 10^6 viable cells/mL. In one example the inoculum was grown by means of a perfusion process using alternating tangential flow filtration.

In one example prior to entering the bioreactor, the cell culture medium is treated using nanofiltration, high temperature short time (HTST), or UV in combination with filtration.

In one example the bioreactor is a production bioreactor. In one example n the bioreactor has a capacity of at least 500L. In a related example the bioreactor has a capacity of at least 500L to 2000L. In a related example the bioreactor has a capacity of at least 1000L to 2000L.

In one example the cell culture medium is a serum-free cell culture medium. In one example the cell culture medium is a serum-free chemically defined cell culture medium. In one example the cell culture medium is a perfusion cell culture medium.

In one example the mammalian cells are Chinese Hamster Ovary (CHO) cells.

In one example the recombinant protein is selected from the group consisting of a human antibody, a humanized antibody, a chimeric antibody, a recombinant fusion protein, or a cytokine.

In one example the recombinant protein is purified from the harvest permeate by one or more of flocculation, precipitation, centrifugation, depth filtration, affinity chromatography, size exclusion chromatography, ion exchange chromatography, mixed mode anion exchange chromatography, hydrophobic interaction chromatography or hydroxyapatite chromatography.

In one example the method above further comprises taking samples during the purification process, evaluating the samples to quantitatively and/or qualitatively monitor characteristics of the recombinant protein and the production process.

In one example the recombinant protein is formulated into a pharmaceutically acceptable formulation.

In one example is provided a recombinant protein produced by the above method.

The disclosure also provides a single unit filter system comprising two or more hollow fiber filter components of different pore sizes or molecular weight cut offs (MWCO), wherein the hollow fiber filter components are secured to one another in series such that a sterile flow path is maintained between the individual hollow fibers and the hollow fiber filter components of different pore size or molecular weight cut offs are isolated from one another with respect to their hollow shell sides from which the permeate is withdrawn, such that permeate can be removed independently from each respective hollow fiber filter component.

In one example at least one hollow fiber filter component has a pore size or molecular weight cut off that retains the recombinant protein in the bioreactor and at least one hollow fiber filter component has a pore size filter that is a hollow fiber filter having a pore size or molecular weight cut off that does not retain the recombinant protein in the bioreactor. In a related example at least one hollow fiber filter component has a molecular weight cutoff of 300 kDa or less and at least one hollow fiber filter component has a molecular weight cutoff of at least 500 kDa. In a related example at least one hollow fiber filter component is an ultrafilter and at least one hollow fiber filter component is a microfilter. In a related example the single unit filter system is contained within a housing. In one example the single filter unit further comprising a spacer between at least two of the hollow fiber filter components.

The method also provides a method for culturing cells expressing a recombinant protein comprising establishing a cell culture by inoculating a bioreactor with mammalian cells expressing a recombinant protein, maintaining the cell culture by perfusing fresh cell culture medium into the bioreactor, passing the cell culture through a single unit filter system and collecting a permeate, wherein the single unit filter system is attached to the bioreactor and the cell culture is drawn out of the bioreactor and into the single unit filter system by a single pumping system, wherein the cell culture passes through the lumen side of the hollow fibers of the single unit filter system and back

into the bioreactor and a permeate is withdrawn from one or more of the hollow fiber filters components.

In one example the method above further comprise taking samples during the cell culture processes, evaluating the samples to quantitatively and/or qualitatively monitor characteristics of the recombinant protein and/or the cell culture process. In a related process the samples are quantitatively and/or qualitatively monitored by process analytical techniques.

In one example the perfusing is continuous perfusion. In one example the rate of perfusion is constant. In one example the perfusing is performed at a rate of less than or equal to 1.0 working volume per day.

In one example the perfusing is accomplished by a peristaltic pump, a double diaphragm pump, a low shear pump or alternating tangential flow. In one example the perfusing is accomplished by alternating tangential flow.

In one example when the permeate is collected from a hollow fiber filter component that has a pore size or molecular weight cut off that does not retain the recombinant protein in the bioreactor, the fresh cell culture medium is formulated with or supplemented to achieve at least 5g/L of a non-ionic block copolymer. In a related example the non-ionic block copolymer is a polyoxypropylene-polyoxyethylene block copolymer. In a related example the non-ionic block copolymer is poloxamer 188.

In one example the method above further comprises subjecting the cell culture to a temperature shift wherein the cells are cultured a) at first temperature for a first period of time and b) at second temperature for a second period of time. In one example the temperature shift occurs at the transition between the growth phase and production phase. In a related example the temperature shift occurs during the production phase. In a related example the temperature shift is in response to a predetermined parameter wherein reaching the predetermined parameter is determined using a capacitance based biomass probe.

In one example the cell culture is established by inoculating the bioreactor with at least 0.1 x 10^6 viable cells/mL. In a related example the inoculum was grown by means of a perfusion process using alternating tangential flow filtration.

In one example prior to entering the bioreactor, the cell culture medium is treated using nanofiltration, high temperature short time (HTST), or UV in combination with filtration.

In one example the bioreactor is a production bioreactor. In a related example the bioreactor has a capacity of at least 500L. In a related example the bioreactor has a capacity of at least 500L to 2000L. In a related example the bioreactor has a capacity of at least 1000L to 2000L.

In one example the cell culture medium is a serum-free cell culture medium. In one example the cell culture medium is a serum-free chemically defined cell culture medium. In one example the cell culture medium is a perfusion cell culture medium.

In one example the mammalian cells are Chinese Hamster Ovary (CHO) cells.

In one example the recombinant protein is selected from the group consisting of a human antibody, a humanized antibody, a chimeric antibody, a recombinant fusion protein, or a cytokine.

In one example the recombinant protein is purified from the harvest permeate by one or more of flocculation, precipitation, centrifugation, depth filtration, affinity chromatography, size exclusion chromatography, ion exchange chromatography, mixed mode anion exchange chromatography, hydrophobic interaction chromatography or hydroxyapatite chromatography.

In one example the method above further comprising taking samples during the purification process, evaluating the samples to quantitatively and/or qualitatively monitor characteristics of the recombinant protein and the production process.

In one example the recombinant protein is formulated into a pharmaceutically acceptable formulation. In one example is provided a recombinant protein produced the method above.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Schematic of single unit filter system, having a single opening where the cell culture fluid enters and exits through a single opening. Filters can be in any orientation, microfilter hollow fiber followed by ultrafilter hollow fiber is shown.

Figure 2 Titer from extended periodic harvest process, n=2 (open square) compared to ultrafilteration process, n=4 (closed circle).

Figure 3 Viable cell density from extended periodic harvest process, n=2 (open square) compared to ultrafiltration process, n=4 (closed circle).

CLAIMS

1. A method for an extended periodic harvest comprising

establishing a cell culture by inoculating a bioreactor with mammalian cells expressing a recombinant protein,

maintaining the cell culture by perfusing fresh cell culture medium into the bioreactor, passing the cell culture through a filter and collecting a permeate, wherein a null permeate is collected using a hollow fiber filter having a pore size or molecular weight cut off that retains the recombinant protein in the bioreactor wherein when the null permeate is collected the host cells and the expressed recombinant proteins remain in the retentate in the bioreactor and the permeate is substantially free of or has significantly less of either,

wherein a harvest permeate is collected using a hollow fiber filter having a pore size or molecular weight cut off that does not retain the recombinant protein in the bioreactor wherein when the harvest permeate is collected the host cells are retained in the bioreactor and the expressed recombinant proteins pass into the permeate, the fresh cell culture medium is formulated with or supplemented to achieve at least 5g/L of a non-ionic block copolymer;

wherein the null permeate is initially collected until a first predetermined parameter is reached, at which time the harvest permeate is collected for a predetermined time, the harvest permeate comprising the recombinant protein,

this is followed by alternately collecting the null permeate until a second predetermined parameter is reached, then collecting the harvest permeate for a predetermined time,

wherein the alternating collection of null permeate and harvest permeate continues until the cell culture is terminated.

2. The method according to claim 1, wherein the predetermined parameter is selected from time, viable cell density, packed cell volume or titer.

3. The method according to claim 1, wherein the first predetermined parameter is at least 12 hours to 25 days following the establishment of the cell culture.

4. The method according to claim 1, wherein the second predetermined parameter is at least 12 to 72 hours following the collection of the harvest permeate.

5. The method according to claim 1, wherein the predetermined time is at least 12 to 72 hours.

6. The method according to claim 1, wherein the filter is a single unit filter system.

7. The method according to claim 1 further comprising taking samples during the cell culture processes, evaluating the samples to quantitatively and/or qualitatively monitor characteristics of the recombinant protein and/or the cell culture process.

8. The method according to claim 1, wherein the perfusing is continuous perfusion.

9. The method according to claim 1, wherein the perfusing is performed at a rate of less than or equal to 1.0 working volume per day.

10. The method according to claim 1, wherein the perfusing is accomplished by a peristaltic pump, a double diaphragm pump, a low shear pump or alternating tangential flow.

11. The method according to claim 1, wherein the method further comprises

subjecting the cell culture to a temperature shift wherein the cells are cultured a) at first temperature for a first period of time and b) at second temperature for a second period of time.

12. The method according to claim 14, wherein the temperature shift is in response to a predetermined parameter wherein achieving the predetermined parameter is determined using a capacitance based biomass probe.

13. The method according to claim 1 wherein the cell culture is established by inoculating the bioreactor with at least 0.1 x 10^6 viable cells/mL.

14. The method according to claim 1, wherein the mammalian cells are Chinese Hamster Ovary (CHO) cells.

15. The method according to claim 1, wherein the recombinant protein is selected from the group consisting of a human antibody, a humanized antibody, a chimeric antibody, a recombinant fusion protein, or a cytokine.

16. The method according to claim 1, wherein the recombinant protein is purified from the harvest permeate by one or more of flocculation, precipitation, centrifugation, depth filtration, affinity chromatography, size exclusion chromatography, ion exchange chromatography, mixed mode anion exchange chromatography, hydrophobic interaction chromatography or hydroxyapatite chromatography

17. The method according to claim 19, further comprising taking samples during the purification process, evaluating the samples to quantitatively and/or qualitatively monitor characteristics of the recombinant protein and the purification process.

18. The method according to claim 20, wherein the samples are quantitatively and/or qualitatively monitored using process analytical techniques.

19. The method according to claim 19, wherein the recombinant protein is formulated into a pharmaceutically acceptable formulation.