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 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 that retains the recombinant protein in the bioreactor and; 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 2, 3, 4, or 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 that does not retain the recombinant protein in the bioreactor and collecting a permeate containing the recombinant protein. 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

CLAIMS

1. 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 that retains the recombinant protein in the bioreactor, and

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 2, 3, 4, or 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 that does not retain the recombinant protein in the bioreactor and

collecting a permeate containing the recombinant protein.

2. The method according to claim 1, wherein 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.

3. The method of claim 1, wherein a concentration of 1 g/L to 7g/L of a non-ionic block copolymer is achieved in the step of maintaining the cell culture.4. The method of claim 1, wherein the non-ionic block copolymer is polyoxypropylene-polyoxyethylene.

5. The method of claim 1, wherein the non-ionic block copolymer is poloxamer 188.

6. The method of claim 1, wherein the cell culture medium further comprises a non-ionic surfactant.

7. The method of claim 1, wherein the cell culture medium further comprises polyvinyl alcohol, polyethylene glycol, alkyl poly(ethylene oxide), copolymers of poly(ethylene oxide), copolymers

of poly(propylene oxide), poly(vinylpyrrolidone), alkyl polyglucosides, fatty alcohols, or cocamides.

8. The method of claims 1 and 2, wherein the hollow fiber filter having a pore size or molecular weight cut off that does not retain the recombinant protein in the bioreactor is a microfilter.

9. The method of claim 8, wherein the molecular weight cutoff of the microfilter is at least 500 kDa.

10. The method of claim 8, wherein the molecular weight cutoff of the microfilter is 750 kDa.

11. The method of claim 8, wherein the pore size of the microfilter is 0.1 micrometers to 10 micrometers.

12. The method of claims 1 and 2, wherein the hollow fiber filter having a pore size or molecular weight cut off that retains the recombinant protein in the bioreactor is an ultrafilter.

13. The method of claim 12, wherein the molecular weight cutoff of the ultrafilter is 300 kDa or less.

14. The method of claim 12, wherein the molecular weight cutoff of the ultrafilter is 30 kDa.

15. The method of claim 12, wherein the pore size of the ultrafilter is 0.01 micrometers to 0.1 micrometers.