MAMMALIAN CELL CULTURE

FIELD OF INVENTION

The invention provides a method for culturing mammalian cells. The method provides greater control over cell growth to achieve high product titer cell cultures.

BACKGROUND OF INVENTION

As the demand for greater and greater quantities of therapeutic recombinant proteins increases, positive increases in cell growth, viability and protein production are sought by implementing new methods to improve cell development, media optimization and process control parameters. Much effort is now being placed on process optimization, particularly methods and strategies for growing, feeding, and maintaining production cell cultures.

New cell culture methods that provide even incremental improvements in recombinant protein production 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, recombinant polypeptide expression, titer, and cell viability that can lead to higher production levels, thereby reducing the costs associated with manufacturing protein therapeutics are needed. The invention fulfills these needs by providing a simple, easy and inexpensive method of controlling cell growth while increasing protein production.

SUMMARY

The present disclosure provides a method of arresting cell growth in a mammalian cell culture expressing a recombinant protein comprising establishing a mammalian cell culture in a serum-free culture medium in a bioreactor; inducing cell growth-arrest by perfusion with a serum-free perfusion medium having an L-asparagine concentration of 5mM or less; maintaining the mammalian cells in a growth-arrested state by perfusion with a serum-free perfusion medium having an L-asparagine concentration of 5mM or less.

The present disclosure also provides a method of increasing recombinant protein production in a mammalian cell culture expressing a recombinant protein comprising establishing a mammalian cell culture in a serum-free culture medium in a bioreactor; inducing cell growth-arrest by perfusion with a serum-free perfusion medium having an L-asparagine concentration of 5mM or less; maintaining the mammalian cells in a growth-arrested state by perfusion with a serum-free perfusion medium having an L-asparagine concentration of 5mM or less. In one aspect, the present disclosure refers to a method of culturing mammalian cells expressing a recombinant protein comprising; establishing a mammalian cell culture in a serum-free culture medium in a bioreactor by inoculating the bioreactor with at least 0.5 x 10^6 to 3.0 x 10^6 cells/mL in a serum-free culture medium; growing the mammalian cells during a growth phase and supplementing the culture medium with bolus feeds of a serum-free feed medium starting perfusion on or about day 5 to on or about day 9 of the cell culture, and maintaining the mammalian cells during a production

phase by perfusion with a serum-free perfusion medium, wherein the packed cell volume during the production phase is less than or equal to 35%. In a related example recombinant protein production in the mammalian cell culture is increased compared to a culture where the cells are not subjected to L-asparagine-induced cell growth-arrest.

The present disclosure also provides a method of limiting a mammalian cell culture expressing a recombinant protein at a desired packed cell volume comprising establishing a mammalian cell culture in a serum-free culture medium in a bioreactor; inducing cell growth-arrest by perfusion with a serum-free perfusion medium having an L-asparagine concentration of 5mM or less; maintaining the mammalian cells in a growth-arrested state by perfusion with a serum-free perfusion medium having an L-asparagine concentration of 5mM or less.

In one example of the present disclosure, in any of the methods above the perfusion with a serum-free perfusion medium having an L-asparagine concentration of 5mM or less begins on or before day 3 of the culture. In another example, in any of the methods above induction of cell growth-arrest takes place prior to a production phase. In another example, in any of the methods above induction of cell growth-arrest takes place during a production phase. In another example, in any of the methods above cell growth-arrest is induced by L-asparagine starvation. In yet another example, any of the methods above further comprise a temperature shift from 36°C to 31°C. In another example, any of the methods above further comprise a temperature shift from 36°C to 33°C. In a related example the temperature shift occurs at the transition between a growth phase and a production phase. In yet another example the temperature shift occurs during a production phase. In another example the methods above further comprise a packed cell volume during a production phase less than or equal to 35%. In a related example the packed cell volume during a production phase is less than or equal to 35%.

The present disclosure also provides a method of culturing mammalian cells expressing a recombinant protein comprising; establishing a mammalian cell culture in a serum-free culture medium in a bioreactor; growing the mammalian cells during a growth phase and supplementing the culture medium with bolus feeds of a serum-free feed medium, and maintaining the mammalian cells during a production phase by perfusion with a serum-free perfusion medium, wherein the packed cell volume during the production phase is less than or equal to 35%. In one example of the present disclosure, perfusion begins on or about day 5 to on or about day 9 of the cell culture. In a related example perfusion begins on or about day 5 to on or about day 7 of the cell culture. In one example perfusion begins when the cells have reached a production phase. In another example the method further comprises inducing cell growth-arrest by L-asparagine starvation followed by perfusion with a serum-free perfusion medium having an L-asparagine concentration of 5 mM or less. In yet another example the method further comprises inducing cell growth-arrest by perfusion with a serum-free perfusion medium having an L-asparagine concentration of 5 mM or less.

In one example of the disclosure the concentration of L-asparagine in the serum-free perfusion medium is less than or equal to 5mM. In another example the concentration of L-asparagine in the serum-free perfusion medium is less than or equal to 4.0 mM. In another

example the concentration of L-asparagine in the serum-free perfusion medium is less than or equal to 3.0 mM. In still another example the concentration of L-asparagine in the serum-free perfusion medium is less than or equal to 2.0 mM. In yet another example the concentration of L-asparagine in the serum-free perfusion medium is less than or equal to 1.0 mM. In yet another example the concentration of L-asparagine in the serum-free perfusion medium is 0 mM. In another example perfusion is performed at a rate that increases during the production phase from 0.25 working volume per day to 1.0 working volume per day during the cell culture. In a related example perfusion is performed at a rate that reaches 1.0 working volume per day on day 9 to day 11 of the cell culture. In another related example perfusion is performed at a rate that reaches 1.0 working volume per day on day 10 of the cell culture. In yet another example the bolus feeds of serum-free feed medium begin on day 3 or day 4 of the cell culture. In another example of the disclosure the method further comprises a temperature shift from 36°C to 31°C. In another example the method further comprises a temperature shift from 36°C to 33°C. 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 one example of the disclosure the L-asparagine concentration of the cell culture medium is monitored prior to and during L-asparagine starvation.

In one example of the disclosure the packed cell volume is less than or equal to 35%. In a related example the packed cell volume is less than or equal to 30%.

In one example of the disclosure the viable cell density of the mammalian cell culture at a packed cell volume less than or equal to 35% is $10x10^6$ viable cells/ml to $80x10^6$ viable cells/ml. In a related example the viable cell density of the mammalian cell culture is $20x10^6$ viable cells/ml to $30x10^6$ viable cells/ml.

In one example of the disclosure perfusion comprises continuous perfusion.

In one example of the disclosure the rate of perfusion is constant.

In one example of the disclosure perfusion is performed at a rate of less than or equal to 1.0 working volumes per day.

In another example of the disclosure the mammalian cell culture is established by inoculating the bioreactor with at least 0.5×10^6 to 3.0×10^6 cells/mL in a serum-free culture medium. In a related example the mammalian cell culture is established by inoculating the bioreactor with at least 0.5×10^6 to 1.5×10^6 cells/mL in a serum-free culture medium.

In another example of the disclosure the perfusion is accomplished by alternating tangential flow.

In another example of the disclosure 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 yet another related example the bioreactor has a capacity of at least 1000L to 2000L.

In another example of the disclosure the mammalian cells are Chinese Hamster Ovary (CHO) cells.

In another example of the disclosure 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 another example of the disclosure any of the methods above further comprise a step of harvesting the recombinant protein produced by the cell culture.

In another example of the disclosure the recombinant protein produced by the cell culture is purified and formulated in a pharmaceutically acceptable formulation.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 Fed-batch start: solid square (■) and solid circle (●) Batch start: open square (□) and open circle (○).

Figure 1A: Viable cell density, Figure 1B: Viability, Figure 1C: Titer

Figure 2 Batch start: open circle (○), Fed-batch start with high agitation: open square (□)

Figure 2A Viable cell density, Figure 2B Viability, Figure 2C Titer, Figure 2D Asparagine concentration

Figure 3 1.0 start perfusion volume, no temperature shift: solid circle. 1.0 start perfusion volume, temperature shift: open circle (○). 0.75 start perfusion volume perfusion volume, no temperature shift: solid square (■). 0.75 start perfusion volume, temperature shift: open square (□).

Figure 3A Viable Cell Density, Figure 3B Viability, Figure 3C Titer

Figure 4 Batch start with low asparagine amount: open triangle (Δ). Batch start with control L-asparagine amount: solid triangle (▲). Fed-batch start with low L-asparagine amount: open diamond (◊). Fed-batch start with control L-asparagine amount: solid diamond (♦). Sparge with drilled pipe: Solid line. Sparge with sintered sparger: dashed line.

What is claimed is:

1. A method of culturing mammalian cells expressing a recombinant protein comprising;

establishing a mammalian cell culture in a serum-free culture medium in a bioreactor by inoculating the bioreactor with at least 0.5 x 10^6 to 3.0 x 10^6 cells/mL in a serum-free culture medium;

growing the mammalian cells during a growth phase and supplementing the culture medium with bolus feeds of a serum-free feed medium

starting perfusion on or about day 5 to on or about day 9 of the cell culture, and

maintaining the mammalian cells during a production phase by perfusion with a serum-free perfusion medium, wherein the packed cell volume during the production phase is less than or equal to 35%.

2. The method according to claim 1, wherein perfusion begins on or about day 5 to on or about day 7 of the cell culture.

3. The method according to claim 1, wherein perfusion begins when the cells have reached a production phase.

4. The method according to claim 1, further comprising (a) inducing cell growth-arrest by L-asparagine starvation followed by perfusion with a serum-free perfusion medium having an L-asparagine concentration of 5 mM or less, or (b) inducing cell growth-arrest by perfusion with a serum-free perfusion medium having an L-asparagine concentration of 5 mM or less.

5. The method according to claim 4, wherein the concentration of L-asparagine in the serum-free perfusion medium is less than or equal to 5mM, less than or equal to 4.0 mM, less than or equal to 3.0 mM, less than or equal to 2.0 mM, less than or equal to 1.0 mM, or is 0 mM.

6. The method according to claim 4(a), wherein the L-asparagine concentration of the cell culture medium is monitored prior to and during L-asparagine starvation.

7. The method according to claim 1, wherein the packed cell volume is less than or equal to 30%.

8. The method according to claim 1, wherein the viable cell density of the mammalian cell culture at a packed cell volume less than or equal to 35% is 10x10^6 viable cells/ml to 80x10^6

viable cells/ml, preferably the viable cell density of the mammalian cell culture is $20x10^6$ viable cells/ml to $30x10^6$ viable cells/ml.

9. The method according to claim 1, wherein perfusion comprises continuous perfusion.

10. The method according to claim 1, wherein the rate of perfusion is constant.

11. The method according to claim 1, wherein perfusion is performed (a) at a rate of less than or equal to 1.0 working volumes per day or (b) at a rate that increases during the production phase from 0.25 working volume per day to 1.0 working volume per day during the cell culture.

12. The method according to claim 1, wherein perfusion is performed at a rate that reaches 1.0 working volume per day on day 9 to day 11 of the cell culture, preferably on day 10 of the cell culture.

13. The method according to claim 1, wherein the bolus feeds of serum-free feed medium begin on day 3 or day 4 of the cell culture.

14. The method according to claim 1, wherein the mammalian cell culture is established by inoculating the bioreactor with at least 0.5×10^6 to 1.5×10^6 cells/mL in a serum-free culture medium.

15. The method according to claim 1, further comprising a temperature shift from 36°C to 31°C or from 36°C to 33°C.

16. The method according to claim 15, wherein the temperature shift occurs at the transition between the growth phase and production phase, or during the production phase.

17. The method according to claim 1, wherein the perfusion is accomplished by alternating tangential flow.

18. The method according to claim 1, wherein the bioreactor has a capacity of at least 500L, preferably at least 500L to 2000L, or at least 1000L to 2000L.

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

20. 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.

21. The method of claim 1, further comprising a step of harvesting the recombinant protein produced by the cell culture.

22. The method of claim 1, wherein the recombinant protein produced by the cell culture is purified and formulated in a pharmaceutically acceptable formulation.