What is claimed is:

1. 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.

2. 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.

3. 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.

4. The method of any of claims 1-3, wherein 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.

5. The method according to any of claims 1-3, wherein induction of cell growth-arrest takes place prior to a production phase.

6. The method according to any of claims 1-3, wherein induction of cell growth-arrest takes place during a production phase.

7. The method according to any of claims 1-3, wherein cell growth-arrest is induced by L-asparagine starvation.

8. The method according to any of claims 1-3, further comprising a temperature shift from 36°C to 31°C.

9. The method according to any of claims 1-3, further comprising a temperature shift from 36°C to 33°C.

10. The method according to claims 8 or 9, wherein the temperature shift occurs at the transition between a growth phase and a production phase.

11. The method according to claims 8 or 9, wherein the temperature shift occurs during a production phase.

12. 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%.

13. The method according to claim 12, wherein perfusion begins on or about day 5 to on or about day 9 of the cell culture.

14. The method according to claim 12 or 13, wherein perfusion begins on or about day 5 to on or about day 7 of the cell culture.

15. The method according to claim 12, wherein perfusion begins when the cells have reached a production phase.

16. The method according to claim 12, further comprising 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.

17. The method according to claim 12, further comprising inducing cell growth-arrest by perfusion with a serum-free perfusion medium having an L-asparagine concentration of 5 mM or less.

18. The method according to any of claims 1-3, 16 and 17, wherein the concentration of L-asparagine in the serum-free perfusion medium is less than or equal to 5mM.

19. The method according to any of claims 1-3 and 16-18, wherein the concentration of L-asparagine in the serum-free perfusion medium is less than or equal to 4.0 mM.

20. The method according to any of claims 1-3 and 16-19, wherein the concentration of L-asparagine in the serum-free perfusion medium is less than or equal to 3.0 mM.

21. The method according to any of claims 1-3 and 16-20, wherein the concentration of L-asparagine in the serum-free perfusion medium is less than or equal to 2.0 mM.

22. The method according to any of claims 1-3 and 16-21, wherein the concentration of L-asparagine in the serum-free perfusion medium is less than or equal to 1.0 mM.

23. The method according to any of claims 1-3 and 16-22, wherein the concentration of L-asparagine in the serum-free perfusion medium is 0 mM.

24. The method according to claim 7 or 16, wherein the L-asparagine concentration of the cell culture medium is monitored prior to and during L-asparagine starvation.

25. The method according to any of claims 1-3, further comprising wherein the packed cell volume during a production phase is less than or equal to 35%

26. The method according to claim 12 or 25, wherein the packed cell volume is less than or equal to 35%.

27. The method according to any of claims 12, 25 and 26, wherein the packed cell volume is less than or equal to 30%.

28. The method according to claim 12 or 25, 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.

29. The method according to claim 28, wherein the viable cell density of the mammalian cell culture is $20x10^6$ viable cells/ml to $30x10^6$ viable cells/ml.

30. The method according to any of claims 1-3 and 12, wherein perfusion comprises continuous perfusion.

31. The method according to any of claims 1-3 and 12, wherein the rate of perfusion is constant.

32. The method according to any of claims 1-3 and 12, wherein perfusion is performed at a rate of less than or equal to 1.0 working volumes per day.

33. The method according to any of claims 1-3 and 12, wherein 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.

34. The method according to claim 12, 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.

35. The method according to claim 12 or 34, wherein perfusion is performed at a rate that reaches 1.0 working volume per day on day 10 of the cell culture.

36. The method according to claim 12, wherein the bolus feeds of serum-free feed medium begin on day 3 or day 4 of the cell culture.

37. The method according to any of claims 1-3 and 12, wherein 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.

38. The method according to any of claims 1-3, 12 and 37, 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.

39. The method according to claim 12, further comprising a temperature shift from 36°C to 31°C.

40. The method according to claim 12, further comprising a temperature shift from 36°C to 33°C.

41. The method according to claim 39 or 40, wherein the temperature shift occurs at the transition between the growth phase and production phase.

42. The method according to claim 39 or 40, wherein the temperature shift occurs during the production phase.

43. The method according to any of claims 1-3 and 12, wherein the perfusion is accomplished by alternating tangential flow.

44. The method according any of claims 1-3 and 12, wherein the bioreactor has a capacity of at least 500L.

45. The method according to any of claims 1-3, 12 and 44, wherein the bioreactor has a capacity of at least 500L to 2000L.

46. The method according to any of claims 1-3, 12, 44 and 45, wherein the bioreactor has a capacity of at least 1000L to 2000L.

47. The method according any of claims 1-3 and 12, wherein the mammalian cells are Chinese Hamster Ovary (CHO) cells.

48. The method according any of claims 1-3 and 12, 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.

49. The method any of claims 1-3 and 12, further comprising a step of harvesting the recombinant protein produced by the cell culture.

50. The method any of claims 1-3 and 12, wherein the recombinant protein produced by the cell culture is purified and formulated in a pharmaceutically acceptable formulation.

51. The method of claim 2, wherein 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.

What is claimed is:

1. A method of (a) arresting cell growth in a mammalian cell culture expressing a recombinant protein, (b) increasing recombinant protein production in a mammalian cell culture expressing a recombinant protein, and/or (c) 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.

2. 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 in 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%.

3.. The method of claim 1, wherein 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.

4. The method according to claim 1, wherein induction of cell growth-arrest takes place prior to a production phase or during a production phase.

5. The method according to claim 1, wherein cell growth-arrest is induced by L-asparagine starvation.

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

7. The method according to claim6, wherein the temperature shift occurs at the transition between a growth phase and a production phase or during a production phase.

8. The method according to claim 2, wherein perfusion begins on or about day 5 to on or about day 9 of the cell culture, preferably perfusion begins on or about day 5 to on or about day 7 of the cell culture.

9. The method according to claim 2, wherein perfusion begins when the cells have reached a production phase.

10. The method according to claim 2, 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.

11. The method according to any of claims 1-2 and 10, 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.

12. The method according to claim 5 or 10(a), wherein the L-asparagine concentration of the cell culture medium is monitored prior to and during L-asparagine starvation.

13. The method according to claim 1, further comprising wherein the packed cell volume during a production phase is less than or equal to 35%, or less than or equal to 30%.

14. The method according to claim 2 or 13, wherein the packed cell volume is less than or equal to 35%, or less than or equal to 30%.

15. The method according to claim 2 or 13, 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.

16. The method according to claim 1or 2, wherein perfusion comprises continuous perfusion.

17. The method according to claim 1or 2, wherein the rate of perfusion is constant.

18. The method according to claim 1 or 2, 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.

19. The method according to claim 2, 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.

20. The method according to claim 2, wherein the bolus feeds of serum-free feed medium begin on day 3 or day 4 of the cell culture.

21. The method according to claim 1 or 2, wherein the mammalian cell culture is established 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 preferably with at least 0.5 x 10^6 to 1.5 x 10^6 cells/mL in a serum-free culture medium.

22. The method according to claim 2, further comprising a temperature shift from 36°C to 31°C or from 36°C to 33°C.

23. The method according to claim 22, wherein the temperature shift occurs at the transition between the growth phase and production phase, or during the production phase.

24. The method according to claim 1 or 2, wherein the perfusion is accomplished by alternating tangential flow.

25. The method according to claim 1or 2, wherein the bioreactor has a capacity of at least 500L.

26. The method according to claim 1 or 2, wherein the mammalian cells are Chinese Hamster Ovary (CHO) cells.

27. The method according to claim 1 or 2, 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.

28. The method of claim 1 or 2, further comprising a step of harvesting the recombinant protein produced by the cell culture.

29. The method of claim 1 or 2, wherein the recombinant protein produced by the cell culture is purified and formulated in a pharmaceutically acceptable formulation.

30. The method of claim 1(b), wherein 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.