

The data described in this example demonstrate an exemplary seed train process involving HD cell banking, disposable single-use bioreactor technology, and perfusion culturing at the N-1 bioreactor stage. The seed train processes provided herein reduce the complexity of conventional seed train processes by decreasing the number of small-scale culturing stages. The data described in this example show that the use of perfusion culture at the N-1 stage can achieve viable cell densities up to 100×10^6 viable cells/mL in 12 days without compromising culture growth characteristics after further expansion steps. Based on these data, the exemplary seed train processes described herein can be used to inoculate at a 500-L production bioreactor at a cell density of 5×10^6 viable cells/mL to reduce the time to steady state cell density by 4-5 days and provide a 10% increase in the overall productivity of a 50-day run. For batch or batch processes that require larger production bioreactors, such as 10,000-L, the seed train processes provided herein can eliminate 1-2 stages from the expansion process by using a single 50-L perfusion bioreactor at the N-1 stage.

WHAT IS CLAIMED IS:

1. A seed train process comprising:
 - (a) disposing a plurality of recombinant mammalian cells into a first culture medium comprised within a vessel to provide a first cell culture;
 - (b) batch culturing the first cell culture to a cell density range of about 1.0×10^6 cells/mL to about 5.0×10^6 cells/mL;
 - (c) disposing a volume of the first cell culture of step (b) into a second culture medium comprised within a perfusion bioreactor to provide a second cell culture with an initial cell density in a range of about 0.25×10^6 cells/mL to about 0.5×10^6 cells/mL;
 - (d) perfusion culturing the second cell culture to a cell density range of between about 5×10^6 cells/mL to about 120×10^6 cells/mL; and
 - (e) disposing a volume of the second cell culture of step (d) into a third culture medium comprised within a production bioreactor to provide a production cell culture with an initial cell density in a range of about 0.25×10^6 cells/mL to about 8×10^6 cells/mL.
2. The process of claim 1, wherein disposing the plurality of recombinant mammalian cells in (a) comprises:
 - thawing a frozen cell bank; and
 - disposing a volume of the thawed cell bank into the first culture medium.
3. The process of claim 2, wherein the frozen cell bank comprises a cell density range of about 10×10^7 cells/mL to about 50×10^7 cells/mL.
4. The process of claim 3, wherein the thawed cell bank contains a percentage of viable cells of at least 60%.
5. The process of claim 4, wherein the thawed cell bank contains a percentage of viable cells of at least 90%.
6. The process of claim 1, wherein disposing the plurality of recombinant mammalian cells in (a) comprises disposing a volume of a third cell culture

comprising the plurality of recombinant mammalian cells into the first culture medium.

7. The process of claim 6, further comprising:

(1) disposing a plurality of the recombinant mammalian cells into a fourth culture medium comprised within a vessel to provide the third cell culture;

(2) batch culturing the third cell culture of (1) to a cell density range of about 1.0×10^6 cells/mL to about 5.0×10^6 cells/mL,

wherein a volume of the third cell culture in (2) is disposed into the first culture medium in (a).

8. The process of claim 7, wherein one or both of the vessel in (a) or the vessel in (1) is a disposable single-use bioreactor.

9. The process of claim 8, wherein the disposable single-use bioreactor comprises a plastic sterile bag.

10. The process of claim 9, wherein disposing the plurality of the recombinant mammalian cells in (1) comprises:

thawing a frozen cell bank; and

disposing a volume of the thawed cell bank into the fourth culture medium.

11. The process of claim 10, wherein the frozen cell bank comprises a cell density range of about 10×10^7 cells/mL to about 50×10^7 cells/mL.

12. The process of claim 11, wherein the thawed cell bank contains a percentage of viable cells of at least 60%.

13. The process of claim 12, wherein the thawed cell bank contains a percentage of viable cells of at least 90%.

14. The process of claim 1, wherein the first cell culture in (a) has a volume range of about 1.0 L to about 50 L.

15. The process of claim 14, wherein the first cell culture in (a) has a volume range of about 5.0 L to about 10.0 L.

16. The process of claim 1, wherein the second cell culture in (c) has a volume range of about 5 L to about 600 L.

17. The process of claim 16, wherein the second cell culture in (c) has a volume range of about 10 L to about 300 L.

18. The process of claim 1, wherein the production cell culture in (e) has a volume range of about 50 L to about 20,000 L.

19. The process of claim 18, wherein the production cell culture in (e) has a volume range of about 100 L to about 10,000 L.

20. The process of claim 7, wherein the fourth culture medium in (1) has a volume range of about 500 mL to about 20 L.

21. The process of claim 7, wherein the fourth culture medium in (1) has a volume range of about 500 mL to about 10 L.

22. The process of claim 1, wherein the vessel in (a) has an internal volume range of about 1.5 L to about 100 L.

23. The process of claim 22, wherein the vessel in (a) has an internal volume range of about 1.5 L to about 50 L.

24. The process of claim 1, wherein the perfusion bioreactor in (c) has an internal volume range of about 7.5 L to about 1,000 L.

25. The process of claim 24, wherein the perfusion bioreactor in (c) has an internal volume range of about 50 L to about 1000 L.

26. The process of claim 1, wherein the production bioreactor in (e) has an internal volume range of about 150 L to about 25,000 L.

27. The process of claim 26, wherein the production bioreactor in (e) has an internal volume range of about 150 L to about 10,000 L.

28. The process of claim 7, wherein the vessel in (1) has an internal volume range of about 1 L to about 40 L.

29. The process of claim 28, wherein the vessel in (1) has an internal volume range of about 1 L to about 20 L.

30. The process of claim 1, wherein the perfusion culturing in (c) is performed using a perfusion bioreactor equipped with an alternating tangential flow filtration device.

31. The process of claim 1, wherein the initial cell density in (e) is in a range of about 2.0×10^6 cells/mL to about 8×10^6 cells/mL

32. The process of claim 1, wherein the initial cell density in (e) is at least 10% of the steady state production cell density.

33. The process of claim 32, wherein the initial cell density in (e) is at least 20% of the steady state production cell density.

34. A method of producing a recombinant protein comprising:

(a) disposing a plurality of recombinant mammalian cells into a first culture medium comprised within a vessel to provide a first cell culture;

(b) batch culturing the first cell culture to a cell density range of about 1.0×10^6 cells/mL to about 5.0×10^6 cells/mL;

(c) disposing a volume of the first cell culture medium of (b) into a second culture medium comprised within a perfusion bioreactor to provide a second cell

culture with an initial cell density in a range of about 0.25×10^6 cells/mL to about 0.5×10^6 cells/mL;

(d) perfusion culturing the second cell culture to a cell density range of between about 5×10^6 cells/mL to about 60×10^6 cells/mL;

(e) disposing a volume of the second cell culture of (d) into a third culture medium comprised within a production bioreactor to provide a production cell culture with an initial cell density in a range of about 0.25×10^6 cells/mL to about 8×10^6 cells/mL;

(f) perfusion culturing the production cell culture under conditions that allow the recombinant mammalian cells to secrete a recombinant protein; and

(g) harvesting the recombinant protein from the production cell culture.

35. The method of claim 34, wherein disposing the plurality of recombinant mammalian cells in (a) comprises:

thawing a frozen cell bank; and

disposing a volume of the thawed cell bank into the first culture medium.

36. The method of claim 35, wherein the frozen cell bank has a cell density range of about 10×10^7 cells/mL to about 50×10^7 cells/mL.

37. The method of claim 36, wherein the thawed cell bank contains a percentage of viable cells of at least 60%.

38. The method of claim 37, wherein the thawed cell bank contains a percentage of viable cells of at least 90%.

39. The method of claim 34, wherein disposing the plurality of recombinant mammalian cells in (a) comprises disposing a volume of a third cell culture comprising the plurality of recombinant mammalian cells into the first culture medium.

40. The method of claim 39, further comprising:

(1) disposing a plurality of the recombinant mammalian cells into a fourth culture medium comprised within a vessel to provide the third cell culture;

(2) batch culturing the third cell culture in (1) to a cell density range of about 1.0×10^6 cells/mL to about 5.0×10^6 cells/mL,
wherein a volume of the third cell culture in (2) is disposed into the first culture medium in (a).

41. The method of claim 40, wherein one or both of the vessel in (a) or the vessel in (1) is a disposable single-use bioreactor.

42. The method of claim 41, wherein the disposable single-use bioreactor comprises a plastic sterile bag.

43. The method of claim 40, wherein disposing the plurality of the recombinant mammalian cells in (1) comprises:
thawing a frozen cell bank; and
disposing a volume of the thawed cell bank into the fourth culture medium.

44. The method of claim 43, wherein the frozen cell bank comprises a cell density range of about 1.0×10^7 cells/mL to about 50×10^7 cells/mL.

45. The method of claim 44, wherein the frozen cell bank has a cell density range of about 10×10^7 cells/mL to about 50×10^7 cells/mL.

46. The method of claim 45, wherein the thawed cell bank contains a percentage of viable cells of at least 60%.

47. The method of claim 46, wherein the thawed cell bank contains a percentage of viable cells of at least 90%.

48. The method of claim 34, wherein the first cell culture in (a) has a volume range of about 1.0 L to about 50 L.

49. The method of claim 48, wherein the first cell culture in (a) has a volume range of about 5.0 L to about 10.0 L.

50. The method of claim 34, wherein the second cell culture in (c) has a volume range of about 5 L to about 600 L.

51. The method of claim 50, wherein the second cell culture in (c) has a volume range of about 10 L to about 300 L.

52. The method of claim 34, wherein the production cell culture in (e) has a volume range of about 50 L to about 20,000 L.

53. The method of claim 52, wherein the production cell culture in (e) has a volume range of about 100 L to about 10,000 L.

54. The method of claim 53, wherein the fourth culture medium in (1) has a volume range of about 500 mL to about 20 L.

55. The method of claim 54, wherein the fourth culture medium in (1) has a volume range of about 500 mL to about 10 L.

56. The method of claim 34, wherein the vessel in (a) has an internal volume range of about 1.5 L to about 100 L.

57. The method of claim 56, wherein the vessel in (a) has an internal volume range of about 1.5 L to about 50 L.

58. The method of claim 34, wherein the perfusion bioreactor in (c) has an internal volume range of about 7.5 L to about 1,000 L.

59. The method of claim 58, wherein the perfusion bioreactor in (c) has an internal volume range of about 50 L to about 1000 L.

60. The method of claim 34, wherein the production bioreactor in (e) has an internal volume range of about 150 L to about 25,000 L.

61. The method of claim 60, wherein the production bioreactor in (e) has an internal volume range of about 150 L to about 10,000 L.

62. The method of claim 61, wherein the vessel in (1) has an internal volume range of about 1 L to about 40 L.

63. The method of claim 62, wherein the vessel in (1) has an internal volume range of about 1 L to about 20 L.

64. The method of claim 34, wherein the perfusion culturing in (c) is performed using a perfusion bioreactor equipped with an alternating tangential flow filtration device.

65. The method of claim 34, wherein the initial cell density in (e) is in a range of about 2.0×10^6 cells/mL to about 8×10^6 cells/mL

66. The method of claim 34, wherein the initial cell density in (e) is at least 10% of the steady state production cell density.

67. The method of claim 66, wherein the initial cell density in (e) is at least 20% of the steady state production cell density.

68. The method of claim 66, wherein the steady state production cell density is between 5×10^6 cells/mL to about 50×10^6 cells/mL.

69. The method of claim 68, wherein the steady state production cell density is between 15×10^6 cells/mL to about 50×10^6 cells/mL.

70. The method of claim 34, wherein the perfusion culturing in (f) results in the production cell culture reaching the steady state production cell density in a period of between about 1 day to about 10 days.

71. The method of claim 70, wherein the perfusion culturing in (f) results in the production cell culture reaching the steady state production cell density in a period of between about 2 days to about 5 days.

72. The method of claim 34, wherein the harvesting in (g) comprises removing culture medium from the production bioreactor.

73. The method of claim 72, wherein the culture medium is continuously removed from the production bioreactor.

74. The method of claim 73, further comprising isolating the recombinant protein from the removed culture medium.

75. The method of claim 74, wherein the isolating is performed using an integrated and continuous process.

76. The method of claim 74, further comprising formulating the isolated recombinant protein into a pharmaceutical agent.