LINKED PERFUSION TO CONTINUOUS-FLOW STIRRED-TANK REACTOR CELL CULTURE SYSTEM

FIELD OF THE SUBJECT TECHNOLOGY

The subject technology relates to methods of protein production in cultured animal cells, preferably mammalian cells, using a linked bioreactor system including a culture bioreactor (N-1 bioreactor) linked to production bioreactor (N bioreactor). More specifically, the subject technology relates to a continuous perfusion culture bioreactor linked to a chemostat or continuous-flow stirred-tank reactor (CSTR) production bioreactor.

BACKGROUND OF THE SUBJECT TECHNOLOGY

Conventional perfusion cell culture systems suffer from disadvantages such as large-volume media consumption, long times to reach peak cell densities and complications with cell retention devices when used at the largest scale.

For example, in the hybrid cell culture systems disclosed in WO 2008/091113 A1, multiple CSTR bioreactors are linked in series, each using cell retention devices (e.g., packed bed). The reference suggests that in an ideal system, the last bioreactor in the series would use complete cell retention, but that the earlier bioreactors would allow some cells to pass out of the bioreactor. In the hybrid cell culture system disclosed in WO 2015/003012 A2, a single 'nurse' or N-1 bioreactor periodically inoculates multiple N-stage production bioreactors which have cell retention devices. In the hybrid cell culture system disclosed in WO 2015/095809 A1, an N-1 perfusion bioreactor is used to prepare an inoculum for a production bioreactor operating in a fed-batch or perfusion mode. However, all the data and examples in this reference suggest that the N-1 is a perfusion bioreactor operating for a short period of time, producing a single inoculum used to inoculate a production bioreactor which operates as a fed-batch. Additionally, nowhere do the authors of this application suggest that the production bioreactor might operate as a chemostat (or CSTR) with no cell retention that produces a continuous harvest to the downstream purification operation.

temperature might slow growth and decrease cellular productivity, so the use of these setpoint control changes would have to be balanced against the need to control lactate formation. The optimal pH setpoint would likely be lower than 7.0 for this purpose, likely between 6.7 and 7.0. The optimal temperature would likely be between 30-35°C

It will be apparent to those skilled in the art that various modifications and variations can be made to the described embodiments without departing from the spirit and scope of the claimed methods. Thus, it is intended that present claimed methods cover the modifications and variations of the embodiments described herein provided that they come within the scope of the appended claims and their equivalents.

INDUSTRIAL APPLICABILITY

The device and methods disclosed herein are useful for linked-perfusion-to-CSTR cell culture biomaufacturing, and thus for improving industrial methods for manufacturing recombinant and/or therapeutic proteins.

ITEMS

In view of the above, it will be appreciated that the present invention also relates to the following items:

1. A method of producing a protein of interest, comprising:
   (a) culturing cells comprising a gene that encodes the protein of interest in a culture bioreactor (N-1 bioreactor);
   (b) inoculating a production bioreactor (N bioreactor) with cells obtained from step (a); and
   (c) culturing the cells in the production bioreactor under conditions that allow production of the protein of interest.
2. The method according to item 1, wherein the method further comprises step (d) harvesting the protein of interest from the production bioreactor.
3. The method according to any of the preceding items, wherein the culture bioreactor is a continuous perfusion culture bioreactor and the production bioreactor is a continuously stirred tank reactor (CSTR) production bioreactor.

4. The method according to any of the preceding items, wherein the production bioreactor has no cell retention device.

5. The method according to any of the preceding items, wherein volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:20 or about 1:1 to about 1:5 or about 1:5.

6. The method according to any of the preceding items, wherein the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor.

7. The method according to item 6, wherein the cell transfer is by cell bleed in continuous or semi-continuous modes.

8. The method according to item 7, wherein the cell transfer is in semi-continuous mode comprising the cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween.

9. The method according to any of the preceding items, wherein step (a) optionally alternates between a first and second culture bioreactors to allow for renewal and continuous production of culture cells for use in step (b).

10. The method according to item 9, wherein the second culture bioreactor is a continuous perfusion culture bioreactor.

11. The method according to any of the preceding items, wherein the production bioreactor operates continuously for a period of greater than 3 weeks or for a period of greater than 4 weeks or for a period of greater than 5 weeks or for a period of greater than 6 weeks.

12. The method according to any of the preceding items, wherein harvesting step (d) is continuous.

13. The method according to any of the preceding items, wherein the cells are CHO cells, HEK-293 cells, VERO cells, NSO cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells or COS cells.

14. The method according to any of the preceding items, wherein the production bioreactor has a volumetric productivity of at least 0.6 grams per liter per day for a period of at least 14 days or for a period of at least 20 days or for a period of at least 30 days.

15. The method according to any of the preceding items, wherein the production bioreactor has a product residence time of about 1 to about 10 days.

16. The method according to any of the preceding items, wherein the production bioreactor has a dilution rate of about 1 to about 0.1 volume per day.

17. A linked culture process, comprising:

(a) culturing cells comprising a gene that encodes the protein of interest in a culture bioreactor (N-1 bioreactor);

(b) inoculating a production bioreactor (N bioreactor) with cells obtained from step (a); and

(c) culturing the cells in the production bioreactor under conditions that allow production of the protein of interest.

18. The linked culture process according to item 17, wherein the linked culture process further comprises step (d) harvesting the protein of interest from the production bioreactor.

19. The linked culture process according to any of items 17-18, wherein the culture bioreactor is a continuous perfusion culture bioreactor and the continuous production bioreactor is a continuously stirred tank reactor (CSTR) production bioreactor.

20. The linked culture process according to any of items 17-19, wherein the production bioreactor has no cell retention device.

21. The linked culture process according to any of items 17-19, wherein volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:20 or about 1:1 to about 1:5 or about 1:5.

22. The linked culture process according to any of items 17-19, wherein the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor.

23. The linked culture process according to item 22, wherein the cell transfer is by cell bleed in continuous or semi-continuous modes.

24. The linked culture process according to item 23, wherein the cell transfer is in semicontinuous mode comprising the cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween.

25. The linked culture process according to any of items 17-24, wherein step (a) optionally alternates between a first and second culture bioreactors to allow for renewal and continuous production of culture cells for use in step (b).

26. The linked culture process according to item 25, wherein the second culture bioreactor is a continuous perfusion culture bioreactor.

27. The linked culture process according to any of items 17-26, wherein the production bioreactor operates continuously for a period of greater than 3 weeks or for a period of greater than 4 weeks or for a period of greater than 5 weeks or for a period of greater than 6 weeks.

28. The linked culture process according to any of items 17-27, wherein harvesting step (d) is continuous.

29. The linked culture process according to any of items 17-28, wherein the cells are CHO cells, HEK-293 cells, VERO cells, NSO cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells or COS cells.

30. The linked culture process according to any of items 17-29, wherein the production bioreactor has a volumetric productivity of at least 0.6 grams per liter per day for a period of at least 14 days or for a period of at least 20 days or for a period of at least 30 days.

31. The linked culture process according to any of items 17-30, wherein the production bioreactor has a product residence time of about 1 to about 10 days.

32. The linked culture process according to any of items 17-31, wherein the production bioreactor has a dilution rate of about 1 to about 0.1 volume per day.

33. A linked culture system, comprising:

a culture bioreactor (N-1 bioreactor) for culturing cells comprising a gene that encodes the protein of interest; and

a production bioreactor (N bioreactor) which is linked to the culture bioreactor and receives cells as inoculum from the culture bioreactor, wherein the production bioreactor operates under conditions that allow production of the protein of interest.

34. The linked culture system according to item 33, wherein the system is linked to a downstream purification system for harvesting the protein of interest from the production bioreactor.

35. The linked culture system according to any of items 33-34, wherein the culture bioreactor is a continuous perfusion culture bioreactor and the production bioreactor is a continuously stirred tank reactor (CSTR) production bioreactor.

36. The linked culture system according to any of items 33-35, wherein the production bioreactor has no cell retention device.

37. The linked culture system according to any of items 33-36, wherein volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:20 or about about 1:1 to about 1:5 or about 1:5.

38. The linked culture system according to any of items 33-37, wherein the inoculation is by transferring cells from the culture bioreactor to the production bioreactor.

39. The linked culture system according to item 38, wherein the cell transfer is by cell bleed in continuous or semi-continuous modes.

40. The linked culture system according to item 39, wherein the cell transfer is in semicontinuous mode comprising the cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween.

41. The linked culture system according to any of items 33-40, wherein the system includes a second culture bioreactor that operates in parallel with a first culture bioreactor and alternates in producing inoculum for transfer to the production bioreactor.

42. The linked culture system according to item 41, wherein the second culture bioreactor is a continuous perfusion culture bioreactor.

43. The linked culture system according to any of items 33-42, wherein the production bioreactor operates continuously for a period of greater than 3 weeks or for a period of greater than 4 weeks or for a period of greater than 5 weeks or for a period of greater than 6 weeks.

44. The linked culture system according to any of items 33-43, wherein the harvesting is continuous.

45. The linked culture system according to any of items 33-44, wherein the cells are CHO cells, HEK-293 cells, VERO cells, NSO cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells or COS cells.

46. The linked culture system according to any of items 33-45, wherein the production bioreactor has a volumetric productivity of at least 0.6 grams per liter per day for a period of at least 14 days or for a period of at least 20 days or for a period of at least 30 days.

47. The linked culture system according to any of items 33-46, wherein the production bioreactor has a product residence time of about 1 to about 10 days.

48. The linked culture system according to any of items 33-47, wherein the production bioreactor has a dilution rate of about 1 to about 0.1 volume per day.

49. A protein of interest produced by a method comprising:

(a) culturing cells comprising a gene that encodes the protein of interest in a culture bioreactor (N-1 bioreactor);

(b) inoculating a production bioreactor (N bioreactor) with cells obtained from step (a); and

(c) culturing the cells in the production bioreactor under conditions that allow production of the protein of interest.

50. The protein of interest according to item 49, wherein the method further comprises step (d) harvesting the protein of interest from the production bioreactor.

51. The protein of interest according to any of items 49-50, wherein the culture bioreactor is a continuous perfusion culture bioreactor and the continuous production bioreactor is a continuously stirred tank reactor (CSTR) production bioreactor.

52. The protein of interest according to any of items 49-51, wherein the production bioreactor has no cell retention device.

53. The protein of interest according to any of items 49-51, wherein volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:20 or about 1:1 to about 1:5 or about 1:5.

54. The protein of interest according to any of items 49-53, wherein the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor.

55. The protein of interest according to item 54, wherein the cell transfer is by cell bleed in continuous or semi-continuous modes.

56. The protein of interest according to any of items 49-55, wherein step (a) optionally alternates between a first and second culture bioreactors to allow for renewal and continuous production of culture cells for use in step (b).

57. The protein of interest according to item 56, wherein the second culture bioreactor is a continuous perfusion culture bioreactor.

58. The protein of interest according to any of items 49-57, wherein the production bioreactor operates continuously for a period of greater than 3 weeks or for a period of greater than 4 weeks or for a period of greater than 5 weeks or for a period of greater than 6 weeks.

59. The protein of interest according to any of items 49-58, wherein harvesting step (d) is continuous.

60. The protein of interest according to any of items 49-59, wherein the protein of interest is an antibody or a fusion protein.

61. The protein of interest according to any of items 49-60, wherein the cells are CHO cells, HEK-293 cells, VERO cells, NSO cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells or COS cells.

CLAIMS

What is claimed is:

1. A method of producing a protein of interest, comprising:
   (a) culturing cells comprising a gene that encodes the protein of interest in a culture bioreactor (N-1 bioreactor);
   (b) inoculating a production bioreactor (N bioreactor) with cells obtained from step (a); and
   (c) culturing the cells in the production bioreactor under conditions that allow production of the protein of interest.
2. The method according to claim 1, wherein the method further comprises step (d) harvesting the protein of interest from the production bioreactor.
3. The method according to any of the preceding claims, wherein the culture bioreactor is a continuous perfusion culture bioreactor and the production bioreactor is a continuously stirred tank reactor (CSTR) production bioreactor and/or

the production bioreactor has no cell retention device.

4. The method according to any of the preceding claims, wherein volume ratio of the culture bioreactor to the production bioreactor is 1:1 to 1:20 or 1:1 to 1:5 or 1:5.
5. The method according to any of the preceding claims, wherein the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor.
6. The method according to claim 5, wherein the cell transfer is by cell bleed in continuous or semi-continuous modes.
7. The method according to claim 6, wherein the cell transfer is in semi-continuous mode comprising the cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween.
8. The method according to any of the preceding claims, wherein step (a) optionally alternates between a first and second culture bioreactors to allow for renewal and continuous production of culture cells for use in step (b).

9. The method according to claim 8, wherein the second culture bioreactor is a continuous perfusion culture bioreactor.
10. The method according to any of the preceding claims, wherein the production bioreactor operates continuously for a period of greater than 3 weeks or for a period of greater than 4 weeks or for a period of greater than 5 weeks or for a period of greater than 6 weeks.
11. The method according to any of the preceding claims, wherein harvesting step (d) is continuous.
12. The method according to any of the preceding claims, wherein the cells are CHO cells, HEK-293 cells, VERO cells, NSO cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells or COS cells.
13. The method according to any of the preceding claims, wherein the production bioreactor has a volumetric productivity of at least 0.6 grams per liter per day for a period of at least 14 days or for a period of at least 20 days or for a period of at least 30 days.
14. The method according to any of the preceding claims, wherein the production bioreactor has a product residence time of about 1 to about 10 days.
15. The method according to any of the preceding claims, wherein the production bioreactor has a dilution rate of about 1 to about 0.1 volume per day.
16. A linked culture process, comprising:

    (a) culturing cells comprising a gene that encodes the protein of interest in a culture bioreactor (N-1 bioreactor);

    (b) inoculating a production bioreactor (N bioreactor) with cells obtained from step (a); and

    (c) culturing the cells in the production bioreactor under conditions that allow production of the protein of interest.

17. A linked culture system, comprising:

    a culture bioreactor (N-1 bioreactor) for culturing cells comprising a gene that encodes the protein of interest; and

    a production bioreactor (N bioreactor) which is linked to the culture bioreactor and receives cells as inoculum from the culture bioreactor, wherein the production bioreactor operates under conditions that allow production of the protein of interest.

18. The linked culture system according to claim 17, wherein the system is linked to a downstream purification system for harvesting the protein of interest from the production bioreactor.

19. A protein of interest produced by a method comprising:

(a) culturing cells comprising a gene that encodes the protein of interest in a culture bioreactor (N-1 bioreactor);

(b) inoculating a production bioreactor (N bioreactor) with cells obtained from step (a); and

(c) culturing the cells in the production bioreactor under conditions that allow production of the protein of interest.

20. The protein of interest according to claim 19, wherein the protein of interest is an antibody or a fusion protein.