The subject technology also relates to a linked culture system, including: 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.

In another aspect, the subject technology relates to 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, wherein the production bioreactor receives cells from the culture bioreactor by cell bleed in semi-continuous mode comprising a cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween.

In one or more examples relating directly or indirectly to the above, the system is linked to a downstream purification system for harvesting the protein of interest from the production bioreactor; the culture bioreactor is a continuous perfusion culture bioreactor and the production bioreactor is a continuously stirred tank reactor (CSTR) production bioreactor; the production bioreactor has no cell retention device; volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:20; volume ratio of the culture bioreactor to the production bioreactor is about 1:1 to about 1:5; volume ratio of the culture bioreactor to the production bioreactor is about 1:5; the inoculation is by transferring cells from the culture bioreactor to the production bioreactor; the cell transfer is by cell bleed in continuous or semi-continuous modes; 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; 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; the second culture bioreactor is a continuous perfusion culture bioreactor; the production bioreactor operates continuously for a period of greater than 3 weeks; the production bioreactor operates continuously for a period of greater than 4 weeks; the production bioreactor operates continuously for a period of greater than 5 weeks; the production bioreactor operates

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, wherein the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor, wherein the cell transfer is by cell bleed 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.
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 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).

6. The method according to claim 5, wherein the second culture bioreactor is a continuous perfusion culture bioreactor.
7. 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.
8. The method according to any of claims 2 to 7, wherein harvesting step (d) is continuous.
9. 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.
10. The method according to any of the preceding claims, wherein the production bioreactor has a product residence time of 1 to 10 days.
11. The method according to any of the preceding claims, wherein the production bioreactor has a dilution rate of 1 to 0.1 volume per day.
12. 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, wherein the inoculation in step (b) is by transferring cells from the culture bioreactor to the production bioreactor, wherein the cell transfer is by cell bleed 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.

13. 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, wherein the production bioreactor receives cells from the culture bioreactor by cell bleed in semi-continuous mode comprising a cell transfer once at every period of time from 2 minutes to 24 hours or at any interval therebetween.

14. The linked culture system according to claim 13, wherein the system is linked to a downstream purification system for harvesting the protein of interest from the production bioreactor.