control of perfusion or HIPCOP" are used to describe the process by which cells control their own rate of perfusion. Volumetric productivities of equal to about, or greater than, 1 gram/L/day for moderate specific productivity cell lines are achieved with very modest medium volumes, comparatively simple bioreactor operations, and a batch length that fits in a standard fed-batch window. The volumetric productivity achieved is more than double what one might achieve with the same cell line in an optimized fed-batch culture.

The subject technology relates to a continuous perfusion culture process comprising monitoring pH in a cell culture in perfusion bioreactor with a pH controller or sensor, activating a medium perfusion pump delivering fresh medium and a permeate perfusion pump removing a nearly equivalent volume of permeate when the pH increases above a setpoint or predetermined value, and deactivating the medium perfusion pump and permeate perfusion pump when the pH decreases below a setpoint or predetermined value. The subject technology relates to a continuous perfusion culture process, including: (a) monitoring pH in a cell culture with a pH sensor; (b) delivering fresh medium and removing permeate when the pH is above a predetermined value; and (c) deactivating the medium delivery and the permeate removal when the pH decreases below the predetermined value. In one aspect, the subject technology relates to a continuous perfusion culture process, comprising: monitoring pH in a cell culture with a pH sensor; delivering fresh medium and removing permeate when the pH is above a predetermined value; and deactivating the medium delivery and the permeate removal when the pH is below the predetermined value, wherein the fresh medium comprises L-lactate. In essence, the pH change in the cell culture medium is a result of the cell metabolism which then triggers the perfusion process. Alternatively, the method may be described as a cell-controlled perfusion process without a need to measure glucose concentration. The pH trigger for turning on and off the perfusion pumps, fresh medium and permeate pumps, is set at a predetermined value. This predetermined value is about pH 7 (e.g., between 6.8-7.4). The medium comprises glucose, L-lactate and a specified ratio of amino acid to glucose. According to this subject technology, the activation and deactivation of the medium perfusion pump and the permeate perfusion pump may occur simultaneously or independently. Also, the permeate may be cell-free or may contain cells.

The described method also comprises adding L-lactate to a fresh perfusion medium used in the continuous culture process. In an embodiment, the L-lactate present in the perfusion medium is in an amount of about 0.1 g/L to about 7.0 g/L. More preferably, the L-lactate present in the perfusion medium is in an amount of about 1 to about 4 g/L, about 1 to about 3 g/L, or

The subject technology also relates to a method for achieving rapid cell growth in a perfusion culture process, comprising: (a) monitoring pH in a cell culture in a perfusion bioreactor with a pH sensor; (b) delivering fresh medium and removing permeate when the pH is above a predetermined value; and (c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value. In another aspect, the subject technology relates to method for achieving rapid cell growth in a continuous perfusion culture process, comprising: monitoring pH in a cell culture with a pH sensor; delivering fresh medium and removing permeate when the pH is above a predetermined value; and (c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value, wherein the fresh medium comprises: (a) between about 0.5 and 40 g/L glucose; (b) between about 0.1 to 7.0 g/L L-lactate; and (c) amino acids in a ratio of between about 0.25 and 1.0 of mole glucose to mole amino acids. In one or more embodiments related, directly or indirectly, to the above to each other, the fresh medium comprises L-lactate; L-lactate is in an amount of about 0.1 g/L to 7.0 g/L; alternatively or in addition to L-lactate, additional sodium bicarbonate is added to the perfusion bioreactor in an amount of about 1 to about 2.5 g/L such that the total sodium bicarbonate in the culture medium is about 2 to about 5.5 g/L and wherein the additional sodium bicarbonate is delivered to the perfusion bioreactor when the pH is above the predetermined value; the additional sodium bicarbonate is added to the perfusion bioreactor such that 1 molar carbonate enters the perfusion bioreactor at a rate of 8.7 mL per 1 liter of perfusion medium utilized; alternatively or in addition to L-lactate, any other physiologically acceptable base such as sodium carbonate, potassium carbonate, or the like is added to the perfusion bioreactor in an amount that would provide an upward influence on the pH in a manner similar to that which would occur by the addition of L-lactate; the fresh medium includes: (a) glucose; (b) L-lactate and/or additional sodium bicarbonate; and (c) amino acids; the fresh medium includes: (a) between about 0.5 to about 40 g/L glucose; (b) between about 0.1 to about 7 g/L L-lactate and/or between about 2 to about 5.5 g/L sodium bicarbonate; and (c) amino acids in a mole-of-glucose-to-mole-of-amino-acids ratio of between about 0.25 to about 1.0 ; the fresh medium includes glucose in an amount equal to about 70 mM of amino acids and about 5.3 grams of glucose per liter of medium; the fresh medium includes glucose at an amino-acids-(in mM)-to-glucose-(in g/L) ratio selected from the group consisting of about 60 to about 4.2; about 90 to about 8; about 100 to about 12; about 120 to about 13; about 240 to about 42 and about 380 to

about 70 per liter of medium; the predetermined pH value is about pH 7 or is about 6.8 to about 7.4; a measurement of glucose concentration in the cell culture or addition of glucose to the cell culture by a glucose pump is not required; a viable cell density of about 60 x 10^6/mL is achieved within 4 days.

The subject technology also relates to a hybrid perfusion/fed-batch culture process. In another aspect, the subject technology relates to a hybrid culture process, comprising: the continuous perfusion culture process as defined herein as the first culture process. This process comprising using the continuous perfusion process described above in combination with a fed-batch process. According to the subject technology, the process would

in a cell culture with a pH sensor; (b) delivering fresh medium and removing permeate when the pH is above a predetermined value; (c) and deactivating the medium delivery and the permeate removal when the pH is below the predetermined value.; and wherein the second continuous perfusion culture process includes steps of (a) adding a concentrated medium; and (b) adding a diluent. In a related embodiment, the hybrid culture process optionally includes an intervening fed-batch step. In this embodiment, the first continuous perfusion culture process is carried out first followed by a fed-batch step which is then followed by the second continuous perfusion culture process. In one or more embodiments related, directly or indirectly, to the above to each other, the continuous perfusion culture process is the first culture process; the continuous perfusion culture process is followed by a fed-batch process; the continuous perfusion culture process is followed by a second continuous perfusion culture process; the second continuous perfusion culture process comprises the steps of: (a) adding a concentrated medium; and (b) adding a diluent; the concentrated medium comprises 600 millimolar amino acids, 90 grams/liter glucose, 0 g/L sodium L-lactate; the diluent is selected from the group consisting of saline and water; the saline or water normalizes osmotic strength of culture medium to between about 0 to 250 and/or 250 to 350 mOsm/kg; the saline comprises 2.0 g/L sodium bicarbonate, 2.4 g/L polyvinyl alcohol, 20 mM potassium chloride, and 80 mM sodium chloride; the hybrid culture process results in volumetric productivity of greater than 1 gram cells/L/day; the hybrid culture process is performed in a culture volume of about 50 and about 150 L; the culture volume is about 70 L.

The subject technology also relates to a bioreactor for conducting a continuous perfusion culture process, wherein the continuous perfusion culture process includes steps of: (a) monitoring pH in a cell culture in a perfusion bioreactor with a pH sensor; (b) delivering fresh medium and removing permeate when the pH is above a predetermined value; (c) and deactivating the medium delivery and the permeate removal when the pH is below the predetermined value. In one or more embodiments related, directly or indirectly, to

the above to each other, the fresh medium comprises: (a) glucose; (b) L-lactate; and (c) amino acids; L-lactate is in an amount of about 0.1 g/L to 7.0 g/L ; alternatively or in addition to L-lactate, additional sodium bicarbonate is added to the perfusion bioreactor in an amount of about 1 to about 2.5 g/L such that the total sodium bicarbonate in the culture medium is about 2 to about 5.5 g/L and wherein the additional sodium bicarbonate is delivered to the perfusion bioreactor when the pH

is above the predetermined value; the additional sodium bicarbonate is added to the perfusion bioreactor such that 1 molar carbonate enters the perfusion bioreactor at a rate of 8.7 mL per 1 liter of perfusion medium utilized; alternatively or in addition to L-lactate, any other physiologically acceptable base such as sodium carbonate, potassium carbonate, or the like is added to the perfusion bioreactor in an amount that would provide an upward influence on the pH in a manner similar to that which would occur by the addition of L-lactate; the fresh medium includes: (a) glucose; (b) L-lactate and/or additional sodium bicarbonate; and (c) amino acids; the fresh medium includes: (a) between about 0.5 to about 40 g/L glucose; (b) between about 0.1 to about 7 g/L L-lactate and/or between about 2 to about 5.5 g/L sodium bicarbonate; and (c) amino acids in a mole-of-glucose-to-mole-of-amino-acids ratio of between about 0.25 to about 1.0 ; the fresh medium includes glucose in an amount equal to about 70 mM of amino acids and about 5.3 grams of glucose per liter of medium; the fresh medium includes glucose at an amino-acids-(in mM)-to-glucose-(in g/L) ratio selected from the group consisting of about 60 to about 4.2; about 90 to about 8; about 100 to about 12; about 120 to about 13; about 240 to about 42 and about 380 to about 70 per liter of medium; the predetermined pH value is about pH 7 or is about 6.8 to about 7.4; a measurement of glucose concentration in the cell culture or addition of glucose to the cell culture by a glucose pump is not required.

In another aspect, the subject technology relates to a perfusion culture process, comprising: (a) monitoring pH in a cell culture in a perfusion bioreactor with a pH sensor; (b) delivering fresh medium to the perfusion bioreactor and removing permeate when the pH is above a predetermined value; and (c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value, wherein the fresh medium comprises L-lactate.

In another aspect, the subject technology relates to a method for achieving rapid cell growth in a perfusion culture process, comprising: (a) monitoring pH in a cell culture in a perfusion bioreactor with a pH sensor; (b) delivering fresh medium to the perfusion bioreactor and removing permeate when the pH is above a predetermined value; and (c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value, wherein the fresh medium comprises L-lactate is in an amount of about 0.1 g/L to 7.0 g/L.

The subject technology also relates to a protein of interest produced by a method including: (a) culturing cells comprising a gene that encodes the protein of interest in a perfusion cell culture bioreactor under conditions that allow production of the protein of interest including: (i) monitoring pH in the cell culture in a perfusion bioreactor with a pH sensor; (ii) delivering fresh medium and removing permeate when the pH is above a predetermined value; and (iii) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value, (b) harvesting the protein of interest from the cell culture bioreactor. In one or more embodiments related, directly or indirectly, to the above to each other, the fresh medium includes: (a) glucose, (b) L-lactate, and (c) amino acids; L-lactate is in an amount of about 0.1 g/L to 7.0 g/L; alternatively or in addition to L-lactate, additional sodium bicarbonate is added to the perfusion bioreactor in an amount of about 1 to about 2.5 g/L such that the total sodium bicarbonate in the culture medium is about 2 to about 5.5 g/L and wherein the additional sodium bicarbonate is delivered to the perfusion bioreactor when the pH is above the predetermined value; the additional sodium bicarbonate is added to the perfusion bioreactor such

CLAIMS

What is claimed is:

1. A continuous perfusion culture process, comprising:

(a) monitoring pH in a cell culture with a pH sensor;
(b) delivering fresh medium and removing permeate when the pH is above a predetermined value; and
(c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value,

wherein the fresh medium comprises L-lactate.

2. The process according to claim 1, wherein the L-lactate is present in the fresh medium in an amount of about 0.1 g/L to 7.0 g/L.

3. The process according to claim 2, wherein the L-lactate is present in the fresh medium in an amount of about 1 to 4 g/L.

4. The process according to claim 3, wherein the L-lactate is present in the fresh medium in an amount of about 1 to 3 g/L.

5. The process according to claim 4, wherein the L-lactate is present in the fresh medium in an amount of about 1 to 2.5 g/L.

6. The process according to claim 1, wherein the L-lactate is sodium L-lactate or potassium L-lactate.

7. The process according to claim 1, wherein the fresh medium comprises:

(a) glucose;
and
(b) amino acids.

8. The process according to claim 1, wherein the fresh medium comprises:

(a) between about 0.5 and 40 g/L glucose;

(b) between about 0.1 and 7 g/L L-lactate; and

(c) amino acids in a ratio of between about 0.25 and 1.0 mole glucose to mole amino acids.

9. The process according to claim 7, wherein the amino acids and glucose are provided in an amount equal to 70 mM amino acids and 5.3 grams of glucose per liter of medium or at ratios selected from the group consisting of 60:4.2; 90:8; 100:12; 120:13; 240:42 and 380:70 mM amino acids:grams/L glucose per liter of medium.

10. The process according to claim 1, wherein the predetermined value is about pH 7.

11. The process according to claim 1, wherein the measurement of glucose concentration in the cell culture or addition of glucose to the cell culture by a glucose pump is not required.

12. A method for achieving rapid cell growth in a continuous perfusion culture process, comprising:

(a) monitoring pH in a cell culture with a pH sensor;

(b) delivering fresh medium and removing permeate when the pH is above a predetermined value; and

(c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value,

wherein the fresh medium comprises:

(a) between about 0.5 and 40 g/L glucose;

(b) between about 0.1 to 7.0 g/L L-lactate; and

(c) amino acids in a ratio of between about 0.25 and 1.0 of mole glucose to mole amino acids.

13. The method of claim 12, wherein a viable cell density of 60 x 10^6/mL is achieved within 4 days.

14. The method of claim 12, wherein the measurement of glucose concentration in the cell culture or addition of glucose to the cell culture by a glucose pump is not required.

15. A hybrid culture process, comprising: the continuous perfusion culture process of claim 1 as the first culture process.

16. The hybrid culture process of claim 15, wherein the continuous perfusion culture process is followed by a fed-batch process.

17. The hybrid culture process of claim 15, wherein the continuous perfusion culture process is followed by a second continuous perfusion culture process.

18. The hybrid culture process of claim 17, wherein the second continuous perfusion culture process comprises the steps of:

(a) adding a concentrated medium; and

(b) adding a diluent.

19. The hybrid culture process of claim 18, wherein the concentrated medium comprises 600 millimolar amino acids, 90 grams/liter glucose, 0 g/L sodium L-lactate.

20. The hybrid culture process of claim 18, wherein the diluent is selected from the group consisting of saline and water.

21. The hybrid culture process of claim 20, wherein the saline or water normalizes osmotic strength of culture medium to between about 0 to 250 and/or 250 to 350 mOsm/kg.

22. The hybrid culture process of claim 20, wherein the saline comprises 2.0 g/L sodium bicarbonate, 2.4 g/L polyvinyl alcohol, 20 mM potassium chloride, and 80 mM sodium chloride.

23. The hybrid culture process of any of claims 15-22, wherein the hybrid culture process results in volumetric productivity of greater than 1 gram cells/L/day.

24. The hybrid culture process of any of claims 15-22, wherein the hybrid culture process is performed in a culture volume of about 50 and about 150 L.

25. The hybrid culture process of claim 24, wherein the culture volume is about 70 L.

26. A perfusion culture process, comprising:

(a) monitoring pH in a cell culture in a perfusion bioreactor with a pH sensor;

(b) delivering fresh medium to the perfusion bioreactor and removing permeate when the pH is above a predetermined value; and

(c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value,

wherein the fresh medium comprises L-lactate.

27. The perfusion culture process according to claim 26, wherein L-lactate is present in the fresh medium in an amount of about 0.1 g/L to 7.0 g/L.

28. The perfusion culture process of according to claim 26, wherein in addition to L-lactate, sodium carbonate is added to the perfusion bioreactor when the pH is above the predetermined value.

29. The perfusion culture process of according to claim 28, wherein the sodium carbonate is added to the perfusion bioreactor such that 1 molar carbonate enters the perfusion bioreactor at a rate of 8.7 mL per 1 liter of perfusion medium utilized.

30. The perfusion culture process of according to claim 26, wherein in addition to L-lactate, a physiologically acceptable base is added to the perfusion bioreactor to provide an upward influence on the pH in a manner similar to that which would occur by the consumption of lactate from the perfusion medium as lactic acid.

31. The perfusion culture process according to claim 26, wherein the L-lactate is sodium L-lactate or potassium L-lactate.

32. The perfusion culture process according to claim 26, wherein the fresh medium comprises:

(a) glucose;
and
(b) amino acids.

33. The perfusion culture process according to claim 32, wherein the fresh medium comprises:

(a) between about 0.5 to about 40 g/L glucose;
(b) between about 0.1 to about 7 g/L L-lactate; and
(c) amino acids in a mole-of-glucose-to-mole-of-amino-acids ratio of about 0.25 to about 1.0.

34. The perfusion culture process according to claim 26, wherein the fresh medium comprises about 5.3 grams of glucose per liter of medium and amino acids in an amount equal to about 70 mM, or wherein the fresh medium comprises glucose at an amino-acids-(in mM)-to-glucose-(in g/L) ratio selected from the group consisting of about 60 to about 4.2; about 90 to about 8; about 100 to about 12; about 120 to about 13; about 240 to about 42 and about 380 to about 70 per liter of medium.

35. The perfusion culture process according to claim 26, wherein the predetermined value is about pH 7.

36. The perfusion culture process according to claim 26, wherein a measurement of glucose concentration in the cell culture or addition of glucose to the cell culture by a glucose pump is not required.

37. A method for achieving rapid cell growth in a perfusion culture process, comprising:

(a) monitoring pH in a cell culture in a perfusion bioreactor with a pH sensor;
(b) delivering fresh medium to the perfusion bioreactor and removing permeate when the pH is above a predetermined value; and
(c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value,

wherein the fresh medium comprises L-lactate is in an amount of about 0.1 g/L to 7.0 g/L.

38. The method of claim 37, wherein the fresh medium comprises:

(a) between about 0.5 to about 40 g/L glucose;
(b) between about 0.1 to about 7.0 g/L L-lactate; and
(c) amino acids in a mole-of-glucose-to-mole-of-amino-acids ratio of about 0.25 to about 1.0.

39. The method of claim 38, wherein in addition to L-lactate, sodium carbonate is added to the perfusion bioreactor when the pH is above the predetermined value.

40. The method of claim 39, wherein the sodium carbonate is added to the perfusion bioreactor such that 1 molar carbonate enters the perfusion bioreactor at a rate of 8.7 mL per 1 liter of perfusion medium utilized.

41. The method of claim 37, wherein a measurement of glucose concentration in the cell culture or addition of glucose to the cell culture by a glucose pump is not required.

42. The method according to any of claims 37-41, wherein a viable cell density of 60 x 10^6/mL is achieved within 4 days.