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.

2. The process according to claim 1, wherein the fresh medium comprises L-lactate.

3. The process according to claim 2, wherein the L-lactate is present in the fresh medium in an amount of about 0.1 g/L to 7.0 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 4 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 3 g/L.

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

7. The process according to claim 2, wherein the L-lactate is sodium L-lactate or potassium L-lactate.

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

(a) glucose;
(b) L-lactate; and
(c) amino acids.

9. 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.

10. The process according to claim 8, 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.

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

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

13. 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.

14. The method of claim 13, 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.

15. The method of claim 13, wherein the rapid cell growth is achieved within 4 days.

16. The method of claim 13, wherein the measurement of glucose concentration in the cell culture or additional of glucose to the cell culture by a glucose pump is not required.

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

18. The hybrid culture process of claim 17, wherein the continuous perfusion culture process is followed by a fed-batch process.

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

20. The hybrid culture process of claim 19, wherein the second continuous perfusion culture process comprises the steps of:

(a) adding a concentrated medium; and

(b) adding a diluent.

21. The hybrid culture process of claim 20, wherein the concentrated medium comprises 600 millimolar amino acids, 90 grams/liter glucose, 0 g/L sodium L-lactate.

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

23. The hybrid culture process of claim 22, wherein the saline or water normalizes osmotic strength of culture medium to between about 0 to 250 and/or 250 to 350 mOsm/kg.

24. The hybrid culture process of claim 22, 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.

25. The hybrid culture process of any of claims 17-24, wherein the hybrid culture process results in volumetric productivity of greater than 1 gram cells/L/day.

26. The hybrid culture process of any of claims 17-24, wherein the hybrid culture process is performed in a culture volume of about 50 and about 150 L.

27. The hybrid culture process of claim 26, wherein the culture volume is about 70 L.

28. A bioreactor for conducting a continuous perfusion culture process, wherein the continuous perfusion culture process comprises the steps of:

(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.

29. The bioreactor of claim 28, wherein the fresh medium comprises:

(a) glucose;
(b) between about 0.1 to 7.0 g/L L-lactate; and
(c) amino acids.

30. The bioreactor of claim 29, wherein the glucose is provided between about 0.5 and 40 g/L.

31. The bioreactor of claim 29, 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.

32. The bioreactor of claim 28, wherein a viable cell density of about 60 x 10^6/mL is achieved within 4 days.

33. The bioreactor of claim 28, wherein the measurement of glucose concentration in the cell culture or additional of glucose to the cell culture by a glucose pump is not required.

34. The bioreactor of claim 28, wherein the predetermined pH value is between 6.8 and 7.4.

35. A perfusion culture process, comprising:

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

36. The perfusion culture process according to claim 35, wherein the fresh medium comprises L-lactate .

37. The perfusion culture process according to claim 36, wherein L-lactate is present in the fresh medium in an amount of about 0.1 g/L to 7.0 g/L.

38. The perfusion culture process of according to claim 36, wherein alternatively or in addition to L-lactate, sodium carbonate is added to the perfusion bioreactor when the pH is above the predetermined value.

39. The perfusion culture process of according to claim 38, 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.

40. The perfusion culture process of according to claim 36, wherein alternatively or 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.

41. The perfusion culture process according to claim 36, wherein the L-lactate is sodium L-lactate or potassium L-lactate.

42. The perfusion culture process according to claim 36, wherein the fresh medium comprises:

(d) glucose;
(e) L-lactate; and
(f) amino acids.

43. The perfusion culture process according to claim 42, wherein the fresh medium comprises:

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

44. The perfusion culture process according to claim 35, wherein the fresh medium comprises glucose in an amount equal to about 70 mM of amino acids and about 5.3 grams of glucose per liter of medium 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.

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

46. The perfusion culture process according to claim 35, wherein a measurement of glucose concentration in the cell culture or additional of glucose to the cell culture by a glucose pump is not required.

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

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

48. The method of claim 47, wherein the fresh medium comprises L-lactate is in an amount of about 0.1 g/L to 7.0 g/L.

49. The method of claim 48, wherein the fresh medium comprises:

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

50. The method of claim 49, wherein alternatively or in addition to L-lactate, sodium carbonate is added to the perfusion bioreactor when the pH is above the predetermined value.

51. The method of claim 50, 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.

52. The method of claim 46, wherein the rapid cell growth is achieved within 4 days.

53. The method of claim 46, wherein a measurement of glucose concentration in the cell culture or additional of glucose to the cell culture by a glucose pump is not required.20. The according to any of claims 46-53, wherein a viable cell density of about 60 x 10^6/mL is achieved within 4 days.

54. A bioreactor for conducting a continuous perfusion culture process, wherein the continuous perfusion culture process comprises the steps of:

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

55. The bioreactor of claim 54, wherein the fresh medium comprises:

(d) glucose;
(e) L-lactate; and
(f) amino acids.

56. The bioreactor of claim 54, wherein L-lactate is in an amount of about 0.1 g/L to 7.0 g/L.

57. The bioreactor of claim 55, wherein alternatively or in addition to L-lactate, sodium carbonate is added to the perfusion bioreactor when the pH is above the predetermined value.

58. The method of claim 57, 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.

59. The bioreactor of claim 54, wherein a measurement of glucose concentration in the cell culture or additional of glucose to the cell culture by a glucose pump is not required.

60. The bioreactor of claim 54, wherein the predetermined pH value is between 6.8 and 7.4.

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

(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 comprising:

i. monitoring pH in the cell culture 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.

62. The protein of interest according to claim 61, wherein the fresh medium comprises:

(g) glucose;

(h) L-lactate; and

(i) amino acids.

63. The protein of interest according to claim 62, wherein L-lactate is in an amount of about 0.1 g/L to 7.0 g/L.

64. The protein of interest according to claim 61, wherein a measurement of glucose concentration in the cell culture or additional of glucose to the cell culture by a glucose pump is not required.

65. The protein of interest according to claim 61, wherein the predetermined pH value is between 6.8 and 7.4.

66. The protein of interest according to claim 62, wherein alternatively or in addition to L-lactate, sodium carbonate is added to the perfusion bioreactor when the pH is above the predetermined value.

67. The protein of interest according to claim 66, 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.