Claims

What is claimed is:

1. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:

providing a cell culture comprising;

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a medium containing glutamine and having a medium characteristic selected from the group consisting of: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (v) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

2. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:

providing a cell culture comprising;

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM; and

said medium containing glutamine; and

said medium having two medium characteristics selected from the group consisting of: (i) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

3. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:

providing a cell culture comprising;

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a medium containing a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; and

said medium containing glutamine; and

said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a

viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

4. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:

providing a cell culture comprising;

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a medium containing a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; and

said medium containing glutamine; and

said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

5. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:

providing a cell culture comprising;

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a medium containing a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; and

said medium containing glutamine; and

said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

6. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:

providing a cell culture comprising;

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a medium containing a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM; and

said medium containing glutamine; and

said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and combinations thereof;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

7. The method of claim 1, wherein said cell culture condition in said changing at least one of the culture conditions step is selected from the group consisting of: (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.

8. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 13 mM.

9. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 10 mM.

10. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 7 mM.

11. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 4 mM.

12. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 13 mM.

13. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 10 mM.

14. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 7 mM.

15. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 4 mM.

16. The method of claim 1, wherein glutamine is only provided in the initial medium at the beginning of the cell culture.

17. The method of claim 1, wherein the concentration of soluble iron in the media is greater than 5 μM.

18. The method of claim 1, wherein viable cell density of said culture is measured on a periodic basis.

19. The method of claim 1, wherein viability of said culture is measured on a periodic basis.

20. The method of claim 1, wherein said lactate levels of said culture is measured on a periodic basis.

21. The method of claim 1, wherein said ammonium levels of said culture is measured on a periodic basis.

22. The method of claim 1, wherein said titer of said culture is measured on a periodic basis.

23. The method of claim 1, wherein osmolarity of said culture is measured on a periodic basis.

24. The method of claims 18-23, wherein said measurements are taken daily.

25. The method of claim 1, wherein the initial density of said mammalian cells is at least $2x10^2$ cells/mL.

26. The method of claim 1, wherein the initial density of said mammalian cells is at least $2x10^3$ cells/mL.

27. The method of claim 1, wherein the initial density of said mammalian cells is at least $2x10^4$ cells/mL.

28. The method of claim 1, wherein the initial density of said mammalian cells is at least $2x10^5$ cells/mL.

29. The method of claim 1, wherein the initial density of said mammalian cells is at least $2x10^6$ cells/mL.

30. The method of claim 1, wherein the initial density of said mammalian cells is at least $5x10^6$ cells/mL.

31. The method of claim 1, wherein the initial density of said mammalian cells is at least $10x10^6$ cells/mL.

32. The method of claim 1, wherein the step of providing comprises providing at least about 1000 L of a culture.

33. The method of claim 1, wherein the step of providing comprises providing at least about 2500 L of a culture.

34. The method of claim 1, wherein the step of providing comprises providing at least about 5000 L of a culture.

35. The method of claim 1, wherein the step of providing comprises providing at least about 8000 L of a culture.

36. The method of claim 1, wherein the step of providing comprises providing at least about 10,000 L of a culture.

37. The method of claim 1, wherein the step of providing comprises providing at least about 12,000 L of a culture.

38. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 30 to 42 degrees Celsius.

39. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 32 to 40 degrees Celsius.

40. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 34 to 38 degrees Celsius.

41. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 36 to 37 degrees Celsius.

42. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 37 degrees Celsius.

43. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 25 to 41 degrees Celsius.

44. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 27 to 38 degrees Celsius.

45. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 35 degrees Celsius.

46. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 33 degrees Celsius.

47. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 30 to 32 degrees Celsius.

48. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 31 degrees Celsius.

49. The method of claim 1, further comprising a second changing step subsequent to first said changing at least one of the culture conditions comprising changing at least one of the culture conditions, so that a third set of conditions is applied to the culture.

50. The method of claim 49, wherein the second changing step comprises changing at least one culture condition selected from the group consisting of: (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.

51. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 25 to 40 degrees Celsius.

52. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 27 to 37 degrees Celsius.

53. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 29 to 34 degrees Celsius.

54. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 30 to 32 degrees Celsius.

55. The method of claim 1, wherein said first period of time is between 0-8 days.

56. The method of claim 1, wherein said first period of time is between 1-7 days.

57. The method of claim 1, wherein said first period of time is between 2-6 days.

58. The method of claim 1, wherein said first period of time is between 3-5 days.

59. The method of claim 1, wherein said first period of time is approximately 4 days.

60. The method of claim 1, wherein said first period of time is approximately 5 days.

61. The method of claim 1, wherein said first period of time is approximately 6 days.

62. The method of claim 1, wherein the total of said first period of time and said second period of time is at least 5 days.

63. The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the lactate level decreases subsequent to the lactate level in the culture reaching a maximal level.

64. The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the ammonium level decreases subsequent to the ammonium level in the culture reaching a maximal level.

65. The method of claim 1, wherein said total amount of said produced polypeptide is at least 1.5-fold higher that the amount of polypeptide produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

66. The method of claim 1, wherein said total amount of said produced polypeptide is at least 2-fold higher that the amount of polypeptide produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

67. The method of claim 1, wherein said cell culture is further provided with supplementary components.

68. The method of claim 67, wherein said supplementary components are provided at multiple intervals.

69. The method of claim 67 wherein said supplementary components are selected from a group consisting of hormones and/or other growth factors, particular ions (such as sodium, chloride, calcium, magnesium, and phosphate), buffers, vitamins, nucleosides or nucleotides, trace elements (inorganic compounds usually present at very low final concentrations), amino acids, lipids, or glucose or other energy source.

70. A method of producing a polypeptide in a large-scale production cell culture comprising steps of;

providing a cell culture comprising;

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a defined medium containing glutamine and having at least two medium characteristics selected from the group consisting of: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

71. A method of producing a polypeptide in a large-scale production cell culture comprising steps of;

providing a cell culture comprising;

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a defined medium containing glutamine and having at least three medium characteristic selected from the group consisting of: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

72. A method of producing a polypeptide in a large-scale production cell culture comprising steps of;

providing a cell culture comprising;

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a defined medium containing glutamine and having at least four medium characteristic selected from the group consisting of: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a

range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

73. A method of producing a polypeptide in a large-scale production cell culture comprising steps of;

providing a cell culture comprising;

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a defined medium containing glutamine, characterized by: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

74. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:

providing a cell culture comprising;

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a medium containing glutamine and having a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that that the polypeptide accumulates in the cell culture.

75. The method of claim 1, wherein said medium comprises a medium containing glutamine and having a medium characteristic selected from the group consisting of:

(i) a starting amino acid concentration greater than about 70 mM, (ii) a molar starting glutamine to starting asparagine ratio of less than about 2, (iii) a molar starting glutamine to starting total amino acid ratio of less than about 0.2, (iv) a molar starting inorganic ion to starting total amino acid ratio between about 0.4 to 1, (v) a combined starting glutamine and starting asparagine concentration greater than about 16 mM, and combinations thereof.

76. The method of any one of claims 1-6 or 70-75, wherein:

lactate levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic;

ammonium levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic; and

total amount of produced polypeptide is at least as high as that observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

77. The method of claim 1, wherein said culture is not supplemented with additional components over the course of producing said polypeptide.

78. The method of claim 1, wherein said culture is not supplemented with additional glutamine over the course of producing said polypeptide.

79. The method of claim 1, wherein the glutamine concentration in said culture is substantially depleted prior to said step of changing to a second set of culture conditions.

80. The method of claim 1, wherein the glutamine concentration in said culture is substantially depleted at approximately the same time as said step of changing to a second set of culture conditions.

81. The method of claim 1, wherein glycylglutamine is substituted for glutamine in said culture.

82. The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (v) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 mM.

83. The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (iv) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 a mM.

84. The method of claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.

85. The method of claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.

86. The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.

87. The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.

88. The method of claim 1, wherein said medium has a medium characteristic selected from the group consisting of:

(i) a cumulative total amount of histidine per unit volume greater than approximately 1.7 mM;

(ii) a cumulative total amount of isoleucine per unit volume greater than approximately 3.5 mM;

(iii) a cumulative total amount of leucine per unit volume greater than approximately 5.5 mM;

(iv) a cumulative total amount of methionine per unit volume greater than approximately 2.0 mM;

(v) a cumulative total amount of phenylalanine per unit volume greater than approximately 2.5 mM;

(vi) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM;

(vii) a cumulative total amount of tryptophan per unit volume greater than approximately 1.0 mM;

(viii) a cumulative total amount of tyrosine per unit volume greater than approximately 2.0 mM; and

(ix) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM.

89. The method of claim 1, wherein said medium has a medium characteristic selected from the group consisting of:

(i) an initial amount of histidine per unit volume greater than approximately 1.7 mM;

(ii) an initial amount of isoleucine per unit volume greater than approximately 3.5 mM;

(iii) an initial amount of leucine per unit volume greater than approximately 5.5 mM;

(iv) an initial amount of methionine per unit volume greater than approximately 2.0 mM;

(v) an initial amount of phenylalanine per unit volume greater than approximately 2.5 mM;

(vi) an initial amount of proline per unit volume reater than approximately 2.5 mM;

(vii) an initial amount of tryptophan per unit volume greater than approximately 1.0 mM;

(viii) an initial amount of tyrosine per unit volume greater than approximately 2.0 mM; and

(ix) an initial amount of proline per unit volume greater than approximately 2.5 mM.

90. The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately 7 mM.

91. The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately 10 mM.

92. The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.

93. The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.

94. The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.

95. The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.

96. The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 2.5 mM.

97. The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 5 mM.

98. The method of claim 1, wherein the cumulative total amount of glutamate per unit volume in said medium is less than approximately 1 mM.

99. The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 8 mg/L.

100. The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 20 mg/L.

101. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 7 mg/L.

102. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 25 mg/L.

103. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 5 mg/L.

104. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 35 mg/L.

105. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 1.0 mg/L.

106. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 2.0 mg/L.

107. The method of claim 1, wherein the cumulative total amount of thiamine hydrochloride per unit volume in said medium is greater than approximately 7 mg/L.

108. The method of claim 1, wherein the cumulative total amount of thiamine hydrochloride per unit volume in said medium is greater than approximately 35 mg/L.

109. The method of any of claims 1-23 or claims 25-75 or claims 77-108, wherein the polypeptide is anti-GDF-8.

110. The method of any of claims 1-23 or claims 25-75 or claims 77-108, wherein the polypeptide is anti-LewY.

111. The method of claim 24, wherein the polypeptide is anti-GDF-8.

112. The method of claim 24, wherein the polypeptide is anti-LewY.

113. The method of claim 76, wherein the polypeptide is anti-GDF-8.

114. The method of claim 76, wherein the polypeptide is anti-LewY.

Claims

1. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:

providing a cell culture comprising:

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a medium containing glutamine, having a cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, and tyrosine per unit volume greater than 25 mM, a cumulative amino acid amount per unit volume greater than 70 mM, a molar cumulative glutamine to cumulative asparagine ratio of less than 2, and having a medium characteristic, wherein said medium characteristic is: (i) a molar cumulative glutamine to cumulative total amino acid ratio of less than 0.2, (ii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iii) a combined cumulative amount of glutamine and asparagine per unit volume of greater than 16 mM, or a combination thereof;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

2. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:

providing a cell culture comprising:

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a medium containing glutamine, having a cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, and tyrosine per unit

volume greater than 25 mM, a cumulative amino acid amount per unit volume greater than 70 mM, a molar cumulative glutamine to cumulative asparagine ratio of less than 2; and

said medium having two medium characteristics, wherein said medium characteristics are: (i) a molar cumulative glutamine to cumulative total amino acid ratio of less than 0.2, (ii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iii) a combined cumulative amount of glutamine and asparagine per unit volume of greater than 16 mM, or combinations thereof;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

3. The method of claim 1, wherein said cell culture condition in said changing at least one of the culture conditions step is: (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, or a combination thereof.

4. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 10 mM.

5. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 4 mM.

6. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 10 mM.

7. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 4 mM.

8. The method of claim 1, wherein glutamine is only provided in the initial medium at the beginning of the cell culture.

9. The method of claim 1, wherein the initial density of said mammalian cells is at least $2x10^5$ cells/mL.

10. The method of claim 1, wherein the initial density of said mammalian cells is at least $2x10^6$ cells/mL.

11. The method of claim 1, wherein the step of providing comprises providing at least about 1000 L of a culture.

12. The method of claim 1, wherein the step of providing comprises providing at least about 10,000 L of a culture.

13. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 30 to 42 degrees Celsius.

14. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 37 degrees Celsius.

15. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 25 to 41 degrees Celsius.

16. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 35 degrees Celsius.

17. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 31 degrees Celsius.

18. The method of claim 1, further comprising a second changing step subsequent to first said changing at least one of the culture conditions comprising changing at least one of the, culture conditions, so that a third set of conditions is applied to the culture.

19. The method of claim 18, wherein the second changing step comprises changing at least one culture condition, wherein the at least one culture condition is: (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, or a combination thereof.

20. The method of claim 18, wherein said third set of conditions comprises a third temperature range that is approximately 27 to 37 degrees Celsius.

21. The method of claim 1, wherein said first period of time is between 1-7 days.

22. The method of claim 1, wherein said first period of time is approximately 4 days.

23. The method of claim 1, wherein the total of said first period of time and said second period of time is at least 5 days.

24. The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the lactate level decreases subsequent to the lactate level in the culture reaching a maximal level.

25. The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the ammonium level decreases subsequent to the ammonium level in the culture reaching a maximal level.

26. The method of claim 1, wherein said total amount of said produced polypeptide is at least 1.5-fold higher that the amount of polypeptide produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

27. The method of claim 1, wherein said total amount of said produced polypeptide is at least 2-fold higher that the amount of polypeptide produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

28. The method of claim 1, wherein said cell culture is further provided with supplementary components, wherein the supplementary components are hormones and/or

other growth factors, particular ions, buffers, vitamins, nucleosides or nucleotides, trace elements, amino acids, lipids, or glucose or other energy source.

29. The method of claim 28, wherein said supplementary components are provided at multiple intervals.

30. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:

providing a cell culture comprising:

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a defined medium containing glutamine, a cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, and tyrosine per unit volume greater than 25 mM, having a cumulative amino acid amount per unit volume greater than 70mM, a molar cumulative glutamine to cumulative asparagine ratio of less than 2, and having a medium characteristic, wherein said medium characteristic is: i) a starting amino acid concentration greater than 70 mM, (ii) a molar glutamine to total amino acid ratio of less than 0.2, (iii) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, or (iv) a combined glutamine and asparagine concentration greater than 16 mM;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

31. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:

providing a cell culture comprising:

mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a defined medium containing glutamine, characterized by: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;

maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

32. The method of claim 1, wherein said medium comprises a medium containing glutamine and having a medium characteristic, wherein said medium characteristic is:

(i) a starting amino acid concentration greater than about 70 mM, (ii) a molar starting glutamine to starting asparagine ratio of less than about 2, (iii) a molar starting glutamine to starting total amino acid ratio of less than about 0.2, (iv) a molar starting inorganic ion to starting total amino acid ratio between about 0.4 to 1, (v) a combined starting glutamine and starting asparagine concentration greater than about 16 mM, or a combination thereof.

33. The method of any one of claims 1, 2 or 31-32, wherein:

lactate levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic;

ammonium levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic; and

total amount of produced polypeptide is at least as high as that observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

34. The method of claim 1, wherein said culture is not supplemented with additional components over the course of producing said polypeptide.

35. The method of claim 1, wherein glycylglutamine is substituted for glutamine in said culture.

36. The method of claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.

37. The method of claim 1, wherein said medium has a medium characteristic that is:

(i) a cumulative total amount of histidine per unit volume greater than approximately 1.7 mM;

(ii) a cumulative total amount of isoleucine per unit volume greater than approximately 3.5 mM;

(iii) a cumulative total amount of leucine per unit volume greater than approximately 5.5 mM;

(iv) a cumulative total amount of methionine per unit volume greater than approximately 2.0 mM;

(v) a cumulative total amount of phenylalanine per unit volume greater than approximately 2.5 mM;

(vi) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM;

(vii) a cumulative total amount of tryptophan per unit volume greater than approximately 1.0 mM; or

(viii) a cumulative total amount of tyrosine per unit volume greater than approximately 2.0 mM.

38. The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately 10 mM.

39. The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.

40. The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.

41. The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 5 mM.

42. The method of claim 1, wherein the cumulative total amount of glutamate per unit volume in said medium is less than approximately 1 mM.

43. The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 20 mg/L.

44. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 25 mg/L.

45. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 35 mg/L.

46. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 2.0 mg/L.

47. The method of claim 1, wherein the cumulative total amount of thiamine hydrochloride per unit volume in said medium is greater than approximately 35 mg/L.

48. The method of any of claims 1-47, wherein the polypeptide is anti-GDF-8.

49. The method of any of claims 1-47, wherein the polypeptide is anti-LewY.