

WHAT IS CLAIMED IS:

1. A method of predicting the effect of a concentration of a sensitizer on cell viability in a production bioreactor, the method comprising:
 - (a) determining cell viability under a plurality of test conditions in a test culture vessel, wherein the plurality of test conditions are defined by a set of parameters; and
 - (b) based on the cell viability in the test culture vessel under the plurality of conditions, predicting the effect of a concentration of a sensitizer on cell viability in a production bioreactor.
2. The method of claim 1, wherein the set of parameters comprises shear stress, concentration of the sensitizer, and concentration of a protectant.
3. The method of claim 1 or claim 2, wherein the plurality of testing conditions are selected based on full factorial screening and/or inscribed center composite design.
4. The method of any one of claims 1-3, wherein the sensitizer is an antifoam agent.
5. The method of claim 4, wherein the antifoam is a polydimethylsiloxane-based antifoam.
6. The method of claim 4, wherein the antifoam is simethicone.
7. The method of any one of claims 1-6, wherein the test culture vessel has a volume of about 5 mL to about 5 L.
8. The method of claim 7, wherein the test culture vessel has a volume of about 10 mL to about 500 mL.

9. The method of any one of claims 1-8, wherein the test culture vessel is a baffled shake flask.

10. The method of any one of claims 1-9, wherein the production bioreactor has a volume of about 5 L to about 20,000 L.

11. The method of claim 10, wherein the production bioreactor has a volume of about 50 L to about 10,000 L.

12. The method of any one of claims 1-11, wherein the production bioreactor is a perfusion bioreactor.

13. The method of any one of claims 1-11, wherein the production bioreactor is a fed batch bioreactor.

14. The method of any one of claims 1-13, further comprising culturing a cell in the production bioreactor using a concentration of the sensitizer predicted to have a specific cell death rate of less 40% per day during the culturing of the cell in the production bioreactor.

15. The method of any one of claims 1-13, further comprising culturing a cell in the production bioreactor using a concentration of the sensitizer predicted to have a specific cell death rate of less than 20% per day during the culturing of the cell in the production bioreactor.

16. The method of any one of claims 1-13, further comprising culturing a cell in the production bioreactor using a concentration of the sensitizer predicted to result in a cell specific LDH production of less than 150 nU/cell/day during the culturing of the cell in the production bioreactor.

17. The method of any one of claims 1-13, further comprising culturing a cell in the production bioreactor using a concentration of the sensitizer predicted to result in a cell specific LDH production of less than 100 nU/cell/day during the culturing of the cell in the production bioreactor.

18. The method of any one of claims 1-13, further comprising culturing a cell in the production bioreactor using a concentration of the sensitizer predicted to result in an apparent growth rate of greater than 1% per day during the culturing of the cell in the production bioreactor.

19. The method of any one of claims 1-13, further comprising culturing a cell in the production bioreactor using a concentration of the sensitizer predicted to result in an apparent growth rate of greater than 5% per day during the culturing of the cell in the production bioreactor.

20. The method of any one of claims 14-19, wherein the cell contains a nucleic acid encoding a recombinant therapeutic protein.

21. The method of claim 20, further comprising collecting the recombinant therapeutic protein.

22. The method of claim 21, further comprising purifying the recombinant therapeutic protein.

23. The method of claim 22, further comprising formulating the purified recombinant therapeutic protein.

24. The method of any one of claims 1-23, wherein the cell is a bacterium, a yeast, or a mammalian cell.

25. The method of any one of claims 1-24, wherein the cell viability is determined by measuring a concentration of lactic acid dehydrogenase (LDH) in the test culture vessel.

26. The method of any one of claims 1-8 and 10-25, wherein the test culture vessel is a baffled shake flask and the plurality of test conditions comprise one or more of:

- (a) a rotary agitation of about 125 RPM to about 400 RPM;
- (b) a concentration of the sensitizer of about 0 ppm to about 120 ppm; and
- (c) a concentration of a protectant that is about 1 g/L to about 10 g/L.

27. The method of claim 2 or 26, wherein the protectant is a poloxamer, a poloxamine, or a pluronic polyol.

28. The method of claim 27, wherein the poloxamer is poloxamer-188, poloxamer-401, poloxamer-402, or poloxamer-407.

29. The method of claim 27, wherein the poloxamine is poloxamine-904 or poloxamine-908.

30. A method of improving cell viability in a production bioreactor, the method comprising:

- (a) determining cell viability under a plurality of test conditions in a test culture vessel, wherein the plurality of conditions are defined by a set of parameters;
- (b) based on the cell viability in the test culture vessel under the plurality of conditions, selecting a condition that is optimal for cell viability in a production bioreactor; and
- (d) culturing a cell in the production bioreactor under the selected condition.

31. The method of claim 30, wherein the set of parameters comprises shear stress, concentration of a sensitizer, and concentration of a protectant.

32. The method of claim 30 or claim 31, wherein the plurality of testing conditions are selected based on full factorial screening and/or inscribed center composite design.

33. The method of claim 31 or 32, wherein the sensitizer is an antifoam agent.

34. The method of claim 33, wherein the antifoam is a polydimethylsiloxane-based antifoam.

35. The method of claim 33, wherein the antifoam is simethicone.

36. The method of any one of claims 30-35, wherein the test culture vessel has a volume of about 5 mL to about 5 L.

37. The method of claim 36, wherein the test culture vessel has a volume of about 10 mL to about 500 mL.

38. The method of any one of claims 30-37, wherein the test culture vessel is a baffled shake flask.

39. The method of any one of claims 30-38, wherein the production bioreactor has a volume of about 5 L to about 20,000 L.

40. The method of claim 39, wherein the production bioreactor has a volume of about 50 L to about 10,000 L.

41. The method of any one of claims 30-40, wherein the production bioreactor is a perfusion bioreactor.

42. The method of any one of claims 30-40, wherein the production bioreactor is a fed batch bioreactor.

43. The method of any one of claims 30-42, wherein step (c) results in a specific cell death rate of less than 40% per day during the culturing of the cell in the production bioreactor.

44. The method of any one of claims 30-42, wherein step (c) results in a specific cell death rate of less than 20% per day during the culturing of the cell in the production bioreactor.

45. The method of any one of claims 30-42, wherein step (c) results in a cell specific LDH production of less than 150 nU/cell/day during the culturing of the cell in the production bioreactor.

46. The method of any one of claims 30-42, wherein step (c) results in a cell specific LDH production of less than 100 nU/cell/day during the culturing of the cell in the production bioreactor.

47. The method of any one of claims 30-42, wherein step (c) results in an apparent growth rate of greater than 1% per day during the culturing of the cell in the production bioreactor.

48. The method of any one of claims 30-42, wherein step (c) results in an apparent growth rate of greater than 5% per day during the culturing of the cell in the production bioreactor.

49. The method of any one of claims 30-48, wherein the cell contains a nucleic acid encoding a recombinant therapeutic protein.

50. The method of claim 49, further comprising collecting the recombinant therapeutic protein.

51. The method of claim 50, further comprising purifying the recombinant therapeutic protein.

52. The method of claim 51, further comprising formulating the purified recombinant therapeutic protein.

53. The method of any one of claims 30-52, wherein the cell is a bacterium, a yeast, or a mammalian cell.

54. The method of any one of claims 30-53, wherein the cell viability is determined by measuring a concentration of lactic acid dehydrogenase (LDH) in the test culture vessel.

55. The method of any one of claims 30-37 and 39-54, wherein the test culture vessel is a baffled shake flask and the plurality of test conditions comprise one or more of:

- (a) a rotary agitation of about 125 RPM to about 400 RPM;
- (b) a concentration of a sensitizer of about 0 ppm to about 120 ppm; and
- (c) a concentration of a protectant that is about 1 g/L to about 10 g/L.

56. The method of claim 31 or 55, wherein the protectant is a poloxamer, a poloxamine, or a pluronic polyol.

57. The method of claim 56, wherein the poloxamer is poloxamer-188, poloxamer-401, poloxamer-402, or poloxamer-407.

58. The method of claim 56, wherein the poloxamine is poloxamine-904 or poloxamine-908.

59. A method for predicting cell viability in a production bioreactor, the method comprising:

- (a) selecting a set of parameters comprising at least two parameters;
- (b) determining cell viability under a plurality of test conditions as defined by the set of parameters in a test culture vessel; and
- (c) based on the cell viability in the test culture vessel under the plurality of conditions, predicting cell viability in the production bioreactor.

60. The method of claim 59, wherein the set of parameters comprises shear stress, concentration of a sensitizer, and concentration of a protectant.

61. The method of claim 59 or claim 60, wherein the plurality of test conditions are selected based on full factorial screening and/or inscribed center composite design.

62. The method of claim 60 or 61, wherein the sensitizer is an antifoam agent.

63. The method of claim 62, wherein the antifoam is a polydimethylsiloxane-based antifoam.

64. The method of claim 62, wherein the antifoam is simethicone.

65. The method of any one of claims 59-64, wherein the test culture vessel has a volume of about 5 mL to about 5 L.

66. The method of claim 65, wherein the test culture vessel has a volume of about 10 mL to about 500 mL.

67. The method of any one of claims 59-66, wherein the test culture vessel is a baffled shake flask.

68. The method of any one of claims 59-67, wherein the production bioreactor has a volume of about 5L to about 20,000 L.

69. The method of claim 68, wherein the production bioreactor has a volume of about 50 L to about 10,000 L.

70. The method of any one of claims 59-69, wherein the production bioreactor is a perfusion bioreactor.

71. The method of any one of claims 59-69, wherein the production bioreactor is a fed batch bioreactor.

72. The method of any one of claims 59-71, further comprising culturing a cell in the production bioreactor using a condition predicted to result in a specific cell death rate of less 40% per day during the culturing of the cell in the production bioreactor.

73. The method of any one of claims 59-71, further comprising culturing a cell in the production bioreactor using a condition predicted to result in a specific cell death rate of less than 20% per day during the culturing of the cell in the production bioreactor.

74. The method of any one of claims 59-71, further comprising culturing a cell in the production bioreactor using a condition predicted to result in a cell specific LDH production of less than 150 nU/cell/day during the culturing of the cell in the production bioreactor.

75. The method of any one of claims 59-71, further comprising culturing a cell in the production bioreactor using a condition predicted to result in a cell specific LDH production of less than 100 nU/cell/day during the culturing of the cell in the production bioreactor.

76. The method of any one of claims 59-71, further comprising culturing a cell in the production bioreactor using a condition predicted to result in an apparent growth rate of greater than 1% per day during the culturing of the cell in the production bioreactor.

77. The method of any one of claims 59-71, further comprising culturing a cell in the production bioreactor using a condition predicted to result in an apparent growth rate of greater than 5% per day during the culturing of the cell in the production bioreactor.

78. The method of any one of claims 72-77, wherein the cell contains a nucleic acid encoding a recombinant therapeutic protein.

79. The method of claim 78, further comprising collecting the recombinant therapeutic protein.

80. The method of claim 79, further comprising purifying the recombinant therapeutic protein.

81. The method of claim 80, further comprising formulating the purified recombinant therapeutic protein.

82. The method of any one of claims 59-81, wherein the cell is a bacterium, a yeast, or a mammalian cell.

83. The method of any one of claims 59-82, wherein the cell viability is determined by measuring a concentration of lactic acid dehydrogenase (LDH) in the test culture vessel.

84. The method of any one of claims 59-66 and 68-83, wherein the test culture vessel is a baffled shake flask and the plurality of test conditions comprise one or more of:

- (a) a rotary agitation of about 125 RPM to about 400 RPM;
- (b) a concentration of the sensitizer of about 0 ppm to about 120 ppm; and
- (c) a concentration of a protectant that is about 1 g/L to about 10 g/L.

85. The method of claim 60 or 84, wherein the protectant is a poloxamer, a poloxamine, or a pluronic polyol.

86. The method of claim 85, wherein the poloxamer is poloxamer-188, poloxamer-401, poloxamer-402, or poloxamer-407.

87. The method of claim 85, wherein the poloxamine is poloxamine-904 or poloxamine-908.

88. A method for culturing a cell in a production bioreactor, comprising a culturing a cell in a liquid culture medium under one or more of the following conditions:

- e) the production bioreactor has a P/V (kW/m^3) from about 1.0 to about 110 (e.g., about 3.0 to about 8.0, about 3.0 to about 7.0, from about 4.0 to about 6.0, from about 5.0 to about 6.0, or about 6.0);
- f) the production bioreactor has a volume of air per volume of liquid per minute (VVM) (min^{-1}) from about 0.01 to about 0.10 (e.g., from about 0.02 to about 0.08, from about 0.04 to about 0.06, or about 0.06);
- g) the medium comprises a sensitizer that has a concentration of about 0 ppm to about 5,000 ppm (e.g., about 0 ppm to about 120 ppm, about 50 ppm to about 110 ppm, from about 80 ppm to about 100 ppm, or about 100 ppm); and
- h) the liquid culture medium comprises a protectant that has a concentration that is from about 1 g/L to about 15 g/L (e.g., from about 2 g/L to about 10 g/L, from about 3 g/L to about 7 g/L, from about 4 g/L to about 6 g/L, or about 5 g/L).

89. The method of claim 88, wherein the sensitizer is an antifoam agent.

90. The method of claim 89, wherein the antifoam is a polydimethylsiloxane-based antifoam.

91. The method of claim 89, wherein the antifoam is simethicone.

92. The method of any one of claims 88-91, wherein the protectant is a poloxamer, a poloxamine, or a pluronic polyol.

93. The method of claim 92, wherein the poloxamer is poloxamer-188, poloxamer-401, poloxamer-402, or poloxamer-407.

94. The method of claim 92, wherein the poloxamine is poloxamine-904 or poloxamine-908.

95. The method of any one of claims 88-94, wherein the production bioreactor has a volume of about 5L to about 20,000 L.

96. The method of claim 95, wherein the production bioreactor has a volume of about 50 L to about 10,000 L.

97. The method of any one of claims 88-96, wherein the production bioreactor is a perfusion bioreactor.

98. The method of any one of claims 88-96, wherein the production bioreactor is a fed batch bioreactor.

99. The method of any one of claims 88-98, wherein the method results in a specific cell death rate of less than 40% per day during the culturing of the cell in the production bioreactor.

100. The method of any one of claims 88-98, wherein the method results in a specific cell death rate of less than 20% per day during the culturing of the cell in the production bioreactor.

101. The method of any one of claims 88-98, wherein the method results in a cell specific LDH production of less than 150 nU/cell/day during the culturing of the cell in the production bioreactor.

102. The method of any one of claims 88-98, wherein the method results in a cell specific LDH production of less than 100 nU/cell/day during the culturing of the cell in the production bioreactor.

103. The method of any one of claims 88-98, wherein the method results in an apparent growth rate of greater than 1% per day during the culturing of the cell in the production bioreactor.

104. The method of any one of claims 88-98, wherein the method results in an apparent growth rate of greater than 5% per day during the culturing of the cell in the production bioreactor.

105. The method of any one of claims 88-98, wherein the cell contains a nucleic acid encoding a recombinant therapeutic protein.

106. The method of claim 105, further comprising collecting the recombinant therapeutic protein.

107. The method of claim 106, further comprising purifying the recombinant therapeutic protein.

108. The method of claim 107, further comprising formulating the purified recombinant therapeutic protein.

109. The method of any one of claims 88-108, wherein the cell is a bacterium, a yeast, or a mammalian cell.

110. The method of any one of claims 88-109, wherein the cell viability is determined by measuring a concentration of lactic acid dehydrogenase (LDH) in the test culture vessel.