

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, wherein the plurality of testing conditions are selected based on full factorial screening and/or inscribed center composite design; ~~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~~the sensitizer on cell viability in ~~a~~the production bioreactor; and;

(c) culturing a cell in the production bioreactor using a concentration of the sensitizer predicted to result in:

a specific cell death rate of less than 40% per day during the culturing of the cell in the production bioreactor;

a cell specific lactic acid dehydrogenase (LDH) production of less than 150 nU/cell during the culturing of the cell in the production bioreactor; or

an apparent growth rate of greater than 1% per day during the culturing of the cell in the 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.~~

~~43.~~ The method of any one of claims 1-~~32~~, wherein the sensitizer is an antifoam agent.

~~54.~~ The method of claim ~~43~~, wherein the antifoam is a polydimethylsiloxane-based antifoam.

~~65.~~ The method of claim ~~43~~, wherein the antifoam is simethicone.

~~76.~~ The method of any one of claims 1-~~65~~, wherein the test culture vessel has a volume of ~~about~~ 5 mL to ~~about~~ 5 L.

87. The method of claim 76, wherein the test culture vessel has a volume of ~~about~~ 10 mL to ~~about~~ 500 mL.

98. The method of any one of claims 1-87, wherein the test culture vessel is a baffled shake flask.

109. The method of any one of claims 1-98, wherein the production bioreactor has a volume of ~~about~~ 5 L to ~~about~~ 20,000 L.

110. The method of claim 109, wherein the production bioreactor has a volume of ~~about~~ 50 L to ~~about~~ 10,000 L.

111. The method of any one of claims 1-110, wherein the production bioreactor is a perfusion bioreactor.

112. The method of any one of claims 1-110, wherein the production bioreactor is a fed batch bioreactor.

113. The method of any one of claims 1-112, 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.

114. The method of any one of claims 1-112, 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.

115. The method of any one of claims 1-112, 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~~16. The method of any one of claims 1-~~13~~12, 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~~17. The method of any one of claims 1-~~13~~12, 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~~18. The method of any one of claims 1-~~13~~12, 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~~19. The method of any one of claims ~~14~~13-~~19~~18, wherein the cell contains a nucleic acid encoding a recombinant therapeutic protein.

~~21~~20. The method of claim ~~20~~19, further comprising collecting the recombinant therapeutic protein.

~~22~~21. The method of claim ~~21~~20, further comprising purifying the recombinant therapeutic protein.

~~23~~22. The method of claim ~~22~~21, further comprising formulating the purified recombinant therapeutic protein.

~~24~~23. The method of any one of claims 1-~~23~~22, wherein the cell is a bacterium, a yeast, or a mammalian cell.

2524. The method of any one of claims 1-2423, wherein the cell viability is determined by measuring a concentration of lactic acid dehydrogenase (LDH) in the test culture vessel.

2625. The method of any one of claims 1-87 and 109-2524, 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.

2726. The method of claim 2 or 2625, wherein the protectant is a poloxamer, a poloxamine, or a pluronic polyol.

2827. The method of claim 2726, wherein the poloxamer is ~~poloxamer-188~~poloxamer-188, ~~poloxamer-401~~poloxamer-401, poloxamer-402, or poloxamer-407.

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