significant impact on the production process. Growth parameters are determined specifically for each cell line and these parameters are measured frequently to assure optimal growth and production conditions.

When the cells grow to sufficient numbers, they are transferred to large-scale production tanks and grown for a longer period of time. At this point in the process, the recombinant protein can be harvested. Typically, the cells are engineered to secrete the polypeptide into the cell culture media, so the first step in the purification process is to separate the cells from the media. Typically, harvesting includes centrifugation and filtration to produce a Harvested Cell Culture Fluid (HCCF). The media is then subjected to several additional purification steps that remove any cellular debris, unwanted proteins, salts, minerals or other undesirable elements. At the end of the purification process, the recombinant protein is highly pure and is suitable for human therapeutic use.

Although this process has been the subject of much study and improvements over the past several decades, the production of recombinant proteins is still not without difficulties. Thus, for example, during the recombinant production of polypeptides comprising disulfide bonds, especially multi-chain polypeptides comprising inter-chain disulfide bonds such as antibodies, it is essential to protect and retain the disulfide bonds throughout the manufacturing, recoveiy and purification process, in order to produce properly folded polypeptides with the requisite biological activity.

SUMMARY

The instant disclosure generally relates to a method for preventing reduction of a disulfide bond in a polypeptide expressed in a recombinant host cell, comprising supplementing the pre-harvest or harvested culture fluid of the recombinant host cell with an inhibitor of thioredoxin or a thioredoxin-like protein.

In one aspect, there is provided a method for preventing reduction of a disulfide bond in a polypeptide expressed in a recombinant host cell, comprising supplementing, following fermentation, a pre-harvest or harvested cell culture fluid of said recombinant host cell with a thioredoxin inhibitor, wherein said thioredoxin inhibitor is: (i) a direct inhibitor of thioredoxin; (ii) a specific inhibitor of thioredoxin reductase; (iii) a metal ion; (iv) an inhibitor of G6PD, which inhibitor is pyridoxal 5 '-phosphate, 1 fluoro-2,4 dinitrobenzene, dehydroepiandrosterone (DHEA) or epiandrosterone (EA); (v) an inhibitor of hexokinase activity, which inhibitor is (a) a chelator of metal ions; or (b) selected from the group consisting of sorbose-1-phosphate, polyphosphates, 6-deoxy-6-fluoroglucose, 2-C-hydroxy-methylglucose, xylose, and lyxose; (vi) an antibody speficailly binding to a thioredoxin reductase; or (vii) a measure

indirectly resulting in the inhibition of thioredoxin activity, which measure is air sparging the harvested culture fluid of said recombinant host cell.

In another aspect, there is provided a method for producing a polypeptide and preventing reduction of a disulfide bond in the polypeptide, the method comprising expressing the polypeptide in a recombinant host cell and supplementing, following fermentation, a pre-harvest or harvested culture fluid of said recombinant host cell with a thioredoxin inhibitor, wherein said thioredoxin inhibitor is: (i) a direct inhibitor of thioredoxin; (ii) a specific inhibitor ofthioredoxin reductase; (iii) is a metal ion; (iv) an inhibitor of G6PD, which inhibitor is pyridoxal 5 '-phosphate, 1 fluoro-2,4 dinitrobenzene, dehydroepiandrosterone (DHEA) or epiandrosterone (EA); (v) an inhibitor of hexokinase activity, which inhibitor is (a) a chelator of metal ions; or (b) selected from the group consisting of sorbose-1-phosphate, polyphosphates, 6-deoxy-6-fluoroglucose, 2-C-hydroxy-methylglucose, xylose, and lyxose; (vi) an antibody specifically binding to a thioredoxin reductase; or (vii) a measure indirectly resulting in the inhibition of thioredoxin activity, which measure is air sparging the harvested culture fluid of said recombinant host cell.

In another aspect, there is provided a method for preventing reduction of a disulfide bond in a polypeptide during processing following fermentation, , comprising expressing the polypeptide in a recombinant CHO cell and following fermentation, lowering pH of the harvested culture fluid of said recombinant CHO cells, and air sparging the harvested culture fluid of said recombinant host cell.

In another aspect, there is provided a method for preventing reduction of a disulfide bond in a polypeptide expressed in a recombinant host cell, comprising, following fermentation, sparging a pre-harvest or harvested culture fluid of said recombinant host cell with air.

In one embodiment, the thioredoxin inhibitor is added to the pre-harvest culture fluid.

In another embodiment, the thioredoxin inhibitor is added to the harvested culture fluid.

In a further embodiment, the thioredoxin inhibitor is a direct inhibitor of thioredoxin.

In all embodiments, the thioredoxin inhibitor may, for example, be an alkyl-2-imidazolyl disulfide or a naphthoquinone spiroketal derivative.

In a further embodiment, the thioredoxin inhibitor is a specific inhibitor of thioredoxin reductase.

In a still further embodiment, the thioredoxin inhibitor is a gold complex, where the gold complex may, for example, be aurothioglucose (ATG) or aurothiomalate (ATM). While

Claims:

1. A method for preventing reduction of a disulfide bond in a polypeptide expressed in a recombinant host cell, comprising supplementing, following fermentation, a pre-harvest or harvested culture fluid of said recombinant host cell with a thioredoxin inhibitor, wherein said thioredoxin inhibitor is:

(i) a direct inhibitor of thioredoxin;

(ii) a specific inhibitor of thioredoxin reductase;

(iii) is a metal ion;

(iv) an inhibitor of G6PD, which inhibitor is pyridoxal 5 '-phosphate, 1 fluoro-2,4 dinitrobenzene, dehydroepiandrosterone (DHEA) or epiandrosterone (EA);

(v) an inhibitor of hexokinase activity, which inhibitor is (a) a chelator of metal ions; or (b) selected from the group consisting of sorbose-1-phosphate, polyphosphates, 6-deoxy-6-fluoroglucose, 2-C-hydroxy-methylglucose, xylose, and lyxose;

(vi) an antibody specifically binding to a thioredoxin reductase; or

(vii) a measure indirectly resulting in the inhibition of thioredoxin activity, which measure is air sparging the harvested culture fluid of said recombinant host cell.

2. A method for producing a polypeptide and preventing reduction of a disulfide bond in the polypeptide, the method comprising expressing the polypeptide in a recombinant host cell and supplementing, following fermentation, a pre-harvest or harvested culture fluid of said recombinant host cell with a thioredoxin inhibitor, wherein said thioredoxin inhibitor is:

(i) a direct inhibitor of thioredoxin;

(ii) a specific inhibitor ofthioredoxin reductase;

(iii) is a metal ion;

(iv) an inhibitor of G6PD, which inhibitor is pyridoxal 5 '-phosphate, 1 fluoro-2,4 dinitrobenzene, dehydroepiandrosterone (DHEA) or epiandrosterone (EA);

(v) an inhibitor of hexokinase activity, which inhibitor is (a) a chelator of metal ions; or (b) selected from the group consisting of sorbose-1-phosphate, polyphosphates, 6-deoxy-6-fluoroglucose, 2-C-hydroxy-methylglucose, xylose, and lyxose;

(vi) an antibody specifically binding to a thioredoxin reductase; or

(vii) a measure indirectly resulting in the inhibition of thioredoxin activity, which measure is air sparging the harvested culture fluid of said recombinant host cell.

3. The method of claim 1 or claim 2 wherein said thioredoxin inhibitor is added to the pre-harvest culture fluid.

4. The method of claim 1 or claim 2 wherein said thioredoxin inhibitor is added to the harvested culture fluid.

5. The method of claim 1(i) or claim 2(i) wherein said thioredoxin inhibitor is an alkyl-2-imidazolyl disulphide or a naphthoquinone spiroketal derivative.

6. The method of claim 1(i) or claim 2(i) wherein said thioredoxin inhibitor is a specific inhibitor of thioredoxin reductase.

7. The method of claim 1(i) or claim 2(i) wherein said thioredoxin inhibitor is a gold complex.

8. The method of claim 7 wherein said gold complex is aurothioglucose (ATG) or aurothiomalate (ATM).

9. The method of claim 8 wherein said ATG or said ATM is added to achieve a concentration between 0.1 mM and 1 mM.

10. The method of claim 8 wherein said ATG or said ATM is added to achieve a concentration at least four-times thioredoxin reductase's concentration in said pre-harvest or harvested culture fluid.

11. The method of claim 1(iii) or claim 2(iii) wherein said metal ion is selected from the group consisting of Hg^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, and Mn^{2+}.

12. The method of claim 11 wherein said metal ion is Cu^{2+} present in the form of cupric sulfate.

13. The method of claim 12 wherein said cupric sulfate is added in the form is pentrahydrate or in anhydrous form.

14. The method of claim 12 wherein said cupric sulfate is added to achieve a concentration between 5 μM and 100 μM in said pre-harvest or harvested culture fluid.

15. The method of claim 12 wherein said cupric sulfate is added to achieve a concentration between 10 μM to 80 μM in said pre-harvest or harvested culture fluid.

16. The method of claim 12 wherein said cupric sulfate is added to achieve a concentration between 15 μM to 50 μM in said pre-harvest or harvested culture fluid.

17. The method of claim 12 wherein said cupric sulfate is added to achieve a concentration at least two-times thioredoxin's concentration in said pre-harvest or harvested culture fluid.

18. The method of claim 17 wherein said DHEA is added to achieve a concentration between 0.05 mM and 5 mM in said pre-harvest or harvested culture fluid.

19. The method of claim 17 wherein said DHEA is added to achieve a concentration between 0.1 mM and 2.5 mM in said pre-harvest or harvested culture fluid.

20. The method of claim 1(v)(a) or claim 2(v)(a) wherein said chelator of metal ions is ethylenediamine tetraacetic acid (EDTA).

21. The method of claim 20 wherein said EDTA is added to achieve a concentration between 5 mM and 60 mM in said pre-harvest or harvested culture fluid.

22. The method of claim 20 wherein said EDTA is added to achieve a concentration between 10 mM and 50 mM in said pre-harvest or harvested culture fluid.

23. The method of claim 20 wherein said EDTA is added to achieve a concentration between 20 mM and 40 mM in said pre-harvest or harvested culture fluid.

24. A method for preventing reduction of a disulfide bond in a polypeptide during processing following fermentation, comprising expressing the polypeptide in a recombinant CHO cell and following fermentation, lowering pH of the harvested culture fluid of said recombinant CHO cells, and air sparging the harvested culture fluid of said recombinant host cell.

25. The method of any one of claims 1 to 23 further comprising the step of lowering the pH of the harvested culture fluid of said recombinant host cells.

26. The method of any one of claims 1 to 25 further comprising the step of adding citrate to the harvested culture fluid of said recombinant host cells.

27. A method for preventing reduction of a disulfide bond in a polypeptide expressed in a recombinant host cell, comprising, following fermentation, sparging a pre-harvest or harvested culture fluid of said recombinant host cell with air.

28. The method of claim 1(vii), claim 2(vii), claim 24 or claim 27, wherein air sparging is continued until the pre-harvest or harvested culture fluid is at least 10% saturated with air.

29. The method of claim 1(vii), claim 2(vii), claim 24 or claim 27, wherein air sparing is continued until the pre-harvest or harvested culture fluid is between 100% saturated to 30% saturated with air.

30. The method of claim 1(vii), claim 2(vii), claim 24 or claim 27, wherein dissolved oxygen (dO_2) in the pre-harvest or harvested cell culture fluid is at least 10%.

31. The method of claim 1(vii), claim 2(vii), claim 24 or claim 27, wherein the amount of dissolved oxygen (dO_2) in the pre-harvest or harvested cell culture fluid is at least 30%.

32. The method of any one of claims 1 to 31 wherein said polypeptide is an antibody, or a biologically functional fragment of an antibody.

33. The method of claim 32 wherein said antibody fragment is selected from the group consisting of Fab, Fab', F(ab')2, scFv, (scFv)2, dAb, complementarity determining region (CDR) fragments, linear antibodies, single-chain antibody molecules, minibodies, diabodies, and multispecific antibodies formed from antibody fragments.

34. The method of claim 32 wherein said antibody or antibody fragment is a therapeutic antibody or a biologically functional fragment thereof.

35. The method of claim 34 wherein said therapeutic antibody is selected from the group consisting of an anti-HER2 antibody; an anti-CD20 antibody; an anti-IL-8 antibody; an anti-

VEGF antibody; an anti-CD40 antibody, an anti-CD11a antibody; an anti-CD18 antibody; an anti-IgE antibody; an anti-Apo-2 receptor antibody; an anti-Tissue Factor (TF) antibody; an anti-human $\alpha_4\beta_7$ integrin antibody; an anti-EGFR antibody; an anti-CD3 antibody; an anti-CD25 antibody; an anti-CD4 antibody; an anti-CD52 antibody; an anti-Fc receptor antibody; an anti-carcinoembryonic antigen (CEA) antibody; an antibody directed against breast epithelial cells; an antibody that binds to colon carcinoma cells; an anti-CD38 antibody; an anti-CD33 antibody; an anti-CD22 antibody; an anti-EpCAM antibody; an anti-GpIIb/IIIa antibody; an anti-RSV antibody; an anti-CMV antibody; an anti-HIV antibody; an anti-hepatitis antibody; an anti-CA 125 antibody; an anti-$\alpha_v\beta_3$ antibody; an anti-human renal cell carcinoma antibody; an anti-human 17-1A antibody; an anti-human colorectal tumor antibody; an anti-human melanoma antibody R24 directed against GD3 ganglioside; an anti-human squamous-cell carcinoma antibody; an anti-human leukocyte antigen (HLA) antibody, and an anti-HLA DR antibody.

36. The method of claim 34 wherein said therapeutic antibody is an antibody binding to a HER receptor, VEGF, IgE, CD20, CD11a, CD40, or DR5.

37. The method of claim 36 wherein said HER receptor is HER1 and/or HER2.

38. The method of claim 37 wherein the HER receptor is HER2.

39. The method of claim 38 wherein said therapeutic antibody comprises a heavy and/or light chain variable domain sequence selected from the group consisting of SEQ ID NOs: 16, 17, 18, and 19.

40. The method of claim 36 wherein said therapeutic antibody is an antibody that binds to CD20.

41. The method of claim 40 wherein said therapeutic antibody comprises a heavy and/or light chain variable domain sequence selected from the group consisting of SEQ ID NOs: 1 to 15.

42. The method of claim 36 wherein said therapeutic antibody is an antibody that binds to VEGF.

43. The method of claim 42 wherein said therapeutic antibody comprises a heavy and/or light chain variable domain sequence selected from the group consisting of SEQ ID NOs: 20 to 25.

44. The method of claim 36 wherein said therapeutic antibody is an antibody that binds to CD11a.

45. The method of claim 44 wherein said therapeutic antibody comprises a heavy and/or light chain variable domain sequence selected from the group consisting of SEQ ID NOs: 26 to 29.

46. The method of claim 36 wherein said therapeutic antibody binds to DR5 receptor.

47. The method of claim 46 wherein said therapeutic antibody is selected from the group consisting of Apomabs 1.1, 2.1, 3.1, 4.1, 5.1, 5.2, 5.3, 6.1, 6.2, 6.3, 7.1, 7.2, 7.3, 8.1, 8.3, 9.1, 1.2, 2.2, 3.2, 4.2, 8.2, 9.2, 1.3, 3.3, 4.3, 9.3, and 25.3.

48. The method of claim 46 or claim 47 wherein said therapeutic antibody is Apomab 8.3 or Apomab 7.3.

49. The method of claim 48 wherein said therapeutic antibody is Apomab 7.3.

50. The method of claim any one of claims 1 to 31, wherein said polypeptide is a therapeutic polypeptide.

51. The method of claim 50 wherein said therapeutic polypeptide is selected from the group consisting of: a growth hormone, optionally human growth hormone or bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; a lipoprotein; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; a clotting factor optionally factor VIIIC, factor IX, tissue factor, or von Willebrands factor; an anti-clotting factor optionally Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, optionally urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha or -beta; enkephalinase; RANTES (regulated on activation normally T -cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin optionally human serum

albumin; Muellerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, optionally beta-lactamase; DNase; IgE; a cytotoxic T-lymphocyte associated antigen (CTLA), optionally CTLA-4; inhibin; activin; vascular endothelial growth factor (VEGF); a receptor for hormones or growth factors; Protein A or D; a rheumatoid factor; a neurotrophic factor optionally bone-derived neurotrophic factor (BDNF), neurotrophin-3,-4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor optionally NGF-β; platelet-derived growth factor (PDGF); a fibroblast growth factor optionally aFGF or bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) optionally TGF-alpha or TGF-beta, optionally TGF-β1,TGF- β2, TGF- β3, TGF- β4, or TGF- β5; insulin-like growth factor-I or -II (IGF-I or IGF-II); des(l-3)-IGF-I (brain IGF-I); an insulin-like growth factor binding protein; a CD protein optionally CD3, CD4, CD8, CD19, CD20, CD34, or CD40; erythropoietin; an osteoinductive factor; an immunotoxin; a bone morphogenetic protein (BMP); an interferon optionally interferon-alpha, -beta, or -gamma; a colony stimulating factor (CSF), optionally M-CSF, GM-CSF, and G-CSF; an interleukin (IL), optionally IL-l to IL-10; superoxide dismutase; a T -cell receptor; a surface membrane protein; decay accelerating factor; a viral antigen optionally a portion of the AIDS envelope; a transport protein; a homing receptor; an addressin; a regulatory protein; an integrin optionally CD11a, CD11b, CD11c, CD18, an ICAM, VLA-4 or VCAM; a tumor associated antigen optionally HER2, HER3 or HER4 receptor; and a fragment of said polypeptide.

52. The method of any one of claims 1 to 23 or 25 to 51 wherein said recombinant host cell is an eukaryotic host cell.

53. The method of claim 52 wherein said eukaryotic host cell is a mammalian host cell.

54. The method of claim 53 wherein said mammalian host cell is a Chinese Hamster Ovary (CHO) cell.

55. The method of any one of claims 1 to 23 or 25 to 51 wherein the recombinant host cell is a prokaryotic host cell.

56. The method of claim 55 wherein the prokaryotic host cell is a bacterial cell.

57. The method of claim 56 wherein the bacterial cell is an *E.*coli cell.

58. The method of any one of claims 1 to 57, further comprising the step of recovering said polypeptide form the harvested culture fluid.

59. The method of any one of claims 1 to 58, further comprising the step of purifying said polypeptide from the harvested culture fluid.