the culture medium is not supplemented with animal products, such as serum, the medium may support only relatively low cell densities. This reduces the yield per volume of medium. However, it is desirable not to supplement the culture medium with animal products in order to reduce the risk of contamination with viruses and other transmissible agents. Animal-protein-free media for the production of FVIII are known from US 6 936 441 (Baxter AG), for example.

The present invention provides processes for producing blood proteins, including rFVIII, in which the yield is improved compared to processes known in the art.

SUMMARY ~~OF THE INVENTION~~

In one aspect, there is provided a method of culturing heterologous protein-secreting mammalian cells in a cell culture supernatant wherein the cell culture supernatant has a dissolved CO_2 concentration of 1 to 10%, wherein the cell density is from $1.4x10^6$ to $2.8x10^6$ cells/ml.

~~A first aspect of the invention~~In one embodiment, the disclosure provides a method of culturing heterologous protein-secreting mammalian cells in a cell culture supernatant wherein the cell culture supernatant is maintained at a temperature that is set at X±0.9 °C wherein X has a value of from 35.1 to 36.5, with the proviso that the temperature is set at less than 37°C.

~~A second aspect of the invention~~In one embodiment, the disclosure provides a method of culturing heterologous protein-secreting mammalian cells in a cell culture supernatant wherein the cell culture supernatant is maintained at a pH that is set at X±0.05 wherein X has a value of from 7.15 to 7.20, with the proviso that the pH is set at greater than 7.10.

~~A third aspect of the invention~~In one embodiment, the disclosure provides a method of culturing heterologous protein-secreting mammalian cells in a cell culture supernatant wherein the cell culture supernatant has a CO_2 concentration of 1-10%.

~~A fourth aspect of the invention~~In one embodiment, the disclosure provides a method of continuous culture of FVIII-secreting mammalian cells in a vessel comprising a cell culture supernatant wherein the density of the cells in the cell culture supernatant is measured by an in-line sensor and the influx of fresh

medium into the vessel is automatically controlled so as to maintain the density of the cells in a desired range.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

In the process of the first aspect of the invention, the cell culture supernatant in which the mammalian cells are cultured is maintained at a temperature that is set

CLAIMS

1. A method of culturing heterologous protein-secreting mammalian cells in a cell culture supernatant wherein the cell culture supernatant is maintained at a temperature that is set at X±0.9°C, wherein X has a value of from 35.1 to 36.5, with the proviso that the temperature is set at less than 37°C.

2. A method according to Claim 1 wherein the temperature is set at 36±0.9°C.

3. A method according to Claim 1 wherein the temperature is set at 36±0.5°C.

4. A method according to Claim 1 wherein the temperature is set at 36±0.2°C.

5. A method according to Claim 1 wherein the temperature is set at 36 °C.

6. A method according to Claim 1 wherein the temperature is set at 35.1±0.9 °C.

7. A method according to Claim 1 wherein the temperature is set at 35.1±0.5 °C.

8. A method according to Claim 1 wherein the temperature is set at 35.1±0.2 °C.

9. A method according to Claim 1 wherein the temperature is set at 35.1 °C.

10. A method according to Claim 1 wherein the temperature is set at 36.5±0.9 °C.

11. A method according to Claim 1 wherein the temperature is set at 36.5±0.5 °C.

12. A method according to Claim 1 wherein the temperature is set at 36.5±0.2 °C.

13. A method according to Claim 1 wherein the temperature is set at 36.5 °C.

14. A method of culturing heterologous protein-secreting mammalian cells in a cell culture supernatant wherein the cell culture supernatant is maintained

at a pH that is set at X±0.05 wherein X has a value of from 7.15 to 7.20, with the proviso that the pH is set at greater than 7.10.

15. A method according to Claim 14 wherein the pH is set at 7.20±0.05.

16. A method according to Claim 14 wherein the pH is set at 7.20±0.03.

17. A method according to Claim 14 wherein the pH is set at 7.20±0.01.

18. A method according to Claim 14 wherein the pH is set at 7.20.

19. A method according to Claim 14 wherein the pH is set at 7.15±0.05.

20. A method according to Claim 14 wherein the pH is set at 7.15±0.03.

21. A method according to Claim 14 wherein the pH is set at 7.15±0.01.

22. A method according to Claim 14 wherein the pH is set at 7.15.

23. A method of culturing heterologous protein-secreting mammalian cells in a cell culture supernatant wherein the cell culture supernatant has a dissolved CO_2 concentration of 1 to 10%.

24. A method according to Claim 23 wherein the CO_2 concentration is 4.0 to 9.0%.

25. A method according to Claim 23 wherein the CO_2 concentration is 5.5 to 8.5%.

26. The method of Claim 23 wherein the cell culture supernatant is maintained at a temperature that is set at X±0.9°C wherein X has a value of from 35.1 to 36.5, with the proviso that the temperature is set at less than 37°C.

27. A method according to Claim 26 wherein the temperature is set at 36±0.9°C.

28. A method according to Claim 26 wherein the temperature is set at 35.1±0.9°C.

29. A method according to Claim 26 wherein the temperature is set at 36.5±0.9°C.

30. The method of Claim 1, 23 or 26 wherein the cell culture supernatant is maintained at a pH that is set at X±0.5 wherein X has a value of from 7.15 to 7.20, with the proviso that the pH is set at greater than 7.10.

31. The method of any preceding claim wherein the cell culture supernatant is buffered with bicarbonate.

32. The method of any preceding claim wherein the supernatant is sparged with air.

33. The method of any preceding claim wherein the cell density is $1.0x10^6$ to $5.0x10^6$ cells/ml.

34. The method of Claim 33 wherein the cell density is $1.0x10^6$ to $3.5x10^6$ cells/ml.

35. The method of Claim 34 wherein the cell density is $1.4x10^6$ to $2.8x10^6$ cells/ml.

36. The method of Claim 35 wherein the cell density is $1.6x10^6$ to $2.6x10^6$ cells/ml.

37. The method of Claim 36 wherein the cell density is $1.8x10^6$ to $2.4x10^6$ cells/ml.

38. The method of any preceding claim wherein the heterologous protein is a blood protein.

39. The method of any one of Claims 2, 15 or 23 wherein the blood protein is Factor VIII.

40. The method of Claim 39 wherein the Factor VIII is co-expressed with von Willebrand Factor.

41. The method of any preceding claim wherein the concentration of Cu^{2+} in the cell culture supernatant is at least 5 ppb.

42. The method of Claim 41 wherein the Cu^{2+} concentration is at least 7, 10, 15 or 25 ppb.

43. A method according to any one of Claims 2, 19 or 23 wherein the blood protein is ADAMTS-13.

44. A method according to any one of Claims 6, 19 or 23 wherein the blood protein is furin.

45. A method according to any one of Claims 10, 15 or 23 wherein the blood protein is Factor VII.

46. The method of any preceding claim wherein the mammalian cell is a rodent cell, such as a CHO cell or a BHK cell.

47. The method of any preceding claim wherein the culture is a batch culture.

48. The method of any of Claims 1 to 45 wherein the culture is a fed-batch culture.

49. The method of any of Claims 1 to 45 wherein the culture is a continuous culture, particularly a repeated batch, chemostat or turbidostat culture.

50. A method of culturing Factor VIII-secreting mammalian cells in a vessel comprising a cell culture supernatant wherein the density of the cells in the cell culture supernatant is measured by an in-line sensor and the influx of fresh medium into the vessel is automatically controlled in order to maintain the density of the cells in a desired range.

51. The method of Claim 50 wherein the cell density is at least 1.2×10^6 cells/ml.

CLAIMS

1. A method of culturing heterologous protein-secreting mammalian cells in a cell culture supernatant wherein the cell culture supernatant has a dissolved CO_2 concentration of 1 to 10%, wherein the cell density is from $1.4x10^6$ to $2.8x10^6$ cells/ml.

2. A method according to Claim 1 wherein the CO_2 concentration is either 4.0 to 9.0% or 5.5 to 8.5%.

3. The method of Claim 1 wherein the cell culture supernatant is maintained at a temperature that is set at X±0.9°C wherein X has a value of from 35.1 to 36.5, with the proviso that the temperature is set at less than 37°C.

4. A method according to Claim 3 wherein the temperature is selected from 36±0.9°C, 35.1±0.9°C, and 36.5±0.9°C.

5. The method of Claim 1 or 3 wherein the cell culture supernatant is maintained at a pH that is set at X±0.05 wherein X has a value of from 7.15 to 7.20, with the proviso that the pH is set at greater than 7.10.

6. The method of any preceding claim wherein the cell culture supernatant is buffered with bicarbonate.

7. The method of any preceding claim wherein the supernatant is sparged with air.

8. The method of any preceding claim wherein the heterologous protein is a blood protein.

9. The method of Claim 1 wherein the heterologous protein is Factor VIII.

10. The method of Claim 9 wherein the Factor VIII is co-expressed with von Willebrand Factor.

11. The method of any preceding claim wherein the concentration of Cu^{2+} in the cell culture supernatant is at least 5, 7, 10, 15 or 25 ppb.

12. A method according to Claim 1 wherein the heterologous protein is ADAMTS-13, furin or Factor VII.

13. The method of any preceding claim wherein the mammalian cell is a rodent cell, such as a CHO cell or a BHK cell.

14. The method of any preceding claim wherein the culture is a batch culture.

15. The method of any of Claims 1 to 12 wherein the culture is a fed-batch culture.

16. The method of any of Claims 1 to 12 wherein the culture is a continuous culture, particularly a repeated batch, chemostat or turbidostat culture.