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

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 culture is a continuous culture maintained at a density of from $1.4x10^6$ to $2.8x10^6$ cells/ml.

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.

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.

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%.

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

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 culture is a continuous culture maintained at a density of from $1.4x10^6$ to $2.8x10^6$ cells/ml.

2. The 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 according to 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. The 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 according to 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 according to any preceding claim wherein the cell culture supernatant is buffered with bicarbonate.

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

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

9. The method according to claim 1 wherein the heterologous protein is Factor VIII.

10. The method according to claim 9 wherein the Factor VIII is co-expressed with von Willebrand Factor.

11. The method according to 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. The method according to claim 1 wherein the heterologous protein is ADAMTS-13, furin or Factor VII.

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

14. The method according to any preceding claim wherein the continuous culture is a chemostat or turbidostat culture.