Claims

1. A process for the production of a recombinant polypeptide comprising culturing CHO cells in a medium under conditions comprising at least one temperature shift and at least one pH shift and expressing the recombinant polypeptide, wherein

- the cells are grown at a first temperature for at least 3 days and the temperature is then shifted to a second temperature which is between about 1 and about 8°C lower than the first temperature and the cells are maintained at said second temperature for a period of at least another 2 days;

- the cells are grown at a first pH value for at least 2 days and the pH is then shifted to a second pH value which is between about 0.05 and about 1 pH units lower than the first pH and the cells are grown at said second pH for at least 1 day.

2. The process according to claim 1 wherein the pH is actively changed between said first and said second pH value.

3. The process according to any of the previous claims wherein the pH is passively changed between said first and said second pH value.

4. The process according to any of the previous claims wherein the first temperature is in the range of between about 33°C and about 38°C.

5. The process according to any of the previous claims wherein the second temperature is in the range of between about 30°C and about 37°C.

6. The process according to any of the previous claims wherein the first pH value is in the range of between about pH 6.8 and about pH 7.5.

7. The process according to any of the previous claims wherein the second pH value is in the range of between about pH 6.0 and about pH 7.1.

8. The process according to any of the previous claims wherein said second pH is actively maintained until the end of culturing.

9. The process according to any of the claims 1 to 8 wherein the first pH shift is followed by a second pH shift after at least 1 day with the third pH value being about 0.05 pH units to about 1 pH unit higher than the second pH value.

10. The process according to claim 9 wherein the pH is actively changed from said second to said third pH value.

11. The process according to claim 9 wherein the pH is passively changed from said second to said third pH value.

12. The process according to any of the previous claims wherein the medium is protein- and serum-free and characterized by a total amino acid content of between about 40 and about 100 mM.

13. The process according to any of the previous claims wherein culturing is done in fed batch mode comprising feeding of at least two nutrient solutions that are added to the culture.

14. The process according to claim 13 wherein one of the feed solutions added to the culture medium is a feed comprising the dipeptide cystine and the amino acid tyrosine.

15. The process according to claim 14 wherein the feed comprises the dipeptide cystine and the amino acid tyrosine at respective concentrations in the

range of about 6.5 g/l and about 8.0 g/l and in the range of about 9 g/l and about 11 g/l in an aqueous solution at a basic pH of above 10.

16. The process according to any of claims 14 and 15 wherein the amount of the feed solution comprising cystine and typrosine is added to the culture medium in the range of about 0.2 and about 0.8 wt % of the initial culture medium weight per day.

17. The process according to any of the previous claims wherein the produced polypeptide is glycosylated.

18. The process according to any of the previous claims, wherein the polypeptide is an antibody or antibody fragment.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A process for the production of a recombinant polypeptide comprising culturing CHO cells in a medium that is protein- and serum-free and characterized by a total amino acid content of between about 40 and about 100 mM under conditions comprising at least one temperature shift and at least one pH shift and expressing the recombinant polypeptide, wherein

- the cells are grown at a first temperature for at least 3 days and the temperature is then shifted to a second temperature which is between about 1 and about 8°C lower than the first temperature and the cells are maintained at said second temperature for a period of at least another 2 days;
- the cells are grown at a first pH value for at least 2 days and the pH is then shifted to a second pH value which is between about 0.05 and about 1 pH units lower than the first pH and the cells are grown at said second pH for at least 1 day.

2. The process according to claim 1, wherein the pH is actively changed between said first and said second pH value.

3. The process according to claim 1 or claim 2, wherein the pH is passively changed between said first and said second pH value.

4. The process according to any one of claims 1 to 3, wherein the first temperature is in the range of between about 33°C and about 38°C.

5. The process according to any one of claims 1 to 4, wherein the second temperature is in the range of between about 30°C and about 37°C.

6. The process according to any one of claims 1 to 5, wherein the first pH value is in the range of between about pH 6.8 and about pH 7.5.

7. The process according to any of claims 1 to 6, wherein the second pH value is in the range of between about pH 6.0 and about pH 7.1.

8. The process according to any one of claims 1 to 7, wherein said second pH is actively maintained until the end of culturing.

9. The process according to any of the claims 1 to 8 wherein the first pH shift is followed by a second pH shift after at least 1 day with the third pH value being about 0.05 pH units to about 1 pH unit higher than the second pH value.

10. The process according to claim 9 wherein the pH is actively changed from said second to said third pH value.

11. The process according to claim 9 wherein the pH is passively changed from said second to said third pH value.

12. The process according to any one of claims 1 to 11, wherein culturing is done in fed batch mode comprising feeding of at least two nutrient solutions that are added to the culture.

13 The process according to claim 12, wherein one of the feed solutions added to the culture medium is a feed comprising the dipeptide cystine and the amino acid tyrosine.

14 The process according to claim 13, wherein the feed comprises the dipeptide cystine and the amino acid tyrosine at respective concentrations in the range of about 6.5 g/l and about 8.0 g/l and in the range of about 9 g/l and about 11 g/l in an aqueous solution at a basic pH of above 10.

15. The process according to claim 13 or claim 14, wherein the amount of the feed solution comprising cystine and tyrosine is added to the culture medium in the range of about 0.2 and about 0.8 wt % of the initial culture medium weight per day.

16. The process according to any one of claims 1 to 15, wherein the produced polypeptide is glycosylated.

17. The process according to any one of claims 1 to 16, wherein the polypeptide is an antibody or antibody fragment.