METHODS OF PRODUCING ADALIMUMAB

Related applications

This application claims the benefit of priority to U.S. Provisional Application No. 60/845158, filed on September 13, 2006, and U.S. Provisional Application No. 60/876374, filed on December 21, 2006. The contents of each of the above priority documents is incorporated by reference herein.

Background of the invention

Recombinant DNA technology has provided a means to produce proteins in amounts which allow for their use in a spectrum of applications, including therapeutic, diagnostic, agricultural, and research purposes.

One goal of recombinant protein production is the optimization of cell culture media and conditions in order to obtain the greatest amount of protein and the most efficient means of productivity. Any improvement, including incremental improvements, can have enormous benefits economically. In the pharmaceutical industry, optimization of protein production for biologics used in therapies for the treatment of disease is advantageous, as any improvement can have significant impact when the biologic is manufactured on a large scale. As such, there remains a need to maximize protein production from cell cultures expressing biologic proteins for use in medicine.

Typically mammalian cell culture media is based on commercially available media formulations, including, for example, DMEM or Ham's F12. Often media formulations are not sufficiently enriched to support increases in both cell growth and biologic protein expression. There remains a need for improved cell culture media, supplements, and cell culture methods for improved protein production.

Summary of the invention

The invention provides methods and compositions for improving protein expression in cell culture, particularly mammalian cell culture. The invention relates to improved cell culture media, including media for growing cells for protein expression and cell culture production media optimized for protein expression.

What is claimed:

1. A method of producing adalimumab, comprising culturing in large-scale mammalian cells that express adalimumab in a cell culture production medium, wherein the pH of the cell culture production medium is adjusted from a first pH to a second pH during said culturing, the second pH being lower than the first pH, and the cell culture production medium is not removed but is supplemented with glucose or one or more other nutrients at least once during adalimumab production; and wherein adalimumab produced by the mammalian cells is purified from the cell culture production medium using a process including Protein A affinity purification.

2. The method of claim 1, wherein the cell culture production medium is supplemented more than once during adalimumab production, optionally wherein the culture medium is supplemented on a schedule comprising supplementation that is continuous, daily, every other day, every two days, and combinations thereof.

3. The method of claim 1, wherein the cell culture production medium is supplemented with glucose when the glucose concentration in the cell culture production medium is determined after monitoring of glucose levels to be below 2 g/L.

4. The method of claim 3, wherein the cell culture production medium is supplemented with glucose to a glucose concentration of at least 2 g/L but less than or equal to 7 g/L.

5. The method of claim 1, wherein during adalimumab production the mammalian cells are initially cultured at a first temperature and then cultured at a reduced temperature, wherein the first temperature is 35° C., 37° C., or 38° C., and the second temperature is 32° C. or 33° C.

6. The method of claim 5, wherein the cell culture production medium comprises a soy-based hydrolysate and a yeast-based hydrolysate.

7. The method of claim 6, wherein the mammalian cells are Chinese Hamster Ovary (CHO) cells and the cell culture production medium is supplemented with a 25x PFCHO solution and a 33× solution of a combination of the soy-based hydrolysate and yeast-based hydrolysate yeastolate.

8. The method of claim 1, wherein the osmolarity of the cell culture production medium is 440 mOsm or less, the cell culture production medium is maintained at about 30% dissolved oxygen, and the mammalian cells are CHO cells.

9. The method of claim 1, wherein after said culturing, the adalimumab yield in the cell culture production medium is at least 2 g/L.

10. The method of claim 1, wherein the first pH is 8 or less, and the second pH is from 6.5 to 7.

11. The method of claim 10, wherein during adalimumab production, if the glucose concentration in the cell culture production medium decreases to below 2 g/L then glucose is added to the cell culture production medium, or glucose is added to the cell culture production medium such that the glucose concentration in the cell culture production medium is at least 2 g/L but less than or equal to 7 g/L.

12. The method of claim 11, wherein after said culturing, adalimumab yield in the cell culture production medium is at least 1.5 g/L.

13. The method of claim 11, wherein after said culturing, adalimumab yield in the cell culture production medium is at least 2 g/L.

14. The method of claim 11, wherein the osmolarity of the cell culture production medium is 440 mOsm or less, the cell culture production medium is maintained at about 30% dissolved oxygen, and the mammalian cells are CHO cells.

15. The method of claim 10, wherein during adalimumab production, the mammalian cells are initially cultured at a first temperature and then cultured at a reduced temperature.

16. The method of claim 15, wherein the first temperature is 35° C., 37° C., or 38° C., and the second temperature is 32° C. or 33° C.

17. The method of claim 10, wherein the cell culture production medium comprises a non-animal-based hydrolysate.

18. The method of claim 10, wherein the cell culture production medium comprises a soy-based hydrolysate and a yeast-based hydrolysate.

19. The method of claim 10, wherein the mammalian cells are CHO cells and the cell culture production medium is supplemented with a 25×PFCHO solution and a 33× solution of a combination of the soy-based hydrolysate and yeast-based hydrolysate, yeastolate.

20. The method of claim 10, wherein the first pH is adjusted to the second pH via a pH ramp.

21. A method of producing adalimumab, comprising culturing in large-scale mammalian cells that express adalimumab in a cell culture production medium, wherein during adalimumab production, the mammalian cells are initially cultured at a first temperature and then cultured at a reduced temperature, and the cell culture production medium is not removed but is supplemented with a nutrient at least once; and the adalimumab produced by the mammalian cells is purified from the cell culture production medium using a process including Protein A affinity purification.

22. The method of claim 21, wherein during adalimumab production, if the glucose concentration in the cell culture production medium decreases to below 2 g/L then glucose is added to the cell culture production medium, or glucose is added to the cell

culture production medium such that the glucose concentration in the cell culture production medium is at least 2 g/L but less than or equal to 7 g/L.

23. The method of claim 21, wherein during said culturing, the mammalian cells are initially cultured at a first pH and then cultured at a reduced pH, wherein the first pH is 8 or less and the reduced pH is 6.5 to 7, and the first pH is adjusted to the reduced pH via a pH ramp.

24. The method of claim 23, wherein the mammalian cells are CHO cells, and the cell culture production medium comprises a soy-based hydrolysate and a yeast-based hydrolysate.

25. The method of claim 23, wherein the mammalian cells are CHO cells and the cell culture production medium is supplemented with a 25×PFCHO solution and a 33× solution of a combination of the soy-based hydrolysate and yeast-based hydrolysate, yeastolate.

26. The method of claim 21, wherein the osmolarity of the cell culture production medium is 440 mOsm or less, the cell culture production medium is maintained at about 30% dissolved oxygen, and the mammalian cells are CHO cells.

27. The method of claim 21, wherein after said culturing, the adalimumab yield in the cell culture production medium is at least 2 g/L.

28. The method of claim 23, wherein after said culturing, adalimumab yield in the cell culture production medium is at least 1.5 g/L.

29. A method of producing adalimumab, comprising culturing in large-scale mammalian cells that express adalimumab in a cell culture production medium, wherein a) the mammalian cells are initially cultured at a first pH and then cultured at a reduced pH, wherein the first pH is 8 or less and the reduced pH is 6.5 to 7; b) during adalimumab production the mammalian cells are initially cultured at a first temperature

and then cultured at a reduced temperature; c) during adalimumab production, the culture medium is not removed but is supplemented with glucose; d) during adalimumab production, the culture medium is supplemented with a nutrient other than glucose at least once; and e) the adalimumab produced by the mammalian cells is purified from the cell culture production medium using a process including Protein A affinity purification.

30. The culturing method of claim 29, wherein the mammalian cells are CHO cells, and the cell culture production medium comprises a soy-based hydrolysate and a yeast-based hydrolysate.