Example 3 : Comparison of Feedback Control and Bolus Fed Strategy

Materials and Methods

Cells were cultured under feedback control or bolus fed strategy as described above.

Results

Figure 11 shows the difference in PTMs in cells cultured using the feedback control or bolus fed strategy. Each pair of columns represents a batch day. For each pair of columns, the left column is the feedback control data, and the right column is the bolus fed data. The feedback control strategy (left column for each pair of columns) was confirmed to reduce the level of %PTM in the subsequent experiment as compared to the bolus feed strategy (right column for each pair of columns). The steady-state addition of small doses of nutrients over time allows for the production of bioproducts having lower levels of PTM in comparison to bolus fed strategy. The %PTM was also reduced from the bolus feed strategy thus demonstrating the ability to control the antibody quality. The data are from a comparison between a bolus fed strategy that does not include Raman Spectroscopy feedback control to a feed system that does include Raman Spectroscopy feedback control.

The disclosed feedback control culture systems and methods provide real-time multi-component analysis without sample removal. Real time data enables automatic feedback control for continuous nutrient addition. Reduced, steady bioreactor concentrations of reactive nutrients results in lower level of antibody PTM by over 50% from standard bolus nutrient feed thus improving product quality and consistency.

While in the foregoing specification this invention has been described in relation to certain embodiments thereof, and many details have been put forth for the purpose of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein can be varied considerably without departing from the basic principles of the invention.

CLAIMS

1. A method for controlling cell culture medium conditions comprising:

quantifying the concentrations of one or more analytes in the cell culture medium using in situ Raman spectroscopy; and

adjusting with a duty cycle feedback controlled continuous nutrient feed the concentrations of the one or more analyte concentrations in the cell culture medium to provide steady-state addition of small doses of nutrients over time near or at the rate the nutrients are taken up or consumed by cells in the culture medium, wherein at least one of the predetermined analyte concentrations ~~that~~ maintain post-translational modifications of proteins in the cell culture medium to 1.0 to 30 percent.

2. The method of claim 1, wherein the post-translational modification comprises glycation.

3. The method of claim 1, wherein proteins in the cell culture comprise an antibody or antigen-binding fragment thereof.

4. The method of claim 1, wherein proteins in the cell culture comprise a fusion protein.

5. The method of claim 1, wherein the cell culture medium comprises mammalian cells.

6. The method of claim 5, wherein the mammalian cells comprise Chinese Hamster Ovary cells.

7. The method of claim 1, wherein the one or more analytes is glucose.

8. The method of claim 7, wherein the predetermined glucose concentration is 0.5 to 8.0 g/L.

9. The method of claim 8, wherein the predetermined glucose concentration is 1.0 g/L to 3.0 g/L.

10. The method of claim 8, wherein the predetermined glucose concentration is 2.0 g/L.

11. The method of claim 8, wherein the predetermined glucose concentration is 1.0 g/L.

12. The method of claim 1, wherein the predetermined analyte concentrations maintain post-translation modifications of proteins in the cell culture medium to 1.0 to 20 percent.

13. The method of claim 1, wherein the predetermined analyte concentrations maintain post-translation modifications of proteins in the cell culture medium to 5.0 to 10 percent.

14. The method of claim 1, wherein the quantifying of one or more analytes is performed continuously.

15. The method of claim 1, wherein the quantifying of one or more analytes is performed intermittently.

16. The method of claim 1, wherein the quantifying of one or more analytes is performed in intervals.

17. The method of claim 1, wherein the quantifying of one or more analytes is performed in 5 minute intervals.

18. 1The method of claim 1, wherein the quantifying of one or more analytes is performed in 10 minute intervals.

19. The method of claim 1, wherein the quantifying of one or more analytes is performed in 15 minute intervals.

20. The method of claim 1, wherein the quantifying of one or more analytes is performed hourly.

21. The method of claim 1, wherein the quantifying of one or more analytes is performed at least daily.

22. The method of claim 1, wherein the adjusting of one or more analyte concentrations is performed automatically.

23. The method of claim 1, wherein at least two different analytes are quantified.

24. The method of claim 1, wherein at least three different analytes are quantified.

25. The method of claim 1, wherein at least four different analytes are quantified.

26. A method for reducing post-translation modifications of a secreted protein comprising:

culturing cells secreting the protein in a cell culture medium comprising 0.5 to 8.0 g/L glucose;

incrementally determining the concentration of glucose in the cell culture medium during culturing of the cells using *in situ* Raman spectroscopy using a duty cycle feedback controlled continuous nutrient feed to provide steady-state addition of small doses of nutrients over time near or at the rate the nutrients are taken up or consumed by cells in the culture medium; and

adjusting the glucose concentration to maintain the concentration of glucose to 0.5 to 8.0 g/L by automatically delivering multiple doses of glucose per hour to maintain post-translational modifications of the secreted protein to 1.0 to 30.0 percent.

27. The method of claim 26, wherein the concentration of glucose is 1.0 to 3.0 g/L.