

CLAIMS

WHAT IS CLAIMED:

1. A method for producing a recombinant protein of interest, the method comprising:

propagating or maintaining CHO cells in a defined cell culture medium, wherein said CHO cells express a recombinant protein of interest;

wherein the defined cell culture medium is supplemented with asparagine in an amount from about 3.6 mM to about 43.2 mM during early fed-batch cell culture and from about 2.6 mM to about 21.6 mM during late fed-batch cell culture, wherein the amount of asparagine supplemented during early fed-batch cell culture is greater than the amount of asparagine supplemented during late fed-batch cell culture, the supplementing begins on day 1 or later of the early fed-batch cell culture, and the supplementing is performed at intervals of about 2 days; and

maintaining said cells in said asparagine supplemented cell culture medium for at least a portion of the early and late fed-batch cell culture to produce said recombinant protein of interest;

wherein said recombinant protein of interest has reduced sequence variants compared to a recombinant protein of interest produced with a method with a lower amount of asparagine supplementation or no asparagine supplementation in early and/or late fed-batch cell culture.

2. The method of claim 1, wherein the asparagine supplement is provided as part of a bulk feed or as a separate asparagine supplement feed in early and/or late fed-batch cell culture.

3. The method of claim 1, wherein the asparagine supplement is provided continuously or as bolus in early and/or late fed-batch cell culture.

4. The method of claim 1, wherein the defined cell culture medium is supplemented with asparagine in an amount from about 7.2 mM to about 21.6 mM during early fed-batch cell culture and about 3.6 mM to about 10.8 mM during late fed-batch cell culture.
5. The method of claim 1, wherein said recombinant protein of interest is an Fc-fusion protein, a receptor-Fc-fusion protein, an antibody, an antibody fragment, or an scFv-Fc fusion protein.
6. The method of claim 1, wherein at least one additional cell culture performance parameter is improved by the asparagine supplementation, as compared to a method with a lower amount of asparagine supplementation or no asparagine supplementation in early and/or late fed batch culture, wherein the at least one additional cell culture performance parameter is selected from the group consisting of increased cell viability, increased cell growth rate, increased cell density, increased titer of the recombinant protein of interest, increased yield of the recombinant protein of interest, reduction in depletions of essential amino acids in at least a portion of the cell culture, reduction in the formation of at least one cell culture by-product in at least a portion of the cell culture, and improvement of at least one indicator of protein quality.
7. The method of claim 6, wherein the at least one cell culture by-product is selected from the group consisting of ammonium ions and alanine.
8. The method of claim 1, wherein the asparagine supplement is provided at least once, at least twice, at least three times, at least four times, at least five times, or continuously for at least a portion of the late fed-batch cell culture.
9. The method of claim 1, wherein the asparagine supplement is provided continuously beginning on day 5 or after of the fed-batch cell culture, and thereafter for at least a portion of the late fed-batch cell culture.
10. The method of claim 6, wherein the continuous asparagine supplement is discontinued on or after day 10 of the fed-batch cell culture.

11. The method of claim 1, wherein the asparagine supplement is provided continuously in early and/or late fed-batch cell culture.
12. The method of claim 1, wherein extracellular amino acid depletions in the cell culture of asparagine, aspartic acid, and glutamic acid are delayed by at least 1 day, at least 2 days, at least 3 days, or at least 4 days or more, as compared to a method with asparagine supplementation provided in early and/or late fed-batch cell culture as a bolus feed.