

CLAIMS

1. A method of producing a product of interest in a fed-batch process comprising:
 - a) providing mammalian cells comprising a nucleic acid encoding a product of interest;
 - b) inoculating the mammalian cells in a basal medium to provide a cell culture;
 - c) adding a feed medium comprising adding one or more feed supplements to the cell culture, wherein the feed medium adds lactate and cysteine at a molar ratio ($\text{mmol} \times \text{L}^{-1} \times \text{day}^{-1} / \text{mmol} \times \text{L}^{-1} \times \text{day}^{-1}$) of lactate/cysteine of about 8:1 to about 50:1 to the basal medium resulting in a cell culture medium or to the resulting cell culture medium, wherein the cysteine is added at 0.225 mM /day or higher;
 - d) culturing the mammalian cells in the cell culture medium under conditions that allow expression of the product of interest; and
 - e) optionally isolating the product of interest.
2. The method according to claim 1, wherein the molar ratio of lactate/cysteine is about 10:1 to 50:1, preferably about 10:1 to about 30:1.
3. The method according to claim 1 or 2, wherein
 - a) the lactate is added at 3 mmol/L/day or higher, at 5 mmol/L/day or higher, at 7 mmol/L/day or higher, or at 10 mmol/L/day or higher;
 - b) the lactate in the cell culture medium is maintained at 0.5 g/L or higher, 1 g/L or higher, 2 g/L or higher, preferably between 2 and 4 g/L;
 - c) the cysteine is provided as cysteine or a salt and/or hydrate thereof, cystine or a salt thereof or a dipeptide or tripeptide comprising cysteine; and/or
 - d) the cysteine is added at 0.25 mM/day or higher, at 0.3 mM/day or higher, or at 0.4 mM/day or higher.
4. The method according to any one of claims 1 to 3, wherein the product of interest is a heterologous protein or a recombinant virus.

5. The method according to any one of claims 1 to 4, wherein the nucleic acid encodes a heterologous protein and
 - (a) the product titers and/or cell specific productivity is increased compared to the product titers and/or cell specific productivity of the heterologous protein produced by the same method, wherein the feed medium adds cysteine at or below 0.19 mM/day in the absence of lactate;
 - (b) the relative amount of high mannose structures in a population of the heterologous protein is reduced compared to a population of the heterologous protein produced by the same method, wherein the feed medium adds cysteine at or below 0.19 mM/day in the absence of lactate, preferably wherein the high mannose structures are mannose 5 structures; and/or
 - (c) the relative amount (of total) of acidic species in a population of the heterologous protein is reduced compared to a population of the heterologous protein produced by the same method, wherein the feed medium adds the same concentration of cysteine in the absence of lactate.
6. The method of any one of claims 1 to 5, wherein the basal medium and the feed medium is serum-free and chemically defined.
7. The method of any one of claims 1 to 6, wherein the heterologous protein is an antibody or an antigen-binding fragment thereof, a bispecific antibody, a trispecific antibody or a fusion protein.
8. A method of culturing mammalian cells in a fed-batch process comprising:
 - a) providing mammalian cells comprising a nucleic acid encoding a product of interest;
 - b) inoculating the mammalian cells in a basal medium to provide a cell culture;
 - c) adding a feed medium comprising adding one or more feed supplements to the cell culture, wherein the feed medium adds lactate and cysteine at a molar ratio ($\text{mmol} \times \text{L}^{-1} \times \text{day}^{-1} / \text{mmol} \times \text{L}^{-1} \times \text{day}^{-1}$) of lactate/cysteine of about 8:1 to about 50:1 to the basal medium resulting in a cell culture medium or to the resulting cell culture medium, wherein the cysteine is added at 0.225 mM /day or higher; and

- d) culturing the mammalian cells in the cell culture medium under conditions that allow expression of the product of interest.
9. A method of reducing acidic species in a heterologous protein produced in a fed-batch process comprising:
- a) providing mammalian cells comprising a nucleic acid encoding a heterologous protein;
 - b) inoculating the mammalian cells in a basal medium to provide a cell culture;
 - c) adding a feed medium comprising adding one or more feed supplements to the cell culture, wherein the feed medium adds lactate and cysteine at a molar ratio ($\text{mmol} \times \text{L}^{-1} \times \text{day}^{-1} / \text{mmol} \times \text{L}^{-1} \times \text{day}^{-1}$) of lactate/cysteine of about 8:1 to about 50:1 to the basal medium resulting in a cell culture medium or to the resulting cell culture medium, wherein the cysteine is added at 0.225 mM /day or higher;
 - d) culturing the mammalian cells in the cell culture medium under conditions that allow expression of the heterologous protein; and
 - e) optionally isolating the heterologous protein;
- wherein the relative amount of acidic species in a population of the heterologous protein is reduced compared to a population of the heterologous protein produced by the same method wherein the feed medium adds the same concentration of cysteine in the absence of lactate.
10. A method of reducing high mannose structures in a heterologous protein produced in a fed-batch process comprising:
- a) providing mammalian cells comprising a nucleic acid encoding a heterologous protein;
 - b) inoculating the mammalian cells in a basal medium to provide a cell culture;
 - c) adding a feed medium comprising adding one or more feed supplements to the cell culture, wherein the feed medium adds lactate and cysteine at a molar ratio ($\text{mmol} \times \text{L}^{-1} \times \text{day}^{-1} / \text{mmol} \times \text{L}^{-1} \times \text{day}^{-1}$) of lactate/cysteine of about 8:1 to about 50:1 to the basal medium resulting in a cell culture medium or to the resulting cell culture medium, wherein the cysteine is added at 0.225 mM /day or higher;

d) culturing the mammalian cells in the cell culture medium under conditions that allow expression of the heterologous protein; and

e) optionally isolating the heterologous protein;

wherein the relative amount of the high mannose structures in a population of the heterologous protein is reduced compared to a population of the heterologous protein produced by the same method wherein the feed medium adds the cysteine at or below 0.19 mM/day in the absence of lactate, preferably wherein the high mannose structures are mannose 5 structures.

11. A method of preventing negative effects of cysteine on product quality characteristics when producing a heterologous protein in a fed-batch process comprising:

a) providing mammalian cells comprising a nucleic acid encoding a heterologous protein;

b) inoculating the mammalian cells in a basal medium to provide a cell culture;

c) adding a feed medium comprising adding one or more feed supplements to the cell culture, wherein the feed medium adds lactate and cysteine at a molar ratio ($\text{mmol} \times \text{L}^{-1} \times \text{day}^{-1} / \text{mmol} \times \text{L}^{-1} \times \text{day}^{-1}$) of lactate/cysteine of about 8:1 to about 50:1 to the basal medium resulting in a cell culture medium or to the resulting cell culture medium, wherein the cysteine is added at 0.225 mM /day or higher;

d) culturing the mammalian cells in the cell culture medium under conditions that allow expression of the heterologous protein; and

e) optionally isolating the heterologous protein from the mammalian cells;

wherein the negative effects on product quality characteristics in a population of the heterologous protein are reduced compared to a population of the heterologous protein produced by the same method wherein the feed medium adds the same concentration of cysteine in the absence of lactate.

12. A heterologous protein produced by the method of any one of claims 9 to 11.

13. Use of lactate in a feed medium for reducing acidic species and/or high mannose structures in a heterologous protein produced in a fed-batch process, wherein the feed medium adds cysteine at 0.225 mM/day or higher.
14. Use of lactate in a feed medium for preventing negative effects of cysteine on product quality characteristics of a heterologous protein produced in a fed-batch process, preferably wherein the negative effects on product quality characteristics are increased high mannose structures, increased low molecular weight species and/or increased acidic species.
15. Use of lactate and cysteine in a feed medium for increasing heterologous protein titer and/or cell-specific productivity in a fed-batch process.
16. The method of any one of claims 1-11 or the use of any one of claims 13 to 15, wherein the fed-batch process comprises culturing a mammalian cell, wherein the mammalian cell is a HEK293 cell or a CHO cell or a HEK293 cell or CHO cell derived cell, preferably the mammalian cell is a CHO cell or a CHO derived cell.
17. A feed medium for mammalian cell fed-batch culture comprising lactate and cysteine at a molar ratio (mM/mM) of lactate/cysteine of about 8:1 to about 50:1, preferably wherein the feed medium comprises one or more feed supplements for separate addition.
18. A kit comprising
 - (a) a concentrated feed medium for mammalian cell fed-batch culture comprising lactate and optionally cysteine, and
 - (b) an aqueous supplement separate from the concentrated feed medium comprising cysteine,wherein the feed medium and the supplement provide a lactate/cysteine molar ratio (mM/mM) of about 8:1 to about 50:1 and cysteine at 0.225 mM/day or higher in a daily addition of less than 5%, preferably less than 3.5% of the cell culture starting volume.