

CLAIMS

1. A method of reducing precipitation in a bacterial fermentation process for producing a fermentation product, wherein the fermentation process comprises the steps:

- a) cultivating host cells for bacterial growth in a batch phase using a batch medium; and
- b) cultivating the host cells for producing the fermentation product in a fed-batch phase using a feed medium;

wherein the feed and batch media are each unprecipitated media solutions after autoclaving,

wherein:

i) the batch medium comprises an amount of calcium salts, magnesium salts, phosphate salts and at least one chelating agent, which is a citrate salt and/or citric acid at an amount of at least 5 mM, and/or EDTA at an amount of at least 1 mM;

ii) the feed medium comprises an amount of calcium salts which is at least 1 mM, and which is at least 5-fold higher as compared to the batch medium (Mol/Mol);

iii) the feed medium comprises an amount of magnesium salts which is at least 3 mM, and which is at least 10-fold higher as compared to the batch medium (Mol/Mol); and

iv) the feed medium comprises phosphate salts in an amount of 0 to less than 10 mM, wherein the amount of phosphate salts is less than 90% of the amount of phosphate salts comprised in the batch medium (Mol/Mol).

2. The method of claim 1, wherein

- a) the amount of calcium salts is up to 1 mM in the batch medium; and up to 5, or up to 4 mM in the feed medium; and/or
- b) the amount of magnesium salts is 1-3 mM in the batch medium; and 3-100 mM, or 30-60 mM in the feed medium; and/or
- c) the amount of phosphate salts is 30-120 mM in the batch medium; and 0-5 mM in the feed medium.

3. The method of claim 1 or 2, wherein both, the batch and feed media further comprise an organic carbon source and trace elements.

4. The method of any one of claims 1 to 3, wherein the batch medium further comprises ammonium salts.

5. The method of any one of claims 1 to 4, wherein:

- 5 a) the batch medium comprises or consists of: 0-1mM calcium salts; 1-3 mM magnesium salts; 10 - 30 g/L glucose and/or glycerol; 50 - 125 mM ammonium; 5 - 50 mM sulphate; 30 - 120 mM phosphate; 5 - 100 mM citrate and/or citric acid, and/or 1 - 30 mM EDTA; trace elements; and optionally an antifoam agent; and
- 10 b) the feed medium comprises or consists of: 1-5 mM calcium salts; 3-100 mM magnesium salts; 0-5 mM phosphate; 500 - 800 g/L glucose and/or glycerol; trace elements; and optionally a chelating agent.

6. The method of claim 5, wherein:

- 15 a) the batch medium comprises:
- (i) calcium salts as CaCl_2 ;
 - (ii) magnesium salts as MgSO_4 ,
 - (iii) 10 - 30 g/L glycerol;
 - (iv) ammonium as one or more of $(\text{NH}_4)_2\text{SO}_4$, NH_4Cl , $\text{NH}_4\text{H}_2\text{PO}_4$, $(\text{NH}_4)_2\text{HPO}_4$, $(\text{NH}_4)_2\text{-H-Citrate}$ or NH_3 ;
 - (v) sulphate as one or more of $(\text{NH}_4)_2\text{SO}_4$, MgSO_4 , or K_2SO_4 ;
 - (vi) phosphate as one or more of KH_2PO_4 , $\text{NH}_4\text{H}_2\text{PO}_4$, $(\text{NH}_4)_2\text{HPO}_4$, H_3PO_4 , NaH_2PO_4 , Na_2HPO_4 or K_2HPO_4 ;
 - (vii) 5 - 100 mM citrate and/or citric acid;
 - 20 (viii) trace elements including one or more of copper, manganese, sodium, boron, zinc, or iron salts; and
 - (ix) an antifoam agent;
- and
- 25 b) the feed medium comprises:
- 30 (i) calcium salts as CaCl_2 ;
 - (ii) magnesium salts as MgSO_4 ;
 - (iii) 0-5 mM phosphate as one or more of KH_2PO_4 , $\text{NH}_4\text{H}_2\text{PO}_4$, $(\text{NH}_4)_2\text{HPO}_4$, H_3PO_4 , NaH_2PO_4 , Na_2HPO_4 or K_2HPO_4 ;
 - (iv) 500 - 800 g/L glycerol;

- (v) trace elements including one or more of copper, manganese, sodium, boron, zinc, or iron salts; and
- (vi) 0 - 20 mM citrate and/or citric acid as a chelating agent.

5 7. The method of any one of claims 1 to 6, wherein the fermentation process is performed at a pH ranging between 6.7 and 7.3.

8. The method of any one of claims 1 to 7, wherein the host cells are grown in the batch phase to a density of at least 10 g cell dry weight per liter, and the host cells are
10 cultured in the fed-batch phase under growth-limiting conditions.

9. The method of any one of claims 1 to 8, wherein the host cells are bacterial production host cells of a species selected from the group consisting of *Escherichia*, *Pseudomonas*, *Bacillus*, *Lactococcus*, *Corynebacterium*, *Clostridium*, *Micrococcus*, and
15 *Streptomyces*.

10. The method of any one of claims 1 to 9, wherein the fermentation product is any one of a protein of interest (POI), RNA, or a recombinant DNA molecule, such as plasmid DNA or a plasmid vector.

20 11. The method of any one of claims 1 to 10, wherein the host cells are bacterial host cells, which comprise a genetic construct to produce and/or express the fermentation product, wherein the genetic construct comprises an inducible promoter, and wherein expression of the genetic construct is induced by limitation of a cell culture component or
25 by addition of an inducer.

12. A media system for use in a bacterial fermentation process comprising a batch medium and a feed medium for cultivating bacterial cells, wherein the media are each unprecipitated media solutions, wherein:

i) the batch medium comprises an amount of calcium salts, magnesium salts, phosphate salts and at least one chelating agent, which is a citrate salt and/or citric acid at an amount of at least 5 mM, and/or EDTA at an amount of at least 1 mM;

ii) the feed medium comprises an amount of calcium salts which is at least 1 mM, and which is at least 5-fold higher as compared to the batch medium (Mol/Mol);

iii) the feed medium comprises an amount of magnesium salts which is at least 3 mM, and which is at least 10-fold higher as compared to the batch medium (Mol/Mol); and

iv) the feed medium comprises phosphate salts in an amount of 0 to less than 10 mM, wherein the amount of phosphate salts is less than 90% of the amount comprised in the batch medium (Mol/Mol).

13. The media system of claim 12, which is characterized by any one or more of the following features:

a) the amount of calcium salts is up to 1 mM in the batch medium; and up to 5, or up to 4 mM in the feed medium;

b) the amount of magnesium salts is 1-3 mM in the batch medium; and 3-100 mM, or 30-60 mM in the feed medium;

c) the amount of phosphate salts is 30-120 mM in the batch medium; and 0-5 mM in the feed medium;

d) the amount of the chelating agent in the batch medium is up to 100 mM citrate salt and/or citric acid; and/or up to 30 mM EDTA;

e) the batch and feed media further comprise an organic carbon source, and trace elements; and

f) the batch medium further comprises a nitrogen source and phosphate.

14. The media system of claim 12 or 13, wherein:

a) the batch medium comprises or consists of: 0-1mM calcium salts; 1-3 mM magnesium salts; 10 - 30 g/L glucose and/or glycerol; 50 - 125 mM ammonium; 5 - 50 mM sulphate; 30 - 120 mM phosphate; 5 - 100 mM citrate and/or citric acid, and/or 1 - 30 mM EDTA; trace elements; and optionally an antifoam agent;
and

b) the feed medium comprises or consists of: 1-5 mM calcium salts; 3-100 mM magnesium salts; 0-5 mM phosphate salts; 500 - 800 g/L glucose and/or glycerol; trace elements; and optionally a chelating agent.

15. The media system of claim 14, wherein:

a) the batch medium comprises:

- (i) calcium salts as CaCl_2 ;
 - (ii) magnesium salts as MgSO_4 ,
 - (iii) 10 - 30 g/L glycerol;
 - (iv) ammonium as one or more of $(\text{NH}_4)_2\text{SO}_4$, NH_4Cl , $\text{NH}_4\text{H}_2\text{PO}_4$, $(\text{NH}_4)_2\text{HPO}_4$, $(\text{NH}_4)_2\text{-H-Citrate}$ or NH_3 ;
 - (v) sulphate as one or more of $(\text{NH}_4)_2\text{SO}_4$, MgSO_4 , or K_2SO_4 ;
 - (vi) phosphate as one or more of KH_2PO_4 , $\text{NH}_4\text{H}_2\text{PO}_4$, $(\text{NH}_4)_2\text{HPO}_4$, H_3PO_4 , NaH_2PO_4 , Na_2HPO_4 or K_2HPO_4 ;
 - (vii) 5 - 100 mM citrate and/or citric acid;
 - (viii) trace elements including one or more of copper, manganese, sodium, boron, zinc, or iron salts; and
 - (ix) an antifoam agent;
- and

b) the feed medium comprises:

- (i) calcium salts as CaCl_2 ;
- (ii) magnesium salts as MgSO_4 ;
- (iii) 0-5 mM phosphate as one or more of KH_2PO_4 , $\text{NH}_4\text{H}_2\text{PO}_4$, $(\text{NH}_4)_2\text{HPO}_4$, H_3PO_4 , NaH_2PO_4 , Na_2HPO_4 or K_2HPO_4 ;
- (iv) 500 - 800 g/L glycerol;
- (v) trace elements including one or more of copper, manganese, sodium, boron, zinc, or iron salts; and

(vi) up to 20 mM citrate and/or citric acid as a chelating agent.

16. Use of the media system of any one of claims 12 to 15 in a method for culturing bacterial host cells in a fed-batch fermentation process.

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17. A method for producing a fermentation product in a bacterial fermentation process, comprising:

a) a batch phase for growing host cells; followed by

b) a fed-batch phase for producing a fermentation product from said host cells;

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and

c) isolating the fermentation product;

employing the media system of any one of claims 12 to 15.

18. The method of claim 17, wherein the fermentation product is any one of a protein of interest (POI), RNA, or a recombinant DNA molecule, such as plasmid DNA or a plasmid vector.

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