

Kuschelewski et al., *Biotechnol. Prog.*, **33**(3) (2017) 759-770 considered the effect of reactive species generated by cell culture media on the overall stability of the media and the behaviour of cells cultured *in vitro*. L-cysteine and other thiol-containing components of cell culture media are likely to be oxidised, forming H₂O₂ or free radical intermediates such as cysteinyl radicals, which can themselves form disulphides in the presence of metal catalysts such as copper or react with oxygen to form cys-SO₂H or react with H₂O₂ to form cys-SO₃H. The generation of oxidation products contributes to an increased cellular stress level.

There is a continuing need to optimise medium formulations and process parameters to improve cell viability, specific productivity and titre. Improvements to medium formulations and/or feeding strategies to result in desirable recombinant protein expression, titre, cell growth and/or cell viability can reduce the costs associated with manufacturing protein therapeutics.

SUMMARY

The method of the present disclosure entails implementing a conditioning period for a medium prior to inoculation with a recombinant protein-producing eukaryotic cell. Eukaryotic cells grown in the resulting medium show increased cell-specific productivity. Furthermore, hydrolytic activity and process specific oxygen demand is improved. Also, the recombinant protein generated by the cell cultured in the resulting medium comprises a decreased fraction of host cell protein impurities.

In one aspect, the present disclosure refers to a method for treatment of a cell culture medium, the method comprising adding to a culture vessel:

(a) a serum-free cell culture medium suitable for growth of a eukaryotic cell and generation of an exogenous protein to a culture vessel, wherein the medium comprises one or more of cystine, cysteine and a cysteine derivative, wherein the cysteine derivative is selected from: S-sulfocysteine, S-sulfocysteinylglycine, N-acetyl cysteine (NAC), cysteine S-linked N-acetyl glucosamine (GlcNAC-cys), homocysteine, L-cysteine mixed disulphides, S-alkylated cysteine with a thiol-protecting group, cysteine with a thiol-protecting group, reduced glutathione (GSH), oxidized GSH, S-sulfoglutathione, γ - glutamylcysteine, cysteinylglycine, N-butanoyl α -glutamyl-cysteinyl-glycine, S-acyl-GSH and S-carboxy-L-cysteine; and

(b) one or more redox active trace metals selected from iron, copper, chromium, cobalt, selenium, manganese, vanadium and molybdenum to the medium; and

holding the cell culture medium comprising the one or more of cystine, cysteine and a cysteine derivative and the one or more redox active trace metals in the vessel under conditions suitable for inoculation of the medium with the eukaryotic cell that has been engineered to recombinantly express the exogenous protein for a period of 10 hours to 96 hours in the absence of cells prior to inoculation.

In yet another aspect, the present disclosure refers to a method of producing a recombinant protein by recombinant expression from a eukaryotic cell, the method comprising:

- subjecting a cell culture medium suitable for cultivating said eukaryotic cell to the method according to the disclosure,
- inoculating the treated cell culture medium with a eukaryotic cell engineered to recombinantly express an exogenous protein to form a cell culture,
- cultivating the cell culture so that the recombinant protein is produced.

FIGURES

Figure 1: Cell specific productivity (qP) from Example 1 for clone 2. For each illustration, the data for the DoE cases “low TES, low CysCys” (A), “low TES, high CysCys” (B), and “high TES, high CysCys” (C) were normalized to the supplementation time “-0 h”.

Figure 2: CHO derived host cell protein (CHO HCP) concentration from Example 1 for clone 2. For each illustration, the data for the DoE cases “low TES, low CysCys” (A), “low TES, high CysCys” (B), and “high TES, high CysCys” (C) were normalized to the supplementation time “-0 h”.

CLAIMS

1. A method for treatment of a cell culture medium, the method comprising adding to a culture vessel:

(a) a serum-free cell culture medium suitable for growth of a eukaryotic cell and generation of an exogenous protein to a culture vessel, wherein the medium comprises one or more of cystine, cysteine and a cysteine derivative, wherein the cysteine derivative is selected from: S-sulfocysteine, S-sulfocysteinylglycine, N-acetyl cysteine (NAC), cysteine S-linked N-acetyl glucosamine (GlcNAC-cys), homocysteine, L-cysteine mixed disulphides, S-alkylated cysteine with a thiol-protecting group, cysteine with a thiol-protecting group, reduced glutathione (GSH), oxidized GSH, S-sulfoglutathione, γ - glutamylcysteine, cysteinylglycine, N-butanoyl α -glutamyl-cysteinyl-glycine, S-acyl-GSH and S-carboxy-L-cysteine; and

(b) one or more redox active trace metals selected from iron, copper, chromium, cobalt, selenium, manganese, vanadium and molybdenum to the medium; and

holding the cell culture medium comprising the one or more of cystine, cysteine and a cysteine derivative and the one or more redox active trace metals in the vessel under conditions suitable for inoculation of the medium with the eukaryotic cell that has been engineered to recombinantly express the exogenous protein for a period of 10 hours to 96 hours in the absence of cells prior to inoculation.

2. The method according to claim 1 wherein the one or more redox active trace metals is added:

(i) at the same time as addition of the medium comprising the one or more of cystine, cysteine and a cysteine derivative to the vessel;

(ii) after addition of the medium comprising the one or more of cystine, cysteine and a cysteine derivative to the vessel and either before or when the vessel is under conditions suitable for inoculation of the medium with the eukaryotic cell; or

(iii) in the vessel, when the vessel is under conditions suitable for inoculation of the medium with the eukaryotic cell.

3. The method according to claim 1 or 2 wherein the medium is held under the conditions suitable for inoculation for a period of at least 24 hours, at least 48 hours, at least 72 hours.

4. The method according to any one of claims 1 to 3, wherein addition of the one or more redox active trace metals takes place after addition of the medium to the vessel.

5. The method according to any one of claims 1 to 4, wherein the vessel is a bioreactor.
6. The method according to any one of claims 1 to 5, wherein the medium comprising the one or more of cystine, cysteine and a cysteine derivative and the one or more redox active trace metals is in a bioreactor at or prior to inoculation thereof.
7. The method according to any one of claims 1 to 6 wherein the medium comprises 0.5 mM to 16 mM of one or more of cystine, cysteine and a cysteine derivative for at least 10 hours at conditions suitable for inoculation.
8. The method according to claim 1, wherein the one or more of iron, copper, chromium, cobalt, selenium, manganese, vanadium and molybdenum is added to the medium as a Trace Element Solution at a cumulative concentration of 6 μ M to 350 μ M.
9. The method according to any one of claims 1 to 8 wherein conditions suitable for inoculation are: a pH of pH 6.0 to pH 8.0, a temperature of 20°C to 39°C; and a % O₂ of 10 to 80%.
10. The method according to any one of claims 1 to 9 wherein the eukaryotic cell is a mammalian cell, a yeast cell or an insect cell.
11. A method for producing a recombinant protein by recombinant expression from a eukaryotic cell, the method comprising:
 - subjecting a cell culture medium suitable for cultivating said eukaryotic cell to the method according to any one of claims 1 to 10,
 - inoculating the treated cell culture medium with a eukaryotic cell engineered to recombinantly express an exogenous protein to form a cell culture,
 - cultivating the cell culture so that the recombinant protein is produced.
12. The method of claim 11 wherein the recombinant protein is an antibody.
13. The method according to claim 11 or 12, further comprising a step of recovering the recombinant protein from the cell culture.