concentration of about 0.1 to 5.0 mM, about 0.2 to 2.0 mM, about 0.2 to 1.0 mM or about 0.4 to 1.0 mM. In certain examples the basal cell culture medium has a total amino acid content of about 30 to about 130, preferably about 35 to about 120, more preferably about 40 to about 100 mM.

The present disclosure also relates to a basal cell culture medium for culturing mammalian cells comprising iron choline citrate at a concentration of about 0.1 to 5.0 mM, about 0.2 to 2.0 mM, about 0.2 to 1.0 mM or about 0.4 to 1.0 mM. In one aspect, the present disclosure relates to a basal cell culture medium for culturing mammalian cells comprising iron choline citrate at a concentration of 0.1 to 5.0 mM, 0.2 to 2.0 mM, 0.2 to 1.0 mM or 0.4 to 1.0 mM.

The present disclosure relates to a feed medium for culturing mammalian cells comprising the following amino acids at a molar ratio relative to isoleucine (mM/mM) of: L-leucine/L-isoleucine of about 2.3–4.2, L-phenylalanine/L-isoleucine of about 0.6-1.1, L-threonine/l-isoleucine of about 1.3-2.4, and L-valine/L-isoleucine of about 1.1-2.0, wherein the feed medium has a total amino acid content of about 100 to 1000 mM. In one example of the present disclosure the feed medium further comprises the following amino acids at a molar ratio relative to isoleucine (mM/mM) of: L-tyrosine/L-isoleucine of about 0.6-1.1, and/or L-lysine/L-isoleucine of about 1.1-2.1. The feed medium according to the disclosure may further comprise at least one of the following amino acids at a molar ratio relative to isoleucine (mM/mM) of: L-tryptophan/L-isoleucine of about 0.3-0.6, L-proline/L-isoleucine of about 0.9-1.8; or L-methionine/L-isoleucine of about 0.4-0.8. Preferably the feed medium comprises L-tryptophan, L-proline and L-methionine each at said molar ratios as defined above. The feed medium is typically a concentrated feed medium. Preferably the feed medium of the disclosure is a serum-free medium, more preferably a chemically defined medium or a chemically defined and protein-free medium. In one example the feed medium additionally comprises iron choline citrate at a concentration of about 0.4 to 5 mM, about 0.4 to 1.0 mM or about 0.5 to 1.0 mM, preferably about 0.5 to 0.6 mM. In one example the feed medium is characterized by a low salt content, preferably a low salt content of about 100 mM or less and more preferably about 50 mM or less. In certain examples the feed medium of the disclosure has a total amino acid content of about 200 to about 900, preferably about 300 to about 800, more preferably about 400 to about 700 mM.

In another aspect, the present disclosure also relates to a feed medium for culturing mammalian cells comprising iron choline citrate at a concentration of 0.4 to 5 mM, 0.4 to 1.0 mM or 0.5 to 1.0 mM, preferably 0.5 to 0.6 mM.

In a related aspect the disclosure relates to a medium platform for culturing mammalian cells comprising a) the basal cell culture medium of the disclosure, and b) the feed medium of the disclosure.

of the disclosure for a period of time and conditions suitable for cell growth and protein production, feeding the cells with said feed medium, harvesting the protein of interest and recovering the protein from the culture medium.

In another aspect, the disclosure relates to a use of iron choline citrate as iron carrier in a mammalian cell culture medium, wherein the iron choline citrate is present in the mammalian cell culture medium at a concentration of 0.2 to 2.0 mM.

DESCRIPTION OF THE FIGURES

Figure 1: RPMI based basal medium with and without optimized amino acid adjustment in a batch experiment at different total amino acid concentrations.

(A-D): CHO2 (CHO-DG44) Rituximab cells were cultured in RPMI based medium 4.1 and medium 5.0 without and with optimized amino acid ratios in duplicates (N=2) with a total amino acid concentration of 44 mM, ◍ culture in medium 5.0, with optimized amino acid ratios (44 mM), ■ culture in medium 4.1, without optimized amino acid ratios (44 mM). Shown are (A) viable cell concentration [1x10^5 cell/mL] CHO2 (CHO-DG44) Rituximab cells, (B) viability [%] CHO2 (CHO-DG44) Rituximab cells and (C) product concentration [mg/L] CHO2 (CHO-DG44) Rituximab, (D) Lactate concentration [g/L] CHO2 (CHO-DG44) Rituximab.

(E-H): CHO2 (CHO-DG44) Rituximab cells were cultured in RPMI based medium 4.2 and medium 5.1 without and with optimized amino acid ratios in duplicates (N=2) with a total amino acid concentration of 66 mM, ◍ culture in medium 5.1, with optimized amino acid ratios (66 mM), ■ culture in medium 4.2, without optimized amino acid ratios (66 mM). Shown are (E) viable cell concentration [1x10^5 cell/mL] CHO2 (CHO-DG44) Rituximab cells, (F) viability [%] CHO2 (CHO-DG44) Rituximab cells and (G) product concentration [mg/L] CHO2 (CHO-DG44) Rituximab, (H) Lactate concentration [g/L] CHO2 (CHO-DG44) Rituximab.

(I): CHO2 (CHO-DG44) Rituximab cells were cultured in RPMI based medium 4.3 and medium 5.2 without and with optimized amino acid ratios in duplicates (N=2) with a total amino acid concentration of 22 or 36 mM, ■ culture in medium 5.2, with optimized amino acid ratios (22 mM), ► culture in medium 4.3, without optimized amino acid ratios (22 mM), ✚ culture in medium 4.0, without optimized amino acid ratios (36 mM). Shown is (I) product concentration [mg/L] CHO2 (CHO-DG44) Rituximab.

(J): CHO2 (CHO-DG44) Rituximab cells were cultured in RPMI based medium 4.2 and medium 5.2 without and with optimized amino acid ratios in duplicates (N=2) with a total amino acid concentration of 22 and 66 mM, ■ culture in medium 5.2, with optimized amino acid ratios (22 mM), ● culture in medium 4.2, without optimized amino acid ratios (66 mM). Shown is (J) product concentration [mg/L] CHO2 (CHO-DG44) Rituximab.

CLAIMS

1. A basal cell culture medium for culturing mammalian cells comprising iron choline citrate at a concentration of 0.1 to 5.0 mM, 0.2 to 2.0 mM, 0.2 to 1.0 mM or 0.4 to 1.0 mM.

2. The basal cell culture medium of claim 1, further comprising the following amino acids at a molar ratio relative to isoleucine (mM/mM) of:
L-leucine/L-isoleucine of 1.2-2.2,
L-phenylalanine/L-isoleucine of 0.5-0.9,
L-tyrosine/L-isoleucine of 1.5-2.7,
L-threonine/l-isoleucine of 1.0-1.9, and
L-valine/L-isoleucine of 1.0-1.9,
wherein the basal cell culture medium has a total amino acid content of 25 to 150 mM, and wherein the medium is a serum-free medium.

3. The basal cell culture medium of claim 2, further comprising L-lysine at a molar ratio relative to isoleucine of 1.6-2.9 (mM/mM).

4. The basal cell culture medium of claims 2 or 3, further comprising at least one of the following amino acids at a molar ratio relative to isoleucine (mM/mM) of:
L-tryptophan/L-isoleucine of 0.3-0.5,
L-proline/L-isoleucine of 1.6-3.0; or
L-methionine/L-isoleucine of 0.4-0.7.

5. A feed medium for culturing cells comprising iron choline citrate at a concentration of 0.4 to 5 mM, 0.4 to 1.0 mM or 0.5 to 1.0 mM, preferably 0.5 to 0.6 mM.

6. The feed medium of claim 5, further comprising the following amino acids at a molar ratio relative to isoleucine (mM/mM) of:
L-leucine/L-isoleucine of 2.3–4.2,
L-phenylalanine/L-isoleucine of 0.6-1.1,
L-threonine/l-isoleucine of 1.3-2.4, and
L-valine/L-isoleucine of 1.1-2.0,
wherein the feed medium has a total amino acid content of 100 to 1000 mM, and wherein the medium is a serum-free medium.

7. The feed medium of claim 6, further comprising the following amino acids at a molar ratio relative to isoleucine (mM/mM) of:

L-tyrosine/L-isoleucine of 0.6-1.1, and/or L-lysine/L-isoleucine of 1.1-2.1, preferably L-tyrosine/L-isoleucine of 0.6-1.1, and L-lysine/L-isoleucine of 1.1-2.1.

8. The feed medium of claims 6 or 7, further comprising at least one of the following amino acids at a molar ratio relative to isoleucine (mM/mM) of:
L-tryptophan/L-isoleucine of 0.3-0.6,
L-proline/L-isoleucine of 0.9-1.8; or
L-methionine/L-isoleucine of 0.4-0.8.

9. A medium platform for culturing mammalian cells comprising:
a) the basal cell culture medium of claims 1 to 4, and
b) the feed medium of claims 5 to 8.

10. A method of culturing a mammalian cell comprising the following steps:
a) providing mammalian cells,
b) culturing the cells in the basal cell culture medium of any one of claims 1 to 4, and
c) optionally adding the feed medium of any one of claims 5 to 8 to the basal cell culture medium;
wherein the cells are cultured under conditions that allow the cells to proliferate.

11. A method of producing a protein of interest comprising the following steps:
a) providing mammalian cells comprising a gene of interest encoding the protein of interest,
b) culturing the cells in the basal cell culture medium of any one of claims 1 to 4, and
c) optionally adding the feed medium of any one of claims 5 to 8 to the basal cell culture medium, and
d) optionally separating and/or isolating and/or purifying said protein of interest from the cell culture;
wherein the cells are cultured under conditions that allow expression of the protein of interest.

12. The method of claim 11, wherein the protein of interest is a secreted protein, preferably the protein of interest is an antibody or Fc-fusion protein.

13. The method of any one of claims 10 to 12, wherein the cell culture is a large-scale cell culture of a working volume of 100 L or more.

14. The cell culture medium of any one of claims 1 to 4, the feed medium of any one of claims 5 to 8, or the method of any one of claims 10 to 13, wherein the mammalian cell is a rodent or human cell, preferably

(a) the rodent cell is a Chinese hamster ovary (CHO) cell such as a CHO-K1 cell, a CHO-DG44 cell, a DuxB11 cell or a CHO GS deficient cell, most preferably the cell is a CHO-DG44 cell or a CHO GS deficient cell; and

(b) the human cell is a PER.C6 cell or a HEK293 cell.

15. Use of iron choline citrate as iron carrier in a mammalian cell culture medium, wherein the iron choline citrate is present in the mammalian cell culture medium at a concentration of 0.2 to 2.0 mM.