Claims

1. A method of cell culture comprising

(i) culturing cells in a cell culture medium, and

(ii) maintaining at least one metabolite selected from aconitic acid (AA), leucinic acid (HICA), cytidine monophosphate (CMP), methylsuccinic acid (MSA), trigonelline (TRI) and N-acetylputrescinium (NAP) below an inhibitory concentration in the cell culture medium for the at least one metabolite.

2. The method of claim 1,

wherein the metabolite is AA and the inhibitory concentration is lower than 100 μM, 440 μM, 880 μM, 3 mM, 5 mM or 10 mM,

wherein the metabolite is HICA and the inhibitory concentration is lower than 10 μM, 23.5 μM, 47 μM, 100 μM, 1 mM, or 3 mM,

wherein the metabolite is CMP and the inhibitory concentration is 5μM, 10 μM, 20 μM, 100 μM, 500 μM, or 1 mM,

wherein the metabolite is MSA and the inhibitory concentration is lower than 1 μM, 3.75 μM, 7.5 μM, 100 μM, 1 mM or 3 mM,

wherein the metabolite is TRI and the inhibitory concentration is lower than 0.1 μM, 0.35 μM, 0.7 μM, 100 μM, 1 mM or 3 mM, or

wherein the metabolite is NAP and the inhibitory concentration is lower than 0.1 μM, 0.3 μM, 0.6 μM, 100 μM, 1 mM or 3 mM.

3. The method of claim 1, wherein the concentration of the at least one metabolite is measured using nuclear magnetic resonance (NMR) spectroscopy, Raman spectroscopy, High/Ultra Performance Liquid Chromatography (H/UPLC), Liquid Chromatography Mass Spectrometer (LC-MS), Gas Chromatography Mass Spectrometer (GC-MS) technology, or a combination thereof.

4. The method of claim 1, wherein step ii) comprises measuring the concentration of the at least one metabolite, and, when the measured concentration of the at least one metabolite is above a predefined value, a concentration of a precursor of the at least one metabolite in the cell culture medium is decreased by reducing the amount of the precursor provided to the cells.

5. The method of claim 4, wherein

the metabolite is AA, the precursor is glutamine, glucose, arginine, asparagine, or a combination thereof;

the metabolite is HICA, the precursor is leucine, isoleucine, or a combination thereof;

the metabolite is CMP, the precursor is glutamine, arginine, aspartate, or a combination thereof;

the metabolite is MSA, the precursor is lysine, isoleucine, serine, glucose, glutamine, or a combination thereof;

the metabolite is TRI, the precursor is aspartate, tryptophan, glutamine, or a combination thereof;

the metabolite is NAP, the precursor is arginine, proline, aspartate glutamine, asparagine, or a combination thereof; or

a combination thereof.

6. The method of claim 1, wherein step ii) comprises measuring the concentration of the at least one metabolite, and, when the measured concentration of the at least one metabolite is above a predefined value, regulating enzyme expression to reduce the synthesis of the metabolite.

7. The method of claim 6,

wherein the metabolite is AA and the enzyme is ADI1, HOGA1, TAD1, or a combination thereof;

wherein the metabolite is HICA and the enzyme is GOT1, D-HicDH, MMUT, AUH, HMGCL, HADHA/B, or a combination thereof;

wherein the metabolite is CMP and the enzyme is UCK1/2, NT5, CMAS, CMPK1, DDYD, CDA, SLC35A1, RRM1, HOGA1, or a combination thereof;

wherein the metabolite is MSA and the enzyme is GOT1, ETHE1, AMT, HADHA/B, MMUT, or a combination thereof;

wherein the metabolite is TRI and the enzyme is NADSYN1, NNMT, CAT, NMNAT1, SULT4A1, or a combination thereof;

wherein the metabolite is NAP and the enzyme is SAT1/2, HOGA1, AMD1, ODC1, GOT1, MAOB, or a combination thereof; or

a combination thereof.

8. The method of claim 6, wherein regulating enzyme expression to reduce the synthesis of the metabolite comprises adding an inhibitory nucleic acid which inhibits expression of a gene encoding the enzyme.

9. The method of claim 6, wherein regulating enzyme expression to reduce the synthesis of the metabolite comprises adding a recombinant DNA molecule to overexpress the regulating enzyme, wherein the regulating enzyme is a downstream enzyme.

10. The method of claim 1, wherein step ii) comprises measuring the concentration of the at least one metabolite, and, when the measured concentration of the at least one metabolite is above a predefined value, regulating enzyme activity to reduce the synthesis of the metabolite.

11. The method of claim 10,

wherein the metabolite is AA and the enzyme is ADI1, HOGA1, TAD1, or a combination thereof;

wherein the metabolite is HICA and the enzyme is D-HicDH, MMUT, AUH, HMGCL, HADHA/B, or a combination thereof;

wherein the metabolite is CMP and the enzyme is UCK1/2, NT5, CMAS, CMPK1, DDYD, CDA, SLC35A1, RRM1, HOGA1, or a combination thereof;

wherein the metabolite is MSA and the enzyme is ETHE1, AMT, HADHA/B, MMUT, or a combination thereof;

wherein the metabolite is TRI and the enzyme is NADSYN1, NNMT, CAT, NMNAT1, SULT4A1, or a combination thereof;

wherein the metabolite is NAP and the enzyme is SAT1/2, HOGA1, AMD1, ODC1, GOT1, MAOB, or a combination thereof; or

a combination thereof.

12. The method of claim 10, wherein regulating enzyme activity to reduce the synthesis of the metabolite comprises adding an inhibitor of enzyme activity to the culture.

13. The method of claim 10, wherein regulating enzyme activity to reduce the synthesis of the metabolite comprises adding a glycolytic pathway activator to the culture.

14. The method of claim 1, wherein maintaining the at least one metabolite below an inhibitory concentration in the cell culture medium for the at least one metabolite comprises controlling temperature, dissolved oxygen level, pH, or a combination thereof.

15. The method of any of the foregoing claims, wherein the cells are CHO cells, HEK 293 cells, HT-1080 cells, engineered T cells, or engineered natural killer cells.

16. The method of any of the foregoing claims, wherein the cell culture is a batch culture, a fed batch culture, or a perfusion culture.

17. The method of any of the foregoing claims, wherein the cells express a recombinant protein, a gene product, or a cell product.

18. The method of claim 17, further comprising obtaining and purifying the recombinant protein, gene product, or cell product.

19. The method of claim 17, wherein the recombinant protein is a monoclonal antibody.

20. The method of any of the foregoing claims, wherein cell growth and/or productivity are increased as compared to a control culture, wherein the control culture is identical to the culture of claim 1 except it is not cultured using step (ii).

21. The method of claim 20, wherein the cell growth is determined by maximum viable cell density and is increased by at least 5% as compared to the control culture.

22. The method of any of the foregoing claims, wherein the maximum viable cell density of the cell culture is greater than 1×10^6 cells/mL, 5×10^6 cells/mL, 1×10^7 cells/mL, 5×10^7 cells/mL, 1×10^8 cells/mL or 5×10^8 cells/mL.

23. The method of any of the foregoing claims, wherein the cell culture method comprises a growth phase and a production phase and step (ii) is applied during the growth phase.