

Ammonia is a by-product of amino acid metabolism and has a negative impact on cell growth. Other state of the art documents like EP 2135946 A1 and Kishishita et al., J. Biosci Bioeng. (2015), 120 (1), 78-84, disclose cell culture processes with culture media comprising i.a. lactate but explicitly teach that this deems to be an unwanted waste product that should be avoided or kept at low concentrations (see EP 2135946 A1 paragraph [0046] and Kishishita et al., p. 81, left hand column, 2nd paragraph, lines 1-3).

[0011] Ritacco F.V. et al, Biotechnol. Prog., 2018, 34(6): 1407-1426 reviews several approaches of CHO cell culture media development. It discloses in the paragraph bridging pages 1408 and 1410 that lactate as a product of glucose consumption can be inhibitory to cell growth in mammalian cell culture. Respective analyses have shown that in exponential phase, CHO cells largely generate energy via aerobic glycolysis and produce lactate regardless of the concentrations of oxygen, while cells in stationary phase mostly perform oxidative phosphorylation and consume lactate. Yet, it can be derived from this disclosure in general that lactate should be avoided. Further it is said that increased asparagine concentrations could be useful for reducing lactate and ammonium (p. 1412, left hand column, 4th paragraph, lines 18 – 20), but a role for cysteine in this context is not discussed.

[0012] In view of the increasing demand in further improved methods for culturing mammalian cells in fed-batch culture to produce high yields of biopharmaceuticals, including heterologous proteins and recombinant virus, with high product quality there is still a need for improved cell culture media and methods using said cell culture media. The aim of the present invention is therefore to provide an improved fed-batch method for the production of a product of interest in mammalian cells.

SUMMARY

[0013] The present disclosure relates to the surprising combinational effect of lactate and cysteine on cell culture performance and/or product quality.

[0014] In one aspect, a method of producing a product of interest in a fed-batch process is provided 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. Preferably the feed medium is added daily, more preferably continuously. The product of interest is preferably a heterologous protein or a recombinant virus and/or the basal medium and the feed medium is preferably serum-free and chemically defined. In certain preferred example, the molar ratio of lactate/cysteine is about 10:1 to 50:1, preferably about 10:1 to about 30:1.

[0015] The lactate may be 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. In certain examples, 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.

[0016] The cysteine may be provided as cysteine or a salt and/or hydrate thereof, as cystine or a salt thereof or a dipeptide or tripeptide comprising cysteine. Irrespective of the form provided, the cysteine may be added at 0.25 mM/day or higher, at 0.3 mM/day or higher, or at 0.4 mM/day or higher.

[0017] In certain examples the nucleic acid encodes a heterologous protein and 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. Alternatively or in addition the nucleic acid encodes a heterologous protein and 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 the high mannose structures are mannose 5 structures. Alternatively or in addition the nucleic acid encodes a heterologous protein and 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.

[0018] The heterologous protein is preferably an antibody or an antigen-binding fragment thereof, a bispecific antibody, a trispecific antibody or a fusion protein. In one

example, the antibody, the bispecific antibody or the trispecific antibody is an IgG1, IgG2a, IgG2b, IgG3 or IgG4 antibody.

[0019] In another aspect, also provided is 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.

[0020] In another aspect a method of reducing acidic species in a heterologous protein produced in a fed-batch process is provided 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.

[0021] In yet another aspect a method of reducing high mannose structures in a heterologous protein produced in a fed-batch process is provided 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.

[0022] In yet another aspect a method of preventing negative effects of cysteine on product quality characteristics when producing a heterologous protein in a fed-batch process is provided 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.

[0023] The mammalian cell used in the methods according to the disclosure may be any mammalian cell or cell line, preferably the mammalian cell is a HEK293 cell or a CHO cell or a HEK293 cell or a CHO cell derived cell, preferably the mammalian cell is a CHO cell or a CHO derived cell.

[0024] Also provided is a heterologous protein produced by any of the methods according to the disclosure, preferably by the method of reducing acidic species in a heterologous protein or of reducing high mannose structures in a heterologous protein

produced in a fed-batch process as described herein. The heterologous protein may also be produced by the method of preventing negative effects of cysteine on product quality characteristics when producing the heterologous protein as described herein. In another aspect, the present disclosure relates to a heterologous protein produced by the method as described herein.

[0025] The disclosure relates to a use of lactate in a feed medium for reducing acidic species in a heterologous protein produced in a fed-batch process, wherein the feed medium adds cysteine at 0.225 mM/day or higher.

[0026] Also provided is a use of lactate in a feed medium for reducing high mannose structures in a heterologous protein produced in a fed-batch process, wherein the feed medium comprises cysteine at 0.225 mM/day or higher. Preferably the high mannose structures are mannose 5 structures. In another aspect, the present disclosure relates to 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.

[0027] In another aspect, also provided is a 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.

[0028] In another aspect, also provided is a use of lactate and cysteine in a feed medium for increasing heterologous protein titer and/or cell-specific productivity in a fed-batch process. Preferably the fed-batch process comprises culturing a mammalian cell, wherein the mammalian cell is preferably 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.

[0029] A feed medium for mammalian cell fed-batch culture is provided comprising lactate and cysteine at a molar ratio (mM/mM) of lactate/cysteine

of about 8:1 to about 50:1. Preferably the feed medium comprises one or more feed supplements for separate addition. In another aspect a feed medium for mammalian cell fed-batch culture is provided 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.

[0030] In yet another aspect a kit is provided 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.