forms. For example, iron, while an essential cofactor for many cellular functions, can be toxic to cells. In undefined media, biological chelators such as transferrin are relied upon to deliver iron without toxicity (Bjare U. Serum-free cell culture. *Pharmacology & Therapeutics.* 1992;53(3):355-374, which is incorporated by reference herein in its entirety). In CDM, non-protein chelators such as citrate must be studied and implemented to prevent iron toxicity. CDM typically contain a large number of components, which can interact in various manners that not necessarily readily apparent. Successful implementation of CDM requires understanding how the components chemically interact and how to keep them in biologically available forms. Understanding about how the components interact is therefore also important in obtaining optimal media and supplements that comprise undefined media.

[0006] There is accordingly a need for improved methods of preparing cell culture media, feeds and supplements, to reduce potential issues due to interactions between components.

SUMMARY OF THE DISCLOSURE

[0007] We have identified that in cell culture media, feeds, or additives comprising cysteine, cysteine oxidation to cystine readily occurs in the presence of trace metals catalysts, primarily copper and iron. When copper catalyzes cysteine oxidation it is converted to an insoluble copper (I) form. Once there is no cysteine left to be oxidized, the copper usually returns to a soluble form. If, however, the solution goes through sterile filtration while cysteine oxidation is occurring, at least a proportion of the copper will typically be in an insoluble form and will be removed from the media, reducing the level of copper in the medium. This is undesirable, as copper is a trace metal that is required in many cell cultures, so the loss of copper from the solution means that the resulting media will provide less copper to any cultured cells that what was intended. This will typically result in lower productivity. An object of the present disclosure is therefore to minimize and/or avoid loss of copper from solutions (such as cell culture medium, cell culture feed or cell culture additive) during filtration.

[0008] In one aspect, the present disclosure provides a method of preventing loss of copper in a solution, wherein the solution is a cell culture medium, cell culture feed or cell culture additive comprising copper (II) and cysteine, wherein the method comprises having substantially the same amount of soluble copper before and after filtering the solution. In a first ~~aspect~~example the present disclosure provides a method of preventing loss of copper in a solution. The solution is a cell culture medium, cell culture feed or cell culture additive comprising copper (II) and cysteine. The method comprises having substantially the same amount of soluble copper before and after filtering the solution.

[0009] In an ~~embodiment~~example, the filtering is performed with the solution comprising a dissolved oxygen (dO_2) at at least a specified level. Maintaining the level of oxygen at at

least a specified level may rapidly convert copper (I) into a soluble copper (II), preventing the accumulation of appreciable amounts of insoluble copper (I). The specified level may be a level of at least about 6% dO_2. The specified level may be a level of at least about 8% dO_2, e.g. at a level of at least about 10% dO_2 or at a level of at least about 12% dO_2. The filtering may be performed with the solution comprising a dissolved oxygen (dO_2) level of at least about 6% dO_2. The method may further comprise maintaining the dO_2 level of the solution at at least about 8% dO_2 (e.g. at a level of at least about 10% dO_2 or at a level of at least about 12% dO_2) for at least about 10 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 6% dO_2 for at least about 10 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 8% dO_2 (e.g. at a level of at least about 10% dO_2 or at a level of at least about 12% dO_2) for at least about 15 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 6% dO_2 for at least about 15 minutes prior to commencing the filtering.

[0010] The specified level may be a level of at least about 0.4 mg/L dO_2, e.g. at a level of at least about 0.6 mg/L dO_2 or at a level of at least about 0.8 mg/L dO_2. The filtering may be performed with the solution comprising a dissolved oxygen (dO_2) level of at least about 0.3 mg/L dO_2. The method may further comprise maintaining the dO_2 level of the solution at at least about 0.4 mg/L dO_2 (e.g. at a level of at least about 0.6 mg/L dO_2 or at a level of at least about 0.8 mg/L dO_2) for at least about 10 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 0.3 mg/L dO_2 for at least about 10 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 0.4 mg/L dO_2 (e.g. at a level of at least about 0.6 mg/L dO_2 or at a level of at least about 0.8 mg/L dO_2) for at least about 15 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 0.3 mg/L dO_2 for at least about 15 minutes prior to commencing the filtering.

[0011] In an ~~embodiment~~example, the dO_2 levels are maintained at at least the required level by oxygenating the solution and / or optimizing the filtering. The required level may be about 6% dO_2, about 8% dO_2, about 10% dO_2 or about 12% dO_2. The required level may be about 8% dO_2. The required level may be about 0.3 mg/L dO_2, about 0.4 mg/L dO_2, about 0.6 mg/L dO_2, or about 0.8 mg/L dO_2. The required level may be about 0.4 mg/L dO_2.

[0012] The oxygenating may comprise sparging the solution with a gas comprising oxygen. The oxygenating may comprise agitating and/or mixing the solution to increase

level of dO_2 may be monitored with an electrochemical oxygen sensor or an optical oxygen sensor. The level of dO_2 may be monitored with an electrochemical oxygen sensor. The level of dO_2 may be monitored with an optical oxygen sensor.

[0024] In ~~embodiment~~examples, the method comprises providing a cysteine solution and a copper (II) solution; and the filtering comprises separately filtering the cysteine solution and the copper (II) solution, and then combining the filtered cysteine solution and the filtered copper (II) solution to form the cell culture medium, cell culture feed or cell culture additive. By keeping the copper (II) solution and cysteine solution separate until after filtering, the copper is retained in soluble copper (II) before and during filtering.

[0025] The cysteine solution may comprise at least the majority of the components of the cell culture medium, cell culture feed or cell culture additive, other than copper. The cysteine solution may comprise substantially all of the components of the cell culture medium, cell culture feed or cell culture additive other than copper and other trace elements such as iron. The cysteine solution may comprise substantially all of the components of the cell culture medium, cell culture feed or cell culture additive other than copper and iron. The cysteine solution may comprise substantially all of the components of the cell culture medium, cell culture feed or cell culture additive other than copper.

[0026] The copper solution may comprise at least the majority of the components of the cell culture medium, cell culture feed or cell culture additive, other than cysteine.

[0027] The concentration of the copper in the copper (II) solution may be in the range of about of about 0.005 µM to about 1.5 M, for example 0.005 µM to about 1 M. The upper limit of this range may be temperature dependent. Thus solutions that are intended for use at a temperature of at least about 30 °C may have a concentration of up to about 1.5 M, while compounds intended for use or storage at less than room temperature (such as a temperature of less than about 20 °C, e.g. at a temperature of about 10 °C) may have a concentration of up to about 1 M. Where the final solution is a cell culture medium, the concentration of copper in the copper (II) solution may be in the range of about 0.005 µM to about 5 µM. Where the final solution is a cell culture feed, the concentration of copper in the copper (II) solution may be in the range of about 0.1 mM to about 150 mM. Where the final solution is a cell culture additive, the concentration of copper in the copper (II) solution may be in the range of about 1 mM to about 1.5 M, e.g. about 1 mM to about 1 M.

[0028] In ~~embodiment~~examples, the filtering comprises sterile filtering. The sterile filtering may comprise use of a filtration medium having a pore size of not more than about 0.5 µm. The sterile filtering may comprise use of a filtration medium having a pore size of not more than

about 0.2 µm. The sterile filtering may comprise use of a filtration medium having a pore size of not more than about 0.1 µm. The sterile filtering may comprise use of a filtration medium having a pore size of not more than about 0.1 µm. In ~~embodiment~~example~~s~~, the filtration medium comprises one or more membranes, hollow fibers, columns, spiral membranes, tubes, capillaries, capsules, cassettes, discs, frits, or plugs.

[0029] In ~~embodiment~~example~~s~~, the cysteine is provided substantially as a cysteine derivative that does not comprise a sulfhydryl group. For example, the cysteine may comprise less than about 1 mM free cysteine, e.g. the cysteine may comprise less than 0.5 mM free cysteine (or less than 0.1 mM free cysteine). Cysteine derivatives that do not comprise sulfhydryl groups do not react with copper (II) to form insoluble copper (I).

[0030] The cysteine may be converted substantially into a cysteine derivative that does not comprise a sulfhydryl group prior to adding the copper (II) to the solution. The cysteine derivative may comprise a disulfide and/or a thiazolidine moiety.

[0031] The cysteine derivative may comprises a disulfide. For example, the cysteine derivative may comprise cystine, S-sulfocysteine, S-sulfocysteinylglycine, L-cysteine mixed disulfides, and/or S-sulfoglutathione. The cysteine derivative may comprise cystine.

[0032] The cysteine derivative may comprise a thiazolidine moiety. For example, the cysteine derivative may comprise 4-carboxy-2-methylthiazolidine-2-carboxylate, 2-methyl-1,3-thiazolidine-2,4-dicarboxylic acid, 2-(2-carboxyethyl)21,3-thiazolidine-2,4-dicarboxylic acid, L-2-oxothiazolidine-4-carboxylic acid, 2-alkyl-thiazolidine-4(R)-carboxylic acid, 2-aryl-thiazolidine-4(R)-carboxylic acid and carbohydrate based thiazolidines (e.g. D-ribose-L-cysteine). The cysteine derivative may comprise 4-carboxy-2-methylthiazolidine-2-carboxylate.

[0033] The cysteine derivative may comprise at least one of cystine, 4-carboxy-2-methylthiazolidine-2-carboxylate, S-sulfocysteine, S-sulfocysteinylglycine, cysteine S-linked N-acetyl glucosamine (GlcNAC-cys), homocystine, L-cysteine mixed disulfides, L-cysteine mixed peptides, S-alkylated cysteine, cysteine with a thiol-protecting group (e.g. FMOC-protected cysteine), 2-methyl-1,3-thiazolidine-2, 4-dicarboxylic acid, 2-(2-carboxyethyl)21,3-thiazolidine-2, 4-dicarboxylic acid, reduced and oxidized glutathione (GSH), S-sulfoglutathione, S-acyl-GSH, S-carboxy-L-cysteine, L-2-oxothiazolidine-4-carboxylic acid, 2-alkyl-thiazolidine-4(R)-carboxylic acid, 2-aryl-thiazolidine-4(R)-carboxylic acid, or carbohydrate based thiazolidines (e.g. D-ribose-L-cysteine).

[0034] In ~~embodiment~~example~~s~~, the copper may be in the form of a copper (II) chelate. The chelate may comprise at least one chelator selected from ethylenediaminetetraacetic acid

(EDTA), diethylenetriamine pentaacetic acid (DTPA), neocuprione, bathocuprione, D-penicillamine, triethylenetetramine (TETA), dimercaprol (BAL), 8-hydroxyquinoline, clioquinol, and 5,7-Dichloro-2-[(dimethylamino)methyl]-8-quinolinol (PBT2).

[0035] In one aspect, the present disclosure provides a method of preparing a cell culture medium, cell culture feed or cell culture additive comprising copper (II), cysteine, and other components, without loss of soluble copper, comprising: providing an aqueous solution comprising cysteine and other components; providing an aqueous solution comprising copper (II); separately filtering the aqueous solution comprising cysteine and other components, and the aqueous solution comprising copper (II); and combining the filtered solutions to provide the cell culture medium, cell culture feed or cell culture additive. In a second ~~aspect~~example, the disclosure provides a method of preparing a cell culture medium, cell culture feed or cell culture additive comprising copper (II), cysteine, and other components, without loss of soluble copper. The method comprises providing an aqueous solution comprising cysteine and other components; providing an aqueous solution comprising copper (II); separately filtering the aqueous solution comprising cysteine and other components, and the aqueous solution comprising copper (II); and combining the filtered solutions to provide the cell culture medium, cell culture feed or cell culture additive.

[0036] Preparation of the aqueous solution comprising cysteine and other components may comprise dissolving and/or solubilizing cysteine and other components in an aqueous solvent system (e.g. water). Preparation of the solution comprising cysteine and other components may comprise dissolving and/or solubilizing cysteine and other components in an initial solvent system comprising acid, base, or water miscible organic solvent, followed by dilution of the initial solvent system with water and optionally other components to form the aqueous solution.

[0037] Preparation of the aqueous solution comprising copper (II) may comprise dissolving a copper (II) salt in an aqueous solvent system (e.g. water). Preparation of the solution comprising copper (II) may comprise dissolving and/or solubilizing a copper (II) salt in an initial solvent system comprising acid, base, or water miscible organic solvent, followed by dilution of the initial solvent system with water and optionally other components to form the aqueous solution.

[0038] The acid may be an inorganic or organic acid. Exemplary inorganic acids include hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like. Exemplary organic acids include relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Exemplary bases include ammonia; hydroxides of sodium, potassium, calcium, ammonium, organic amino, or magnesium and the like; carbonates or bicarbonates of sodium, potassium, or ammonium. Exemplary water miscible organic solvents include ethanol, methanol, acetone, dimethylsulfoxide (DMSO) and dimethylformadine.

[0039] The concentration of the copper in the copper (II) solution may be in the range of about of about 0.005 µM to about 1 M. Where the final solution is a cell culture medium, the concentration of copper in the copper (II) solution may be in the range of about 0.005 µM to about 5 µM. Where the final solution is a cell culture feed, the concentration of copper in the copper (II) solution may be in the range of about 0.1 mM to about 150 mM. Where the final solution is a cell culture additive, the concentration of copper in the copper (II) solution may be in the range of about 1 mM to about 1 M.

[0040] In an ~~embodiment~~example, the filtering is performed with the solution comprising a dissolved oxygen (dO_2) at at least a specified level. Maintaining the level of oxygen at at least a specified level may rapidly convert copper (I) into a soluble copper (II), preventing the accumulation of appreciable amounts of insoluble copper (I). The specified level may be a level of at least about 6% dO_2. The specified level may be a level of at least about 8% dO_2, e.g. at a level of at least about 10% dO_2 or at a level of at least about 12% dO_2. The filtering may be performed with the solution comprising a dissolved oxygen (dO_2) level of at least about 6% dO_2. The method may further comprise maintaining the dO_2 level of the solution at at least about 8% dO_2 (e.g. at a level of at least about 10% dO_2 or at a level of at least about 12% dO_2) for at least about 10 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 6% dO_2 for at least about 10 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 8% dO_2 (e.g. at a level of at least about 10% dO_2 or at a level of at least about 12% dO_2) for at least about 15 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 6% dO_2 for at least about 15 minutes prior to commencing the filtering.

[0041] The specified level may be a level of at least about 0.4 mg/L dO_2, e.g. at a level of at least about 0.6 mg/L dO_2 or at a level of at least about 0.8 mg/L dO_2. The filtering may be performed with the solution comprising a dissolved oxygen (dO_2) level of at least about 0.3 mg/L dO_2. The method may further comprise maintaining the dO_2 level of the solution at at least about 0.4 mg/L dO_2 (e.g. at a level of at least about 0.6 mg/L dO_2 or at a level of at least about 0.8 mg/L dO_2) for at least about 10 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 0.3 mg/L dO_2 for at least about 10 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 0.4 mg/L dO_2 (e.g. at a level of at least about 0.6 mg/L dO_2 or at a level of at least about 0.8 mg/L dO_2) for at least about 15 minutes prior to commencing the filtering. The method

may further comprise maintaining the dO_2 level of the solution at at least about 0.3 mg/L dO_2 for at least about 15 minutes prior to commencing the filtering.

[0042] In an ~~embodiment~~example, the dO_2 levels are maintained at at least the required level by oxygenating the solution and / or optimizing the filtering. The required level may be about 6% dO_2, about 8% dO_2, about 10% dO_2 or about 12% dO_2. The required level may be about 8% dO_2. The required level may be about 0.3 mg/L dO_2, about 0.4 mg/L dO_2, about 0.6 mg/L dO_2, or about 0.8 mg/L dO_2. The required level may be about 0.4 mg/L dO_2.

[0043] The oxygenating may comprise sparging the solution with a gas comprising oxygen. The oxygenating may comprise agitating and/or mixing the solution to increase contact of the solution with gas comprising oxygen. The oxygenating may comprise sparging the solution with a gas comprising oxygen and agitating or mixing the solution to increase contact of the solution with gas comprising oxygen. The gas comprising oxygen may be atmosphere.

[0044] Optimizing the filtering may comprise increasing filtration rate, and/or filter size, and/or filter capacity, such that the dO_2 levels do not fall below a specified level during filtering. The specified level may be about 6% dO_2, about 8% dO_2, about 10% dO_2 or about 12% dO_2. The specified level may be about 8% dO_2. The specified level may be about 0.3 mg/L dO_2, about 0.4 mg/L dO_2, about 0.6 mg/L dO_2, or about 0.8 mg/L dO_2. The specified level may be about 0.4 mg/L dO_2.

[0045] The increasing may comprise increasing the filtration rate (and optionally the filter size, and/or optionally the filter capacity) by at least about 10%. The increasing may comprise increasing the filtration rate (and optionally the filter size, and/or optionally the filter capacity) by at least about 15%. The increasing may comprise increasing the filtration rate (and optionally the filter size, and/or optionally the filter capacity) by at least about 20%. The increasing may comprise increasing the filtration rate (and optionally the filter size, and/or optionally the filter capacity) by at least about 25%. The increasing may comprise increasing the filtration rate (and optionally the filter size, and/or optionally the filter capacity) by at least about 30%. The increasing may comprise increasing the filtration rate (and optionally the filter size, and/or optionally the filter capacity) by at least about 50%. The increasing may comprise increasing the filtration rate (and optionally the filter size, and/or optionally the filter capacity) by at least about 75%. The increasing may comprise increasing the filtration rate (and optionally the filter size, and/or optionally the filter capacity) by at least about 100%. The increasing may comprise increasing the filter size (and optionally the filter capacity) by at least about 10%. The increasing may comprise increasing the filter size (and optionally the filter capacity) by at least about 15%.

The increasing may comprise increasing the filter size (and optionally the filter capacity) by at least about 20%. The increasing may comprise increasing the filter size (and optionally the filter capacity) by at least about 25%. The increasing may comprise increasing the filter size (and optionally the filter capacity) by at least about 30%. The increasing may comprise increasing the filter size (and optionally the filter capacity) by at least about 50%. The increasing may comprise increasing the filter size (and optionally the filter capacity) by at least about 75%. The increasing may comprise increasing the filter size (and optionally the filter capacity) by at least about 100%. The increasing may comprise increasing the filter capacity by at least about 10%. The increasing may comprise increasing the filter capacity by at least about 15%. The increasing may comprise increasing the filter capacity by at least about 20%. The increasing may comprise increasing the filter capacity by at least about 25%. The increasing may comprise increasing the filter capacity by at least about 30%. The increasing may comprise increasing the filter capacity by at least about 50%. The increasing may comprise increasing the filter capacity by at least about 75%. The increasing may comprise increasing the filter capacity by at least about 100%.

[0046] The method may comprise monitoring the level of oxygen prior to commencing the filtering, for example monitoring the level of oxygen for at least about 10 minutes (e.g. for at least about 15 minutes) prior to commencing the filtering. The method may comprise monitoring the level of dO_2 during filtering. The method may comprise monitoring the level of oxygen prior to commencing the filtering and monitoring the level of dO_2 during filtering. When the method comprises monitoring the level of oxygen, maintaining the level of oxygen at at least the specified level may comprise adding oxygen to the solution in response to the monitored level of oxygen approaching the specified level. When the method comprises monitoring the level of oxygen, maintaining the level of oxygen at at least the specified level may comprise increasing oxygenating the solution and/or optimizing the filtering in response to the monitored level of oxygen approaching the specified level. The level of dO_2 may be monitored using any suitable technique. The level of dO_2 may be monitored with an electrochemical oxygen sensor or an optical oxygen sensor. The level of dO_2 may be monitored with an electrochemical oxygen sensor. The level of dO_2 may be monitored with an optical oxygen sensor.

[0047] In ~~embodiment~~example**s**, the cysteine is provided substantially as a cysteine derivative that does not comprise a sulfhydryl group. For example, the cysteine may comprise less than about 1 mM free cysteine, e.g. the cysteine may comprise less than 0.5 mM free cysteine (or less than 0.1 mM free cysteine). Cysteine derivatives that do not comprise sulfhydryl groups do not react with copper (II) to form insoluble copper (I).

[0048] The cysteine may be converted substantially into a cysteine derivative that does not comprise a sulfhydryl group prior to adding the copper (II) to the solution. The cysteine derivative may comprise a disulfide and/or a thiazolidine moiety.

[0049] The cysteine derivative may comprises a disulfide. For example, the cysteine derivative may comprise cystine, S-sulfocysteine, S-sulfocysteinylglycine, L-cysteine mixed disulfides, and/or S-sulfoglutathione. The cysteine derivative may comprise cystine.

[0050] The cysteine derivative may comprise a thiazolidine moiety. For example, the cysteine derivative may comprise 4-carboxy-2-methylthiazolidine-2-carboxylate, 2-methyl-1,3-thiazolidine-2,4-dicarboxylic acid, 2-(2-carboxyethyl)21,3-thiazolidine-2,4-dicarboxylic acid, L-2-oxothiazolidine-4-carboxylic acid, 2-alkyl-thiazolidine-4(R)-carboxylic acid, 2-aryl-thiazolidine-4(R)-carboxylic acid and carbohydrate based thiazolidines (e.g. D-ribose-L-cysteine). The cysteine derivative may comprise 4-carboxy-2-methylthiazolidine-2-carboxylate.

[0051] The cysteine derivative may comprise at least one of cystine, 4-carboxy-2-methylthiazolidine-2-carboxylate, S-sulfocysteine, S-sulfocysteinylglycine, cysteine S-linked N-acetyl glucosamine (GlcNAC-cys), homocystine, L-cysteine mixed disulfides, L-cysteine mixed peptides, S-alkylated cysteine, cysteine with a thiol-protecting group (e.g. FMOC-protected cysteine), 2-methyl-1,3-thiazolidine-2, 4-dicarboxylic acid, 2-(2-carboxyethyl)21,3-thiazolidine-2, 4-dicarboxylic acid, reduced and oxidized glutathione (GSH), S-sulfoglutathione, S-acyl-GSH, S-carboxy-L-cysteine, L-2-oxothiazolidine-4-carboxylic acid, 2-alkyl-thiazolidine-4(R)-carboxylic acid, 2-aryl-thiazolidine-4(R)-carboxylic acid, or carbohydrate based thiazolidines (e.g. D-ribose-L-cysteine).

[0052] In ~~embodiment~~example**s**, the copper may be in the form of a copper (II) chelate. The chelate may comprise at least one chelator selected from ethylenediaminetetraacetic acid (EDTA), diethylenetriamine pentaacetic acid (DTPA), neocuprione, bathocuprione, D-penicillamine, triethylenetetramine (TETA), dimercaprol (BAL), 8-hydroxyquinoline, clioquinol, and 5,7-Dichloro-2-[(dimethylamino)methyl]-8-quinolinol (PBT2).

[0053] In one aspect, the present disclosure provides a method of preparing a cell culture medium, cell culture feed or cell culture additive comprising copper (II), cysteine, and other components, without loss of soluble copper, comprising: filtering a solution comprising the copper (II), cysteine, and other components to provide the cell culture medium, cell culture feed or cell culture additive. In third ~~aspect~~example, the disclosure provides a method of preparing a cell culture medium, cell culture feed or cell culture additive comprising copper (II), cysteine, and other components, without loss of soluble copper. The method comprises filtering a solution comprising the copper (II), cysteine, and other components to provide the cell culture medium, cell culture feed or cell culture additive.

[0054] The filtering may be completed within about 24 hours of adding copper (II) to the solution. The filtering may be completed within about 18 hours of adding copper (II) to the

filtering may be performed at a rate of from about 40 L/min to about 130 L/min. The filtering may be performed at a rate of from about 50 L/min to about 130 L/min. The filtering may be performed at a rate of from about 10 L/min to about 100 L/min. The filtering may be performed at a rate of from about 20 L/min to about 100 L/min. The filtering may be performed at a rate of from about 30 L/min to about 100 L/min. The filtering may be performed at a rate of from about 40 L/min to about 100 L/min. The filtering may be performed at a rate of from about 50 L/min to about 100 L/min.

[0061] The filtering may be performed at a rate of at least 0.1% of the total volume per minute. The filtering may be performed at a rate of at least 0.25% of the total volume per minute. The filtering may be performed at a rate of at least 0.5% of the total volume per minute. The filtering may be performed at a rate of at least 1% of the total volume per minute. The filtering may be performed at a rate of at least 2.5% of the total volume per minute. The filtering may be performed at a rate of at least 5% of the total volume per minute.

[0062] In ~~embodiment~~example**s**, the filtering comprises sterile filtering. The sterile filtering may comprise use of a filtration medium having a pore size of not more than about 0.5 µm. The sterile filtering may comprise use of a filtration medium having a pore size of not more than about 0.2 µm. The sterile filtering may comprise use of a filtration medium having a pore size of not more than about 0.1 µm. The sterile filtering may comprise use of a filtration medium having a pore size of not more than about 0.1 µm.

[0063] In an ~~embodiment~~example, the filtering is performed with the solution comprising a dissolved oxygen (dO_2) at at least a specified level. Maintaining the level of oxygen at at least a specified level may rapidly convert copper (I) into a soluble copper (II), preventing the accumulation of appreciable amounts of insoluble copper (I). The specified level may be a level of at least about 6% dO_2. The specified level may be a level of at least about 8% dO_2, e.g. at a level of at least about 10% dO_2 or at a level of at least about 12% dO_2. The filtering may be performed with the solution comprising a dissolved oxygen (dO_2) level of at least about 6% dO_2. The method may further comprise maintaining the dO_2 level of the solution at at least about 8% dO_2 (e.g. at a level of at least about 10% dO_2 or at a level of at least about 12% dO_2) for at least about 10 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 6% dO_2 for at least about 10 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 8% dO_2 (e.g. at a level of at least about 10% dO_2 or at a level of at least about 12% dO_2) for at least about 15 minutes prior to commencing the filtering. The method may further

comprise maintaining the dO_2 level of the solution at at least about 6% dO_2 for at least about 15 minutes prior to commencing the filtering.

[0064] The specified level may be a level of at least about 0.4 mg/L dO_2, e.g. at a level of at least about 0.6 mg/L dO_2 or at a level of at least about 0.8 mg/L dO_2. The filtering may be performed with the solution comprising a dissolved oxygen (dO_2) level of at least about 0.3 mg/L dO_2. The method may further comprise maintaining the dO_2 level of the solution at at least about 0.4 mg/L dO_2 (e.g. at a level of at least about 0.6 mg/L dO_2 or at a level of at least about 0.8 mg/L dO_2) for at least about 10 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 0.3 mg/L dO_2 for at least about 10 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 0.4 mg/L dO_2 (e.g. at a level of at least about 0.6 mg/L dO_2 or at a level of at least about 0.8 mg/L dO_2) for at least about 15 minutes prior to commencing the filtering. The method may further comprise maintaining the dO_2 level of the solution at at least about 0.3 mg/L dO_2 for at least about 15 minutes prior to commencing the filtering.

[0065] In an ~~embodiment~~example, the dO_2 levels are maintained at at least the required level by oxygenating the solution and / or Optimizing the filtering. The required level may be about 6% dO_2, about 8% dO_2, about 10% dO_2 or about 12% dO_2. The required level may be about 8% dO_2. The required level may be about 0.3 mg/L dO_2, about 0.4 mg/L dO_2, about 0.6 mg/L dO_2, or about 0.8 mg/L dO_2. The required level may be about 0.4 mg/L dO_2.

[0066] The oxygenating may comprise sparging the solution with a gas comprising oxygen. The oxygenating may comprise agitating and/or mixing the solution to increase contact of the solution with gas comprising oxygen. The oxygenating may comprise sparging the solution with a gas comprising oxygen and agitating or mixing the solution to increase contact of the solution with gas comprising oxygen. The gas comprising oxygen may be atmosphere.

[0067] Optimizing the filtering may comprise increasing filtration rate, and/or filter size, and/or filter capacity, such that the dO_2 levels do not fall below a specified level during filtering. The specified level may be about 6% dO_2, about 8% dO_2, about 10% dO_2 or about 12% dO_2. The specified level may be about 8% dO_2. The specified level may be about 0.3 mg/L dO_2, about 0.4 mg/L dO_2, about 0.6 mg/L dO_2, or about 0.8 mg/L dO_2. The specified level may be about 0.4 mg/L dO_2.

[0068] The increasing may comprise increasing the filtration rate (and optionally the filter size, and/or optionally the filter capacity) by at least about 10%. The increasing may

oxygen at at least the specified level may comprise adding oxygen to the solution in response to the monitored level of oxygen approaching the specified level. When the method comprises monitoring the level of oxygen, maintaining the level of oxygen at at least the specified level may comprise increasing oxygenating the solution and/or optimizing the filtering in response to the monitored level of oxygen approaching the specified level. The level of dO_2 may be monitored using any suitable technique. The level of dO_2 may be monitored with an electrochemical oxygen sensor or an optical oxygen sensor. The level of dO_2 may be monitored with an electrochemical oxygen sensor. The level of dO_2 may be monitored with an optical oxygen sensor.

[0070] In ~~embodiment~~examples, the cysteine is provided substantially as a cysteine derivative that does not comprise a sulfhydryl group. For example, the cysteine may comprise less than about 1 mM free cysteine, e.g. the cysteine may comprise less than 0.5 mM free cysteine (or less than 0.1 mM free cysteine). Cysteine derivatives that do not comprise sulfhydryl groups do not react with copper (II) to form insoluble copper (I).

[0071] The cysteine may be converted substantially into a cysteine derivative that does not comprise a sulfhydryl group prior to adding the copper (II) to the solution. The cysteine derivative may comprise a disulfide and/or a thiazolidine moiety.

[0072] The cysteine derivative may comprises a disulfide. For example, the cysteine derivative may comprise cystine, S-sulfocysteine, S-sulfocysteinylglycine, L-cysteine mixed disulfides, and/or S-sulfoglutathione. The cysteine derivative may comprise cystine.

[0073] The cysteine derivative may comprise a thiazolidine moiety. For example, the cysteine derivative may comprise 4-carboxy-2-methylthiazolidine-2-carboxylate, 2-methyl-1,3-thiazolidine-2,4-dicarboxylic acid, 2-(2-carboxyethyl)21,3-thiazolidine-2,4-dicarboxylic acid, L-2-oxothiazolidine-4-carboxylic acid, 2-alkyl-thiazolidine-4(R)-carboxylic acid, 2-aryl-thiazolidine-4(R)-carboxylic acid and carbohydrate based thiazolidines (e.g. D-ribose-L-cysteine). The cysteine derivative may comprise 4-carboxy-2-methylthiazolidine-2-carboxylate.

[0074] The cysteine derivative may comprise at least one of cystine, 4-carboxy-2-methylthiazolidine-2-carboxylate, S-sulfocysteine, S-sulfocysteinylglycine, cysteine S-linked N-acetyl glucosamine (GlcNAC-cys), homocystine, L-cysteine mixed disulfides, L-cysteine mixed peptides, S-alkylated cysteine, cysteine with a thiol-protecting group (e.g. FMOC-protected cysteine), 2-methyl-1,3-thiazolidine-2, 4-dicarboxylic acid, 2-(2-carboxyethyl)21,3-thiazolidine-2, 4-dicarboxylic acid, reduced and oxidized glutathione (GSH), S-sulfoglutathione, S-acyl-GSH, S-carboxy-L-cysteine, L-2-oxothiazolidine-4-

carboxylic acid, 2-alkyl-thiazolidine-4(R)-carboxylic acid, 2-aryl-thiazolidine-4(R)-carboxylic acid, or carbohydrate based thiazolidines (e.g. D-ribose-L-cysteine).

[0075] In ~~embodiment~~example~~s~~, the copper may be in the form of a copper (II) chelate. The chelate may comprise at least one chelator selected from ethylenediaminetetraacetic acid (EDTA), diethylenetriamine pentaacetic acid (DTPA), neocuprione, bathocuprione, D-penicillamine, triethylenetetramine (TETA), dimercaprol (BAL), 8-hydroxyquinoline, clioquinol, and 5,7-Dichloro-2-[(dimethylamino)methyl]-8-quinolinol (PBT2).

[0076] In ~~embodiments of~~ any of the first, second or third ~~aspect~~example~~s~~, the other components comprise at least one carbon source, and/or at least one nitrogen source, and/or at least one vitamin, and/or at least one buffering agent, and/or at least one inorganic salt, and/or at least one trace metal other than copper, and/or at least one polyamine, and/or at least one (essential) fatty acid, and/or at least one antioxidant, and/or at least one corticosteroid, and/or at least one chelator, and/or at least one lipase inhibitor. The other components may comprise at least one carbon source, and/or at least one nitrogen source, and/or at least one vitamin, and/or at least one buffering agent, and/or at least one inorganic salt, and/or at least one trace metal other than copper, and/or at least one polyamine, and/or at least one (essential) fatty acid. The other components may comprise at least one carbon source. The other components may comprise at least one nitrogen source. The other components may comprise at least one vitamin. The other components may comprise at least one buffering agent. The other components may comprise at least one inorganic salt. The other components may comprise at least one trace metal other than copper. The other components may comprise at least one polyamine. The other components may comprise at least one (essential) fatty acid. The other components may comprise at least one carbon source, at least one nitrogen source, at least one vitamin, at least one buffering agent, at least one inorganic salt, at least one trace metal other than copper, at least one polyamine, and at least one (essential) fatty acid.

[0077] The at least one carbon source may comprise a sugar, optionally a monosaccharide or disaccharide. The sugar may comprise at least one (e.g. two or more of) glucose, galactose, maltose, fructose, ribose and deoxyribose. The sugar may comprise glucose and/or deoxyribose.

[0078] The at least one nitrogen source (e.g. two or more nitrogen sources) may comprise at least one amino acid and/or ammonia/ammonium. The at least one nitrogen source may comprise at least one amino acid. The at least one amino acid may be selected from L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cystine, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-

[0084] The at least one (essential) fatty acid (e.g. at least two fatty acids) may be selected from n-3, n-6, n-9 fatty acids and cholesterol. The at least one (essential) fatty acid (e.g. at least two fatty acids) may be selected from linoleic acid (ALA), linolenic acid (LA), doscosahexenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA). For example, the at least one essential fatty acid (e.g. at least two fatty acids) may be selected from ALA and linolenic acid LA.

[0085] The at least one antioxidant (e.g. two or more antioxidants) may comprise at least one of ascorbic acid, taurine, hypotaurine, a tocopherol, and a tocotrienol.

[0086] The at least one corticosteroid (e.g. two or more corticosteroids) may comprise at least one glucocorticoid (e.g. hydrocortisone) or mineralocorticoid (e.g. aldosterone).

[0087] The at least one metal chelator (e.g. two or more chelators) may comprise at least one of ethylenediaminetetraacetic acid (EDTA), diethylenetriamine pentaacetic acid (DTPA), neocuprione, bathocuprione, D-penicillamine, triethylenetetramine (TETA), dimercaprol (BAL), 8-hydroxyquinoline, clioquinol, and 5,7-Dichloro-2-[(dimethylamino)methyl]-8-quinolinol (PBT2), 1,2- bis(2-Aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), 1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester) (BAPTA-AM), deferoxamine mesylate, .

[0088] In one aspect, the present disclosure provides a method of culturing a cell in a medium, the method comprising: preventing loss of copper in the medium according to a method as disclosed herein, or preparing the medium according to a method as disclosed herein, contacting the cell with the medium, and culturing the cell in the medium. In a fourth ~~aspect~~example, the disclosure provides a method of culturing a cell in a medium. The method comprises preventing loss of copper in the medium according to a method of the first ~~aspect~~example, or preparing the medium according to a method of the second or third ~~aspect~~example; contacting the cell with the medium; and culturing the cell in the medium.

[0089] The cell may be a eukaryotic, a prokaryotic or an archaea cell. The eukaryotic cell may be a mammalian cell. The cell may be selected from hybridoma cells, CHO cells, COS cells, VERO cells, HeLa cells, HEK 293 cells, PER-C6 cells, K562 cells, MOLT-4 cells, Ml cells, NS-1 cells, COS-7 cells, MDBK cells, MDCK cells, MRC-5 cells, WI-38 cells, WEHI cells, SP2/0 cells, BHK cells (including BHK-21 cells) and derivatives thereof.

[0090] The culturing the cell in the medium may comprise multiplying the cell. The method may further comprise isolating and/or purifying the multiplied cell.

[0091] In one aspect, the disclosure provides a method of making a biological product, comprising: preventing loss of copper in the medium according to a method as disclosed herein or preparing a medium according to a method as disclosed herein, contacting a cell configured to express said biological product with the medium, and culturing the cell in the medium under conditions such that the cell expresses the biological product. In a fifth ~~aspect~~example, the disclosure provides a method of making a biological product. The method comprises preventing loss of copper in the medium according to the first ~~aspect~~example or preparing a medium according to the second or third ~~aspect~~example; contacting a cell configured to express said biological product with the medium; and culturing the cell in the medium under conditions such that the cell expresses the biological product.

[0092] The biological product may be a polypeptide, protein, peptide, hormone, virus or virus like particle, nucleic acid or a fragment thereof; optionally an antibody, or a biologically functional fragment of an antibody.

[0093] The cell may be a eukaryotic, a prokaryotic or an archaea cell. The eukaryotic cell may be a mammalian cell. The cell may be selected from hybridoma cells, CHO cells, COS cells, VERO cells, HeLa cells, HEK 293 cells, PER-C6 cells, K562 cells, MOLT-4 cells, Ml cells, NS-1 cells, COS-7 cells, MDBK cells, MDCK cells, MRC-5 cells, WI-38 cells, WEHI cells, SP2/0 cells, BHK cells (including BHK-21 cells) and derivatives thereof.

[0094] The method may further comprise isolating and/or purifying the product.

[0095] In one aspect, the present disclosure provides a cell culture medium, cell culture feed, or cell culture additive obtainable or obtained by the method as disclosed herein. In a sixth ~~aspect~~example, the method provides a cell culture medium, cell culture feed, or cell culture additive obtainable or obtained by a method of the second or third ~~aspect~~example.

[0096] In one aspect, the present disclosure provides use of a cell culture medium, cell culture feed, or cell culture additive as disclosed herein for culturing cells, optionally wherein the use comprises producing a biological product from said cells and / or optionally wherein the use comprises multiplying said cells. In a seventh ~~aspect~~example, the disclosure provides use of a cell culture medium, cell culture feed, or cell culture additive of the sixth ~~aspect~~example for culturing cells. The culturing may comprise producing a biological product from said cells. The culturing may comprise multiplying said cells. The culturing may comprise multiplying said cells and producing a biological product from said cells. The use may further comprise isolating the biological product.

BRIEF DESCRIPTION OF THE DRAWINGS

[0097] Embodiments of the invention are further described hereinafter with reference to the accompanying drawings, in which:

Figure 1 illustrates a time course of copper removal by filtration in Genentech Essential Medium. Aliquots (n=3) were filtered at each time-point and the copper content was compared to unfiltered media.

Figure 2 provides an illustration of how several parameters varied over time in exemplary media after trace metals addition. a) provides the concentration profile for cysteine monomer, b) provides the profile for dissolved oxygen (dO_2) concentration compared to % saturation, c) provides the redox potential, and d) provides an overlay of the results for copper removal, cysteine monomer, and dissolved oxygen, allowing comparison of the relative levels of each of these parameters.

Figure 3 illustrates copper removal extended by a) increasing the starting cysteine concentration in an unmixed system b) increasing the starting cysteine concentration in a mixed system c) sparging with nitrogen after cysteine depletion d) sparging with nitrogen prior to cysteine depletion.