LAYERED CELL CULTURE PARTICLES AND METHODS OF MAKING THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application no. 62/272,828, filed 30 December, 2015. The entire content of the aforementioned application is incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The present invention relates to dry cell culture media, feeds, supplements, or concentrates comprising layered particles useful in culturing cells and other such applications. The invention relates to processes for preparing such layered compositions and methods of making cell culture compositions that are stable, for e.g., thermally, photo-chemically and/or to gamma irradiation. The invention also relates to the use of such layered particle preparations to produce proteins and polypeptides, and to increase cell growth and protein titers thereby.

BRIEF SUMMARY OF THE INVENTION

[0003] In one aspect, the invention is directed in part to methods of making a media or a feed composition for culturing cells, comprising: i) subjecting a dry powder to suspension in a moving column of a gas in a fluid bed apparatus; ii) introducing at least one solvent using a sprayer into the dry powder of step (i) to form a layered base particle; and iii) drying the layered base particle.

[0004] In another aspect, the invention is also directed in part to methods of making a media or a feed composition for culturing cells, comprising: i) subjecting a dry powder to suspension in a moving column of a gas in a fluid bed apparatus; ii) introducing a first solvent using a first sprayer onto the dry powder of step (i) to form a base granule; iii) introducing at least one second solvent using a second sprayer onto the base granule of step (ii) to form a layered base granule; iv) drying the layered base granule.

[0005] Also disclosed is that the dry powder may be a basal media powder, a complete media powder, a cell culture feed, a cell culture supplement, a cell culture media or feed concentrate or an amino acid mixture.

[0006] Also disclosed is that the first sprayer may be selected from the group consisting of: a top sprayer, a bottom sprayer and a tangential sprayer. Alternately, the first or the second sprayer may be selected from

the group consisting of: a top sprayer, a bottom sprayer and a tangential sprayer. In a preferred example, the second sprayer may be a bottom sprayer.

[0007] Also disclosed is that the second sprayer may spray a plurality of solvents on to the base granule to form a multilayered base granule or multilayered base particle respectively. Alternately, the second sprayer may spray the plurality of solvents sequentially, such that there are intermittent drying steps between each spraying step. And alternately, the second sprayer may spray the plurality of solvents sequentially without any intermittent drying steps between each spraying step.

[0008] Also disclosed is that each solvent in the plurality of solvents may have one component; or, the same components; or, each solvent may have different components. For example, each solvent may have a mixture of components. Also disclosed is that the solvent comprises a reactive species. Also disclosed is that the solvent comprises a sensitive species.

[0009] Also disclosed is that the components to be sprayed through the solvent are segregated into reactive species and into sensitive species.

[0010] Also disclosed is that the reactive species and the sensitive species are sprayed separately to give a layered granule. Also disclosed is that the reactive species may be selected from the group consisting of one or more trace elements, one or more reactive amino acids, one or more reactive amino acids, one or more metal salts, one or more acid soluble reactive groups, one or more alcohol soluble reactive groups and one or more pH modifier groups.

[0011] Also disclosed is that the sensitive species may be selected from the group consisting of one or more polyamines, one or more vitamins, one or more growth factor and one or more labile amino acids.

[0012] Also disclosed is that an inlet gas is used in the methods which may be at about temperature 20° C to 30° C. Also disclosed is that he inlet gas used in the method may be at about temperature 25° C.

[0013] Also disclosed is that the vitamins that may be sensitive are selected from the group consisting of: vitamin B12, biotin, choline, folic acid, niacinamide, pyridoxine, riboflavin, thiamine, ascorbic acid and para-aminobenzoic acid (PABA).

[0014] Also disclosed is that the drying step may be at about temperature 50° C to 60° C.

[0015] Also disclosed is that the base particle or the base granule may be dried to a moisture content of about 0.5% to 10%.

[0016] Also disclosed is that the solvent additionally comprises at least one antioxidant, or at least one neutral species, or both.

[0017] Also disclosed is that the layered base particle or the layered base granule is obtained through any of the methods described above. In yet another aspect, the present invention provides the layered base particle or the layered base granule obtained herein.

[0018] Also disclosed is that the methods are directed to producing a nutritive medium layered particle, a nutritive medium supplement layered particle, a nutritive medium subgroup layered particle, the method comprising making a layered media particle according to any of the methods described above and wherein at least one layer on the base particle or base granule comprises a reactive species and/or at least one layer on the base particle or base granule comprises a sensitive species. In yet another aspect, the present invention provides a method of producing a nutritive medium layered particle, a nutritive medium supplement layered particle, a nutritive medium subgroup layered particle, the method comprising making a layered media particle according to any of the preceding claims, wherein at least one layer on the base particle or base granule comprises a reactive species and/or at least one layer on the base particle or base granule comprises a sensitive species. Also disclosed is that the reactive species may be selected from the group consisting of one or more trace elements, one or more reactive amino acids, one or more reactive amino acids, one or more metal salts, one or more acid soluble reactive groups, one or more alcohol soluble reactive groups and one or more pH modifier groups. Alternately, the sensitive species may be selected from the group consisting of one or more polyamines, one or more vitamins, one or more growth factor and one or more labile amino acids. In some examples, the dry powder may be selected from the group consisting of vitamins, bulk inorganic salts and sugars.

[0019] Also disclosed is that the methods are directed to culturing a cell in a liquid reconstituted from the layered media made as described above, the method comprising: i) reconstituting the layered media particle in a suitable liquid or buffer; wherein said layered media particle may be a cell culture medium, feed, supplement or concentrate; and ii) culturing the cell may be the reconstituted liquid under conditions favorable for the growth of the cell. In yet another aspect, the present invention provides a method of culturing a cell in a liquid reconstituted from the layered media particle of any of the preceding claims, comprising: i) reconstituting the layered media particle in a suitable liquid or buffer;

wherein said layered media particle is a cell culture medium, feed, supplement or concentrate; ii) culturing the cell is the reconstituted liquid under conditions favorable for the growth of the cell. Also disclosed is that the culturing of the cell may be used to produce increased amount of a polypeptide. Also disclosed is that the polypeptide may be a recombinant polypeptide. Alternately, the culturing increases product production, increasing cell growth, as compared to a culture with liquid media not made from the layered media particles.

[0020] In yet another aspect, the present invention provides a kit comprising: i) a first container comprising a layered particle or granule according any one of the above claims; wherein said layered particle or granule may be a cell culture medium, feed, supplement or concentrate; and ii) instructions for using the layered particle or granule for cell culture.

[0021] In yet another aspect, the present invention provides a system comprising: a liquid medium reconstituted from a layered media particles according to the methods described above, and a cell. Also disclosed is that the reconstituted liquid medium may be used to cultivate a cell that produces a recombinant polypeptide, a virus, a secreted protein, or to cultivate a cell in suspension or a cell that may be attached.

[0022] Also disclosed are methods of making a media or a feed composition for culturing cells as described above, or, a kit as described above, or, a system as described above, wherein amino acids are used and the amino acids are selected from one or more of the twenty amino acids, their salts or derivatives thereof. Also disclosed is that the amino acids are selected from one or more of glycine, alanine, arginine, aspartic acid, glutamic acid, histidine, isoleucine, methionine, phenylalanine, proline, hydroxyproline, serine, threonine, tryptophan, valine, tyrosine, cysteine and lysine.

[0023] In yet another aspect, the present invention provides use of the layered particles or granules obtained from the methods disclosed herein for culturing a cell. Also disclosed are layered particles or granules obtained through the methods described above used for culturing a cell. Also disclosed is that the cell may be a mammalian cell. Also disclosed is that the cell may be selected from the group consisting of CHO, BHK, HEK, 293, and VERO cells. Also disclosed is that the cell may be a plant cell, an animal cell, a eukaryotic cell, a eukaryotic cell, an algal cell, a fungal cell, and a bacterial cell.

INCORPORATION BY REFERENCE

[0024] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0026] Figure 1: Schematic of Process for Making Culture Media Layered Granule Formulations. Bottom spray coating was performed with a GPCG 1.1 with a 6" Wurster Insert, 2 Liter Granulator/Dryer/Coater. Bottom Spray Layering- Layer composition varies for each granulation.

[0027] Figure 1a: Schematic diagram of exemplary single layer, single ingredient cell culture media layered granules – Layered Granule Prototype A.