PELLETS USED IN CELL CULTURE 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. 62/269,031, filed 17 December, 2015. The entire content of the aforementioned application is incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The present invention relates to pelletized dry cell culture media, feeds, supplements, concentrates or media buffers useful in culturing cells and other such applications. The invention relates to processes for preparing such pelletized compositions and methods to tune the pellet to a desired particle size. The invention also relates to the use of such pellet preparations to produce proteins and polypeptides, and to increase cell growth and protein titers thereby.

SUMMARY

[0003] A first aspect of the invention is directed to a method of making a pelletized cell culture medium, the method comprising: a) subjecting a first dry powder to suspension in an upwardly moving column of a gas in a fluid bed apparatus, and to spinning in a disc rotor; b) introducing a solvent and/or a second dry powder into the fluid bed apparatus at step (a), such that the pellet is formed; and c) drying the pellet, wherein the first dry powder, the second dry powder, or the solvent comprises a binder, an excipient, or both and wherein the first dry powder and/or the second dry powder is selected from the group consisting of a basal media powder, a complete media powder, a feed, a supplement, a media or feed concentrate and an amino acid mixture that can support the cultivation of a cell in culture.

[0003a] A second aspect of the invention is directed to a method of making a pelletized base powder module, the method comprising: a) subjecting a first dry powder to suspension in an upwardly moving column of a gas in a fluid bed apparatus, and to spinning in a disc rotor; b) introducing a solvent and optionally, a second dry powder into the fluid bed apparatus at step (a), such that the pellet of the based powder module is formed; and c) drying the pellet, wherein the first dry powder, the second dry powder, or the solvent comprises a binder, an excipient, or both; optionally wherein the module is selected from the group consisting of one or more water soluble vitamins, one or more acid soluble vitamins, one or more neutral soluble vitamins, one or more acid soluble amino acids, one or more base soluble amino acids, one or more neutral water soluble amino acids, one or more water soluble, inorganic salts, one or more inorganic, acid soluble salts, one or more sugars, one or more acid soluble, trace elements, one or more alcohol soluble polyamines, one or more alcohol soluble lipids, and one or more buffer salts; and or

optionally wherein the water soluble or acid or base or neutral soluble vitamin module, the water soluble or acid or base or neutral soluble amino acid module, or the water soluble or acid or base or neutral soluble salts module comprises at least one water insoluble excipient or water insoluble binder or both.

[0003b] A third aspect of the invention is directed to a method of producing a nutritive medium pellet, a nutritive medium supplement pellet, a nutritive medium subgroup pellet or a buffer pellet, the method comprising: subjecting a dry powder medium, supplement, medium subgroup, or medium buffer with a solvent and/or the powder and forming a pellet using a rotating disk in a fluid bed, wherein the pellet comprises a binder or excipient; optionally wherein the binder or excipient is sprayed in through the solvent, or is blended into the dry powder; and/or optionally wherein said dry powder is selected from the group consisting of one or more water soluble groups, one or more base soluble groups, one or more acid soluble groups, one or more acid soluble reactive groups, one or more alcohol soluble groups, one or more alcohol soluble reactive groups and one or more pH modifier groups; and/or wherein the water soluble group is selected from the group consisting of vitamins, bulk inorganic salts and sugars; and/or wherein the sugar is D-glucose.

[0004] In one example, the first dry powder of step (a) may be pre-wetted, and optionally, no solvent may be introduced in step (b).

[0005] In some examples, the first and second dry powders can be the same, and in other examples, the first and second dry powders can be different.

[0006] In a particular example, the first dry powder may be selected from the group consisting of a basal media powder, a complete media powder, a feed, a supplement, a media or feed concentrate and an amino acid mixture that can support the cultivation of a cell in culture. In another example, the second dry powder may be selected from the group consisting of a basal media powder, a complete media powder, a

40% amino acids, 20-65% binders, 1-5% vitamins, 2-10% salts, 0.01-0.05% trace components. In a particular example, the pellet compositions comprises one or more of 31-32% amino acids, 59-60% binders, 25% vitamins, 6-7% salts, 0.01-0.05% trace components. In a preferred composition the binder is D-glucose.

[0027] In an eleventh example, the disclosure provides methods of producing a nutritive medium pellet, a nutritive medium supplement pellet, a nutritive medium subgroup pellet or a buffer pellet, the method comprising: agglomerating a dry powder medium, supplement, medium subgroup, or medium buffer with a solvent and forming a pellet using a rotating disk in a fluid bed, wherein the pellet comprises a binder or excipient.

[0028] Another example of the disclosure provides methods of producing a nutritive medium pellet, a nutritive medium supplement pellet, a nutritive medium subgroup pellet or a buffer pellet, wherein the binder or excipient may be sprayed in through the solvent, or may be blended into the dry powder before pelletization. In a particular example, the binder may be a sugar, and in a preferred example, the sugar comprises D-glucose. The percent of binder, for e.g. glucose can be varied to achieve differential pellet size, or variable bulk density, or variable angle of repose, or variable stickiness or dryness. For example, in some examples, the amount could be varied from, 0-60%, or 10-16%, or 20-60% , preferably 30-40%, 40-50%, 30-35%, 30-45%, 40-50%, 40-55% and so on, to achieve the desired characteristics.

[0029] In another example, the disclosure may be directed to pellets obtained by, or obtainable by any one of the methods above, in all variations described above.

[0030] In a twelfth example, the disclosure provides methods of making a pellet for culturing cells comprising: preparing a microsuspension of a dry powder medium or module; extruding the microsuspension through an extruding device to form droplets; drying the droplets to pellets. An example may be directed to the pellets obtained thereby.

[0031] A thirteenth example may be directed to use of the pellets obtained by the methods of any of the above claims for culturing a cell. In an example, the cell may be selected from the group consisting of eukaryotic, prokaryotic, animal, plant, fungal, algal, insect, yeast, or wherein the cell may be used for the cultivation of a virus or a viral particle. In a particular example, the cell may be an animal cell. In yet another example, the cell may be a mammalian cell. In a particular example, the mammalian cell may be selected from the group consisting of CHO, BHK, HEK 293, VERO, etc.

dibasic and/or tribasic buffer salts are other examples of pH opposing components. Typically, mono, dibasic and tribasic phosphate salts are used, but other salts may also be used in proper ratios.

[0089] "Microsuspensions": Cell culture media component(s) or the base powder may be dissolved, suspended, colloided or otherwise introduced into a solvent at a desired concentration, prior to use for pelletization (see Example 1).

[0090] Methods of growing cells employing the media supplements described herein such that they can be included in medium at the start of culture, or can be added in a fed-batch or in a continuous manner. The resulting media can be used in various cultivation methods including, but not limited to, batch, fed-batch, chemostat and perfusion, and with various cell culture hardware including, but not limited to, stationary flasks, agitated flasks, spinner flasks, stirred fermentors, airlift fermentors, membrane reactors (including hollow fiber, flat membrane plate, external loop perfusion), reactor s with cells retained on a solid support or immobilized/entrapped as in microporous beads, and any other configuration appropriate for the growth or maintenance of the desired cell line.

[0091] Cell lines that can be grown in reconstituted liquid media from pellets may be eukaryotic, prokaryotic, animal, plant, fungal, algal, insect, or yeast cells or cells used for the cultivation of a virus or a viral particle. Exemplary cells include but are not limited to keratinocytes, endothelial cells, hepatocytes, melanocytes, CHO cells, BHK cells, VERO, HEK 293 cells, PerC6, hybridomas, hematopoetic cells, embryonic cells, neural cells etc.

[0092] Additional components to be added in low amounts to the culture media of the invention may or may not be included as needed, for example, serum or serum fractions, growth factors (*e.g.*, EGF, aFGF, bFGF, KGF, HGF, IGF-1, IGF-2, NGF, insulin, and the like), interleukins, colony-stimulating factors, interferons, attachment factors, extracellular matrix components (*e.g.*, collagens, laminins, proteoglycans, glysoaminoglycans, fibronectin, vitronectin, and the like), lipids (such as phospholipids, cholesterol, bovine cholesterol concentrate, fatty acids, sphingolipids and the like), peptides (for *e.g.*: di, tri, tetra peptides), purified or recombinantly expressed proteins (*e.g.:* recombinant albumin, recombinant insulin), hydrolysates (*e.g.*, plant like rice, yeast, corn, potato), etc.

EXAMPLES

[0093] 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 examples in which the principles of the invention are utilized. The skilled artisan will

CLAIMS

1. A method of making a pelletized cell culture medium, the method comprising:

a) subjecting a first dry powder to suspension in an upwardly moving column of a gas in a fluid bed apparatus, and to spinning in a disc rotor;

b) introducing a solvent and/or a second dry powder into the fluid bed apparatus at step (a), such that the pellet is formed; and

c) drying the pellet,

wherein the first dry powder, the second dry powder, or the solvent comprises a binder, an excipient, or both; and

wherein the first dry powder and/or the second dry powder is selected from the group consisting of a basal media powder, a complete media powder, a feed, a supplement, a media or feed concentrate and an amino acid mixture that can support the cultivation of a cell in culture.

2. The method of making pelletized cell culture medium of claim 1, wherein the first dry powder of step (a) is pre-wetted, and optionally, no solvent is introduced in step (b); and/or wherein the first and second dry powders are the same or wherein the first and second dry powders are different.

3. The method of making pelletized cell culture medium of claim 1 or claim 2, wherein the binder or the excipient is selected from the group consisting of a sugar, a natural substance, a synthetic substance and a semisynthetic substance;

optionally wherein the natural substance is a microcrystalline cellulose; or

optionally wherein the sugar is selected from the group consisting of glucose, sucrose, trehalose, a monosaccharide, a disaccharide and an oligosaccharide; further optionally wherein the % composition of glucose in the pellet is from 0.1 to 100%.

4. The method of making the pelletized cell culture medium of any preceding claims, wherein said pellet size is 0.05mm to 7 mm; optionally wherein said pellet size is 0.05 mm to 0.5mm, or 0.05 mm to 1mm, or 0.05 mm to 2mm, or 0.05 mm to 3mm, or 0.05 mm to 4mm, or 0.05 mm to 5mm, or 0.05 mm to 6mm, or 0.05 mm to 0.1mm, or 0.05 mm to 0.2mm, or 0.05 mm to 0.3mm, or 0.05 mm to 0.4mm, or 0.1mm to 1mm, or 0.1mm to 2mm, or 0.1mm to 3mm, or 0.1mm to 4mm, or 0.1mm to 5mm, or 0.1mm to 6mm, or 0.5mm to 1mm, or 0.5mm to 2mm, or 0.5mm to 3mm, or 0.5mm to 4mm, or 0.5mm to 5mm, or 0.5mm to 6mm, or 0.5mm to 7mm, or 1mm to 2mm, or 1 mm to 3mm, or 1 mm to 4mm, or 1 mm to 5mm, or 1 mm to 6mm, or 1 mm to 7mm; and/or

wherein said pellet size is larger than about 0.1 mm, or larger than about 0.2 mm, or larger than about 0.3 mm, or larger than about 0.4mm, or larger than about 0.5 mm, or larger than about 0.6 mm, or larger than about 0.7 mm, or larger than about 0.8 mm, or larger than about 0.9mm, or larger than about 1mm, or larger than about 2mm.

5. The method of making the pelletized cell culture medium of medium of any preceding claim, wherein, the disc rotor spins at variable speeds; and/or

wherein the pellet is dried to a moisture content of 0.5% to 3%.

6. The method of making the pelletized cell culture medium of medium of any of the above claims, wherein the solvent is introduced into step (a) through a tangential spray, a top spray, or a bottom spray.

7. The method of making the pelletized cell culture medium of medium of any of the above claims, wherein the rate of the solvent introduced into step (a) is between 1 gm/min up to 30 gm/ min;

optionally wherein the rate of the solvent introduced into step (a) is about 5 gm/min.

8. The method of making the pelletized cell culture medium of medium of any preceding claims, wherein a temperature of an inlet gas is at 20° C to 30° C, optionally wherein the temperature of the inlet gas is at about 25° C; and/or

wherein the pellet temperature is maintained at 20° C to 30° C; and/or

wherein the temperature for drying the pellet is 50° C to 60° C.

9. A method of making a pelletized base powder module, the method comprising:

a) subjecting a first dry powder to suspension in an upwardly moving column of a gas in a fluid bed apparatus, and to spinning in a disc rotor;

b) introducing a solvent and optionally, a second dry powder into the fluid bed apparatus at step (a), such that the pellet of the based powder module is formed; and

c) drying the pellet,

wherein the first dry powder, the second dry powder, or the solvent comprises a binder, an excipient, or both;

optionally wherein the module is selected from the group consisting of one or more water soluble vitamins, one or more acid soluble vitamins, one or more neutral soluble vitamins, one or more

acid soluble amino acids, one or more base soluble amino acids, one or more neutral water soluble amino acids, one or more water soluble, inorganic salts, one or more inorganic, acid soluble salts, one or more sugars, one or more acid soluble, trace elements, one or more alcohol soluble polyamines, one or more alcohol soluble lipids, and one or more buffer salts; and or

optionally wherein the water soluble or acid or base or neutral soluble vitamin module, the water soluble or acid or base or neutral soluble amino acid module, or the water soluble or acid or base or neutral soluble salts module comprises at least one water insoluble excipient or water insoluble binder or both.

10. A method of producing a nutritive medium pellet, a nutritive medium supplement pellet, a nutritive medium subgroup pellet or a buffer pellet, the method comprising: subjecting a dry powder medium, supplement, medium subgroup, or medium buffer with a solvent and/or the powder and forming a pellet using a rotating disk in a fluid bed, wherein the pellet comprises a binder or excipient;

optionally wherein the binder or excipient is sprayed in through the solvent, or is blended into the dry powder; and/or

optionally wherein said dry powder is selected from the group consisting of one or more water soluble groups, one or more base soluble groups, one or more acid soluble groups, one or more acid soluble reactive groups, one or more alcohol soluble groups, one or more alcohol soluble reactive groups and one or more pH modifier groups; and/or

wherein the water soluble group is selected from the group consisting of vitamins, bulk inorganic salts and sugars; and/or

wherein the sugar is D-glucose.

11. The method of any of claims 1 to 10, further comprising:

i) reconstituting the pellet in a suitable liquid or buffer; wherein said pellet is a cell culture medium, feed, supplement or concentrate; and

ii) culturing a cell in the reconstituted liquid under conditions favorable for the growth of the cell.

12. The method of claim 11, wherein the cell is selected from the group consisting of eukaryotic, prokaryotic, animal, plant, fungal, algal, insect, yeast, or wherein the cell is used for the cultivation of a virus or a viral particle; and/or

wherein the cell culture is used to produce increased amount of a polypeptide, further optionally wherein the polypeptide is a recombinant polypeptide; and/or

the culturing increases product production, increasing cell growth, as compared to a culture with liquid media not made from pellets.

13. The method of any of claims 1 to 10, further comprising assembling a kit comprising:

i) a first container comprising one or more said pellets ; and

ii) instructions for using said pellets.

14. The method of any of claims 1 to 10, further comprising reconstituting the pellet, and providing a liquid medium comprising the reconstituted pellet and a cell.

15. The method of claim 14, wherein the cell is selected from the group consisting of eukaryotic, prokaryotic, animal, plant, fungal, algal, insect, yeast, or wherein the cell is used for the cultivation of a virus or a viral particle; and/or

wherein the reconstituted liquid medium is used to cultivate a cell that produces a recombinant polypeptide, a virus, a secreted protein, or a cell either in suspension or an attached cell.

16. The method of claim 15, wherein the cell is selected from the group consisting of CHO, BHK, HEK 293, and VERO.