transfer to the culture at the membrane-culture contact surface.[10-12]

[0004] While porous and non-porous membranes have been applied in cell culture, only low densities have been achieved (i.e., <20e6 cells/mL) due to limitations in membrane design and challenges associated with membrane operation.[13] For example, in the case of porous membranes, the cell culture can be injured due to the formation of micron-sized bubbles that can emerge if the gas and culture pressures are improperly balanced with one another. Conversely, in the case of non-porous membranes, the mass transfer of gas molecules there through is slow thereby preventing sufficient gas exchange to support high density cultures.

[0005] To overcome the many challenges associated with sparging-based gas exchange processes in high-density cell culture, alternate methods and materials for bubble-free gas exchange are needed.

SUMMARY

[0006] Generally, this disclosure relates to bioreactors, and to bioprocessing methods using bioreactors. The present disclosure relates in part to the surprising finding that by replacing the sparger (e.g., used for oxygenation and/or introduction of gasses into a cell culture) of a high density cell culture bioreactor with one or more hollow-fiber membrane modules, sustained levels of dissolved oxygen (e.g., 60% DO) could be achieved at high cell densities (e.g., at least 120 X 10^6 cells/mL) without severe effects on culture health during operation. As a result, cells used in bioprocessing are maintained in a viable condition throughout the process, thereby increasing the productivity of a bioreactor.

[0007] ~~In one aspect, the~~The disclosure provides a method of culturing cells in a bioreactor comprising providing a mass transfer of a gas to/from the bioreactor without generating bubbles inside the bioreactor. In one aspect, the disclosure provides a method of culturing cells in a bioreactor comprising providing mass transfer of gas to/from the bioreactor without generating bubbles inside the bioreactor, wherein the mass transfer of gas to/form the

bioreactor is provided by gas transfer modules, wherein the bioreactor comprises two or more gas transfer modules, wherein the gas transfer modules comprise non-porous membranes, and wherein the bioreactor comprises a cell density of at least 20 x 10^6 cells/ml. In certain examples, the bioreactor is a perfusion bioreactor. The bioreactor can also be a batch-fed bioreactor.

[0008] In certain examples, the mass transfer of a gas to/from the bioreactor is provided by a gas transfer module. In various examples, the gas transfer module comprises a non-porous membrane, which can be, for example, a polymer, metal, or ceramic. Polymers can include silicone gum homopolymer, polydimethylsiloxane (PDMS), or silicone-

requires no anti-foaming agent during cell culture.

[00016] In other examples, the bioreactor comprises no headspace or substantially no headspace.

[00017] In various examples, the bioreactor comprises two or more gas transfer modules. The two or more gas transfer modules can provide mass transfer of different gases comprising oxygen, carbon dioxide, or nitrogen gas, and even air.

[00018] ~~In another aspect, the~~The disclosure provides a bioreactor system for high-density cell culture comprising a bioreactor vessel for growing cells in cell culture media and a liquid flow path in fluid communication with the bioreactor vessel, wherein the liquid flow path forms a circuit exterior to the bioreactor vessel for translocating cell culture media through a first and a second membrane gas transfer module. In another aspect, the disclosure provides a bioreactor system for high-density cell culture comprising a bioreactor vessel for growing cells in cell culture media and a liquid flow path in fluid communication with the bioreactor vessel, wherein the mass transfer of gas to/from the bioreactor is provided by gas transfer modules, wherein the bioreactor comprises two or more gas transfer modules, wherein the liquid flow path forms a circuit exterior to the bioreactor vessel for translocating cell culture media through a first and a second membrane gas transfer module, wherein the gas transfer modules comprise non-porous membranes, and wherein the bioreactor comprises a cell density of at least 20 x 10^6 cells/ml.

[00019] In various examples, the first membrane gas transfer module is for adding oxygen to the cell culture media.

[00020] In other examples, the second membrane gas transfer module is for stripping carbon dioxide from the cell culture media.

[00021] In still other examples, the first membrane gas transfer module comprises a oxygen flow path and a cell culture medium flow path, wherein the gas flow path and cell culture flow path are separated by a gas-permeable membrane.

[00022] In still other examples, the first membrane gas transfer module comprises an

air/carbon dioxide flow path and a cell culture medium flow path, wherein the air/carbon dioxide flow path and cell culture flow path are separated by a gas-permeable membrane.

[00023] The liquid flow path of the bioreactors described herein forms a circuit further comprising one or more additional elements, which can include a cell harvesting filter, a perfusion pump, a source of oxygen, a source of air/carbon dioxide, an oxygen sensor and/or a pH sensor.

[00024] In various examples of the bioreactors described herein, the cells of the high-density cell culture are at a density of about 20 x 10^6 cells/ml, about 30 x 10^6 cells/ml, about 40 x 10^6 cells/ml, about 50 x 10^6 cells/ml, about 60 x 10^6 cells/ml, about 70 x 10^6 cells/ml, about 80 x 10^6 cells/ml, about 90 x 10^6 cells/ml, about 100 x 10^6 cells/ml, about 110 x 10^6 cells/ml, about 120 x 10^6 cells/ml, about 130 x 10^6 cells/ml, about 140 x 10^6 cells/ml,

CLAIMS

1. A method of culturing cells in a bioreactor comprising providing ~~a~~ mass transfer of ~~a~~ gas to/from the bioreactor without generating bubbles inside the bioreactor,
wherein the mass transfer of gas to/form the bioreactor is provided by gas transfer modules,
wherein the bioreactor comprises two or more gas transfer modules,
wherein the gas transfer modules comprise non-porous membranes, and
wherein the bioreactor comprises a cell density of at least 20 x 10^6 cells/ml.

2. The method of claims 1, wherein the bioreactor is a perfusion bioreactor or a batch-fed bioreactor.

3. The method of claim 1 or 2, ~~wherein the mass transfer of a gas to/from the bioreactor is provided by a gas transfer module, preferably wherein the gas transfer module comprises a non-porous membrane, more preferred~~ wherein the non-porous membranes are ~~is~~ a polymer, metal, or ceramic, most preferred wherein the polymer comprises silicone gum homopolymer, polydimethylsiloxane (PDMS), or silicone-polycarbonate copolymer and/or wherein the non-porous membrane comprises a plurality of hollow fibers.

4. The method of claim 3, wherein the gas transfer module comprises a first flow path through the hollow fibers for passage of one or more gases and a second flow path around the hollow fibers for a flow of cell culture media and/or cells, preferably wherein the gas transfer modules are ~~is~~ located outside of the bioreactor , more preferred wherein the plurality of hollow fibers provide a flow path for culture media and cells to travel through spaces separating the hollow fibers, most preferred wherein the spaces are homogenous or heterogenous.

5. The method of claim 4, wherein the spaces are of sufficient size to allow passage of a cell without causing shear forces on the cell, preferably wherein the spaces comprise a distance of 15 µm to 2000 µm, more preferred wherein the spaces comprise a distance 15-30 µm, 20-40 µm, 30-60 µm, 40-80 µm, 60-120 µm, 80-160 µm, 100-200 µm, 150-

300 µm, 200-400 µm, 200-500 µm, 200-600 µm, 200-700 µm, 200-800 µm, 200-900 µm, 200-1000 µm, or 500-2000 µm, or a combination thereof.

6. The method of claim 4, wherein the flow of cell culture media and/or cells comprises tangential, axial flow or a combination thereof and/or wherein the flow of the cell culture media and/or cells is at a rate that is sufficient to maintain culture homogeneity without causing shear forces on the cells.

7. The method of one or more of claims 1 to ~~7~~6, wherein the gas is air, carbon dioxide, oxygen, and/or nitrogen, or a mixture thereof.

8. The method of one or more of claims 1 to ~~8~~7, wherein the bioreactor comprises a cell density of ~~20 x 10^6 cells/ml,~~ at least 30 x 10^6 cells/ml, 40 x 10^6 cells/ml, 50 x 10^6 cells/ml, 60 x 10^6 cells/ml, 70 x 10^6 cells/ml, 80 x 10^6 cells/ml, 90 x 10^6 cells/ml, 100 x 10^6 cells/ml, 110 x 10^6 cells/ml, 120 x 10^6 cells/ml, 130 x 10^6 cells/ml, 140 x 10^6 cells/ml, 150 x 10^6 cells/ml, 160 x 10^6 cells/ml, 170 x 10^6 cells/ml, or 180 x 10^6 cells/ml.

9. The method of one or more of claims 1 to ~~9~~8, wherein the method avoids production of foam.

10. The method of one or more of claims 1 to ~~10~~9, wherein the bioreactor comprises no headspace or substantially no headspace.

11. The method of one or more of claims 1 to ~~11~~10, wherein the method requires no anti-foaming agent during cell culture.

12. The method of ~~claim 3~~one or more of claims 1 to 11, ~~wherein the bioreactor comprises two or more gas transfer modules, preferably~~ wherein the two or more gas transfer modules provide mass transfer of different gases comprising air, oxygen, carbon dioxide, or nitrogen gas.

13. The method of one or more of claims 1 to 12, wherein the two or more gas transfer

modules allow the addition of O_2 to the cell culture and removal of CO_2 from the cell culture.

~~13.~~14. A bioreactor system for high-density cell culture comprising a bioreactor vessel for growing cells in cell culture media and a liquid flow path in fluid communication with the bioreactor vessel,

wherein the mass transfer of gas to/from the bioreactor is provided by gas transfer modules,

wherein the bioreactor comprises two or more gas transfer modules,

wherein the liquid flow path forms a circuit exterior to the bioreactor vessel for translocating cell culture media through a first and a second membrane gas transfer module,

wherein the gas transfer modules comprise non-porous membranes, and

wherein the bioreactor comprises a cell density of at least 20 x 10^6 cells/ml.

~~14.~~15. The bioreactor system of claim ~~13~~14, wherein the first membrane gas transfer module is for adding oxygen to the cell culture media, preferably wherein the first membrane gas transfer module comprises a oxygen flow path and a cell culture medium flow path, and/wherein the gas flow path and cell culture flow path are separated by a gas-permeable membrane or further comprising an oxygen sensor that regulates the level of oxygen introduced into the cell culture media through the first membrane gas transfer module.

~~15.~~16. The bioreactor system of claim ~~13 or 14~~14 or 15, wherein the second membrane gas transfer module is for stripping carbon dioxide from the cell culture media, preferably wherein the first membrane gas transfer module comprises an air/carbon dioxide flow path and a cell culture medium flow path, and/or wherein the air/carbon dioxide flow path and cell culture flow path are separated by a gas-permeable membrane or further comprising a pH sensor that regulates the level of carbon dioxide introduced into the cell culture media through the second membrane gas transfer module.

~~16.~~17. The bioreactor system of one or more of claims ~~13 to 15~~14 to 16, wherein the liquid flow path that forms a circuit further comprises one or more additional elements, preferably wherein the one or more additional elements is a cell harvesting filter and/or wherein the one or more additional elements is a perfusion pump.

~~17.~~18. The bioreactor system of one or more of claims ~~13 to 16~~14 to 17, further comprising a source of oxygen or further comprising a source of an air/carbon dioxide mixture.

~~18.~~19. The bioreactor system of one or more of claims ~~13 to 17~~14 to 18, wherein the cells of the high-density cell culture are at a density of ~~20 x 10^6 cells/ml,~~ at least 30 x 10^6 cells/ml, 40 x 10^6 cells/ml, 50 x 10^6 cells/ml, 60 x 10^6 cells/ml, 70 x 10^6 cells/ml, 80 x 10^6 cells/ml, 90 x 10^6 cells/ml, 100 x 10^6 cells/ml, 110 x 10^6 cells/ml, 120 x 10^6 cells/ml, 130 x 10^6 cells/ml, 140 x 10^6 cells/ml, 150 x 10^6 cells/ml, 160 x 10^6 cells/ml, 170 x 10^6 cells/ml, or 180 x 10^6 cells/ml.

~~19.~~20. The bioreactor system of one or more of claims ~~13 to 18~~14 to 19, wherein the first and second membrane gas transfer modules add and/or remove gasses to/from the cell culture medium without forming bubbles.

21. The bioreactor system of one or more of claims 14 to 20, wherein the two or more gas transfer modules allow the addition of O_2 to the cell culture and removal of CO_2 from the cell culture.