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] 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 Chinese Hamster Ovary (CHO) 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/from the

bioreactor is provided by two or more gas transfer modules comprising a plurality of hollow fibers, wherein at least one gas transfer module comprises an oxygen flow path through the hollow fibers and a cell culture medium flow path around the hollow fibers separated by a non-porous membrane comprising PDMS, wherein at least one gas transfer module comprises an air/carbon dioxide flow path through the hollow fibers and a cell culture medium flow path around the hollow fibers separated by a non-porous membrane comprising PDMS, wherein the bioreactor is a perfusion bioreactor, and wherein the bioreactor comprises a CHO 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] 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.

[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

percent viability using the membrane and traditional sparging are comparable during the course of culture. FIG. 4C shows that LDH (lactate dehydrogenase activity assay) of the bioreactor operated with membrane gas exchange matches that of the bioreactor operated with traditional sparging. The increase in LDH for the membrane culture after day 13 may be attributed to the increased cell density.

[00036] FIGs. 5A-5C show DO (dissolved oxygen) levels during culture with membrane gas exchange during steady-state and unsteady-state operation, e.g., using the bioreactor system of FIG. 1B, FIG. 1C, or FIG. 1D. FIG. 5A shows that baseline DO profile established during ramp-up to steady-state density. Microsparging started at day 1-2 (*) and stabilized by day 2-3 when a density of 40e6 cells/mL was achieved. FIG. 5B shows that the O_2 line was switched over to the membrane on day 7 (*). Fluctuations were controlled by adjusting the gain and integral (* to **) and a mixture of air (0.25 LPM) and O_2 (0.25 LPM) was sparged through the drilled hole sparger, rather than 0.5 LPM O_2 so that the fluctuations in DO would further stabilize as the membrane provided too much oxygen to the culture (**). FIG. 5C shows that cell bleed was lowered on day 11 and density was increased to 120e6 cells/mL. Fluctuations in the DO may have been caused by wetting of the fibers and the gas outlet vent filter, causing blockage and loss of gas transfer during operation.

[00037] FIGs. 6A-6C show VCD (viable cell density), percent viability, and LDH (lactate dehydrogenase activity assay) of CHO1 cells cultured during ramp-up to high cell densities, e.g., using the bioreactor system of FIG. 1B, FIG. 1C, or FIG. 1D. FIG. 6A shows that densities of ~130e6 cells/mL were reached during ramp-up to high cell density with traditional sparging, while densities of ~100 X 10^6 cells/mL were reached with membrane gas exchange. Shear from the membrane may affect culture health during ramp-up to high densities. FIG. 6B shows that cells had comparable viability for both membrane and sparger aeration during the course of culture (>85%). FIG. 6C shows that LDH was comparable for both membrane gas exchange and traditional sparging. Higher LDH would be expected for the culture with traditional sparging due to higher cell density, indicating that the membrane exchanger may be causing shear and damage to cells during operation.

[00038] FIGs. 7A-7B show DO control during ramp-up to high cell densities using a bioreactor system of FIG. 1B, FIG. 1C, or FIG. 1D. FIG. 7A shows that initially, DO is

controlled with sparging through the drilled hole sparger. As density increases, additional O_2 is sent through the membrane to sustain DO at 60% (*). MFC failure led to wide fluctuations and issues controlling DO during the membrane startup phase. Once control was restored to the MFC, DO fluctuated around setpoint, eventually reaching steady operation day 6-7 at cell densities around 80e6 cells/mL. FIG. 7B shows that at peak densities, DO control through the membrane remained stable, with several fluctuations occurring during temporary MFC failure.

[00039] FIGs. 8A-8C show VCD (viable cell density), percent viability, and LDH (lactate dehydrogenase activity assay) of CHO2 cells cultured in a bioreactor operated with membranes for bubble-free aeration, e.g., using a bioreactor system of FIG. 1B, FIG. 1C, or FIG. 1D. FIG. 8A shows that viable cell densities of 25-30e6 cells/mL were achieved for weeks in culture. Membrane cultures took an additional day to reach steady-state operating density indicating there may be some effect of the membrane on the culture health. FIG. 8B shows that the viability of the membrane cultures was comparable to, but slightly lower than traditional sparging, further indicating effects of membrane on the cells. FIG. 8C shows that the LDH of the membrane culture was higher than the bubble-sparged bioreactor, indicating that cell shear and lysis may occur due to the densely-packed fibers in the current membrane unit design.

[00040] FIGs. 9A-9B shows DO (dissolved oxygen) control with bubble-free gas exchange through silicone-based membranes, e.g., using a bioreactor system of FIG. 1B, FIG. 1C, or FIG. 1D. FIG. 9A shows DO controlled around 60% during culture supporting CHO2 densities of 25-30e6 cells/mL. On day 7, recirculation was increased to support oxygen demand at high cell density (*). On day 9, control was switched to manual for better control over fluctuations in DO (**).FIG. 9B shows daily pO_2 and pCO_2 readings monitored offline during the entire culture period, demonstrating comparable levels of dissolved oxygen in cultures controlled with sparging or membrane gas exchange. Note that the increase in pCO_2 at Day 6 was likely due to 100% pure O_2 flow to both membranes. To address high pCO_2, air was mixed with O_2 through the first sparger and pCO_2 returned to levels comparable to the

CLAIMS

1. A method of culturing Chinese Hamster Ovary (CHO) 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/from the bioreactor is provided by two or more gas transfer modules comprising a plurality of hollow fibers,
wherein at least one gas transfer module comprises an oxygen flow path through the hollow fibers and a cell culture medium flow path around the hollow fibers separated by a non-porous membrane comprising PDMS,
wherein at least one gas transfer module comprises an air/carbon dioxide flow path through the hollow fibers and a cell culture medium flow path around the hollow fibers separated by a non-porous membrane comprising PDMS,
wherein the bioreactor is a perfusion bioreactor, and
wherein the bioreactor comprises a CHO cell density of at least 20 x 10^6 cells/ml.
2. The method of claim 1, wherein the two or more gas transfer modules are located outside of the bioreactor.

3. The method of claim 1 or 2, wherein the plurality of hollow fibers provide a flow path for culture media and cells to travel through spaces separating the hollow fibers.

4. The method of claim 3, wherein the spaces are homogenous or heterogenous.

5. The method of claim 3 or 4, wherein the spaces are of sufficient size to allow passage of a cell without causing shear forces on the cell.

6. The method of any one of claims 3 to 5, wherein the spaces comprise a distance of 15 µm to 2000 µm.

7. The method of claim 6, wherein the spaces comprise a distance of 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.

8. The method of any one of claims 2 to 7, wherein the flow of cell culture media and/or cells comprises tangential, axial flow or a combination thereof.

9. The method of any one of claims 2 to 8, 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.

10. The method of any one of claims 1 to 9, wherein the bioreactor comprises a cell density of about 30 x 10^6 cells/ml.

11. The method of any one of claims 1 to 10, wherein the method avoids production of foam.

12. The method of any one of claims 1 to 11, wherein the bioreactor comprises no headspace or substantially no headspace.

13. The method of any one of claims 1 to 12, wherein the method requires no anti-foaming agent during cell culture.

14. The method of any one of claims 1 to 13, wherein a dissolved oxygen content in the bioreactor is maintained at around 60%.

15. The method of any one of claims 1 to 14, wherein hollow fibers have a wall thickness of 55 μm.