allow the cultivation of mammalian cells under optimal conditions, even if grown in large scale volumes and therefore allow a process performance and product quality independent of the size of the single-use bioreactor.

[0025] It is also an object of the present disclosure to provide single-use bioreactors with proportions that can be scaled from laboratory scale to industry scale bioreactors and vice versa. The design of the single-use bioreactors according to the present disclosure has this flexibility because it maintains geometric similarity.

[0026] In general, the present disclosure is directed to a bioreactor system and method. The present disclosure is also directed to a single use bioreactor that is well suited to incubating cell cultures and thereafter being disposed. The single use bioreactor of the present disclosure can be scaled to any suitable size and is designed to fit in pre-existing stainless steel structures. The bioprocess system and method of the present disclosure contain many unique aspects and features.

[0027] According to the disclosure, a single-use bioreactor is provided. The single-use bioreactor may include a bioprocess container, a shell, at least one agitator, at least one sparger, at least one gas filter inlet port for the sparger(s) and headspace overlay, at least one fill port, at least one harvest port, at least one sample port, and at least one probe.

[0027a] According to one aspect, there is provided a bioreactor comprising:

a bioprocess container made from a liquid impermeable and flexible shape-conforming material, the bioprocess container having a top, a bottom, and at least one side wall therebetween, the bioprocess chamber defining a hollow enclosure for receiving a culture media; and

a mixing device comprising a rotatable shaft coupled to at least one top impeller and at least one bottom impeller that extend into the hollow enclosure of the bioprocess container, the top impeller and the bottom impeller both being made from a polymer material.

[0027b] According to another aspect, there is provided a bioreactor comprising:

a bioprocess container made from a liquid impermeable and flexible shape-conforming material, the bioprocess container having a top, a bottom, and at least one side wall therebetween, the bioprocess chamber defining a hollow enclosure for receiving a culture media;

at least one inlet port for feeding materials into the hollow enclosure of the bioprocess container;

a mixing device comprising a rotatable shaft coupled to at least one agitator, the shaft and agitator extending into the hollow enclosure of the bioprocess container; and

at least one baffle being configured to extend adjacent to the side wall of the bioprocess container in a longitudinal direction, the baffle having a shape that extends radially inward from the side wall an amount sufficient to affect fluid flow in the hollow enclosure during mixing of a culture media by the mixing device.

[0027c] According to another aspect, there is provided a bioreactor system comprising:

a bioprocess container made from a liquid impermeable and flexible shape-conforming material, the bioprocess container having a top, a bottom, and at least one side wall therebetween, the bioprocess chamber defining a hollow enclosure for receiving a culture media;

a plurality of inlet ports for feeding materials into the hollow enclosure of the bioprocess container;

a drain line positioned at the bottom of the bioprocess container for draining fluids from the bioprocess container;

a mixing device comprising a rotatable shaft coupled to at least one agitator, the shaft and agitator extending into the hollow enclosure of the bioprocess container;

at least one sensor in operative association with the bioprocess container for monitoring at least one parameter within the hollow enclosure, the at least one sensor comprising a pH sensor, a dissolved carbon dioxide sensor, a dissolved oxygen sensor, or a load cell; and

a controller in communication with the at least one sensor, the controller being configured to receive information from the at least one sensor and, based on the information, to control a fluid supply for varying a flow rate of a fluid from the fluid supply into the hollow enclosure of the bioprocess container for maintaining the at least one parameter of a culture media contained within the hollow enclosure within preset limits.

[0027d] According to another aspect, there is provided a bioreactor comprising:

a bioprocess container made from a liquid impermeable and flexible shape-conforming material, the bioprocess container having a top, a bottom, and at least one side wall

therebetween, the bioprocess chamber defining a hollow enclosure for receiving a culture media;

a mixing device comprising a rotatable shaft coupled to at least one agitator, the shaft and agitator extending into the hollow enclosure of the bioprocess container; and

at least one feed line that extends into the hollow enclosure for feeding fluids into the bioprocess container, the feed line including a subsurface fluid outlet positioned adjacent the agitator, the fluid outlet being associated with a fluid control device that only permits fluid to flow out of the fluid outlet and prevents fluid flow in an opposite direction.

[0027e] According to another aspect, there is provided a bioreactor comprising:

a bioprocess container made from a liquid impermeable and flexible shape-conforming material, the bioprocess container having a top, a bottom, and at least one side wall therebetween, the bioprocess chamber defining a hollow enclosure for receiving a culture media;

a mixing device comprising a rotatable shaft coupled to at least one agitator, the shaft and agitator extending into the hollow enclosure of the bioprocess container; and

at least one feed line positioned at the top of the bioprocess container, the feed line including a supersurface fluid discharge positioned above a volume of culture media residing in the bioprocess container, the supersurface fluid discharge being located such that a fluid flowing through the fluid discharge makes direct contact with a culture media contained within the bioprocess container without contacting the side wall.

[0027f] According to another aspect, there is provided a method for producing a single use bioreactor comprising:

constructing a bioprocess container from a liquid impermeable and flexible shape-conforming material, the bioprocess container having a top, a bottom, and at least one side wall therebetween, the bioprocess chamber defining a hollow enclosure for receiving a culture media, the hollow enclosure having a volume of from about 10 liters to about 20,000 liters, the bioprocess container including a plurality of inlet ports for feeding materials into the hollow enclosure of the bioprocess container, each inlet port having a diameter;

inserting a mixing device into the hollow enclosure of the bioprocess container, the mixing device comprising a rotatable shaft coupled to at least one agitator;

inserting at least one sparger into the hollow enclosure of the bioprocess container, the

sparger comprising a gas tube that has a longitudinal portion and a lateral portion, the longitudinal portion extending vertically into the hollow enclosure, the lateral portion being located at an end of the longitudinal portion below the agitator, the lateral portion defining a plurality of holes for releasing a gas into a culture media contained within the bioprocess container, the plurality of holes having a diameter;

connecting a drain line to the bottom of the bioprocess container, the drain line having a cross-sectional area; and wherein the diameter of the inlet ports, the diameter of the plurality of holes on the sparger, and the cross-sectional area of the drain line are proportional to the volume of the hollow enclosure, and wherein the drain line has a cross-sectional area of from about 0.3 mm^2 to about 0.7 mm^2 per liter of volume of the hollow enclosure.

[0027g] According to another aspect, there is provided a bioreactor comprising:

a bioprocess container made from a liquid impermeable and flexible shape-conforming material, the bioprocess container having a top, a bottom, and at least one side wall therebetween, the bioprocess chamber defining a hollow enclosure for receiving a culture media;

at least one inlet port for feeding materials into the hollow enclosure of the bioprocess container;

a mixing device comprising a rotatable shaft coupled to a plurality of agitators, the shaft and agitators extending into the hollow enclosure of the bioprocess container;

a cell retention chamber in fluid communication with the hollow enclosure of the bioprocess container;

a filtrate outlet in fluid communication with the cell retention chamber, the filtrate outlet including a biofilter that is permeable to liquids but impermeable to biological materials contained in a culture media, the filtrate outlet for removing liquids from the cell retention chamber; and

a flow regulator that is configured to alternate flow of a culture media between the hollow enclosure of the bioprocess container and the cell retention chamber.

[0027h] According to another aspect, there is provided a bioreactor comprising:

a bioprocess container made from a liquid impermeable and flexible shape-conforming material, the bioprocess container having a top, a bottom, and at least one side wall

therebetween, the bioprocess chamber defining a hollow enclosure for receiving a culture media;

at least one inlet port for feeding materials into the hollow enclosure of the bioprocess container; and

a mixing device comprising a rotatable shaft coupled to at least one agitator, the shaft and agitator extending into the hollow enclosure of the bioprocess container, the agitator being collapsible onto the rotating shaft.

[0028] In one embodiment, the present disclosure is directed to a bioreactor comprising a bioprocess container. The bioprocess container is made from a liquid impermeable and flexible shape-conforming material. For instance, the bioprocess container can be made from a flexible film, such as a multi-layer film. In one embodiment, for instance, the film is comprised of a polyethylene polymer, such as a low density polyethylene that has been modified to form a hydrophilic surface. The hydrophilic surface is for contact with cell cultures within the bioreactor and improves wettability. In one embodiment, the polyethylene polymer is modified by being subjected to irradiation, photo or plasma induction, or oxidation.

[0029] The bioprocess container can have a top, a bottom, and at least one side wall therebetween. The bioprocess chamber can define a hollow enclosure for receiving a culture media. The hollow enclosure can have any suitable volume, such as 100 mL, 250 mL, 500 mL, 750 mL, 1 liter, 2 liters, 3 liters, 4 liters, 5 liters, 6 liters, 7 liters, 8 liters, 9

hollow enclosure of the bioprocess container. In accordance with the present disclosure, the agitator can be collapsible onto the rotating shaft. For instance, the agitator can comprise an impeller comprising at least one blade element. The blade element can be foldable towards the rotatable shaft. In one embodiment, the rotatable shaft is coupled to a first impeller and a second impeller and both impellers can include at least one blade element that is foldable. A retaining ring can be positioned on the shaft. The retaining ring can include an agitator engaging position and an agitator disengaging position for holding the agitator in an upright position during mixing or in a collapsed and folded position respectively.

[0063] In one embodiment, the rotatable shaft comprises a metallic reinforcing rod surrounded by a shaft sleeve. The metallic reinforcing rod, which can be made from stainless steel, can be made from multiple pieces that are attached together. The top of the reinforcing rod can include a magnetic member for magnetically engaging a motor. The shaft sleeve can be comprised of a polymeric material. The agitator on the shaft can also be made from a polymeric material, such as a hydrophilic polymer. For example, the shaft sleeve and the agitator can comprise a polyethylene polymer that has been modified by being subjected to irradiation, photo or plasma induction, or oxidation.

[0064] In some embodiments, the single-use bioreactor can be configured for growing mammalian, insect, plant, other eukaryotic cells; microbial cells, including bacteria, yeast, and protozoan cells, and viruses; tissues; proteins; cellular products, such as organelles, enzymes, lipids, carbohydrates, and cell fractionates; exozymes; and cocultured organisms.

[0065] According to the disclosure, the cultivated cells are eukaryotic cells, such as animal cells, such as mammalian cells. The mammalian cells can be for example human cell lines, mouse myeloma (NS0)-cell lines, Chinese hamster ovary (CHO)-cell lines or hybridoma-cell lines. In one embodiment, the mammalian cells are CHO-cell lines.

[0066] In one embodiment, the cultivated cells are used to produce antibodies, including monoclonal or polyclonal antibodies, and/or recombinant proteins, such as recombinant proteins for therapeutic use. Of course the cells may also produce vesicles, exosomes, organelles, peptides, amino acids, fatty acids or other useful biochemical intermediates or metabolites. In addition, in some embodiments, the cultivated cells or

tissues formed therefrom may be the desired end product. The target concentration of the products produced by the cultivated cells may vary. For example, in one particular embodiment, the target concentration of the proteins produced by the cultivated cells can be more than 0.01g/l, such as more than 0.1 g/l, such as more than 0.5 g/l, such as more than 2.0 g/l, such as more than 10.0 g/l depending on culture volume. The method according to the disclosure can be used as a batch, fed-batch, perfusion, or draw and fill process. Although the cell-culture-medium used in the method according to the disclosure is preferably protein free medium, the design does not exclude the use of protein containing streams.

[0067] In one embodiment, a single use bioreactor system according to the instant disclosure comprises: a single use cell culture bioprocess container ("SUB"), a reusable shell in which the SUB is held during operation, and a controller that controls the operation of the SUB and associated sub-systems and processes. Associated sub-systems include an agitation system, a baffle system, a sparger system, a feeding system, a harvesting system, a monitoring system, control system(s), and a fill system.

[0068] In one embodiment, each of cell culture contacting and process fluid contacting surface of the SUB are preferably animal derived component free.

[0069] According to the disclosure, a single-use bioreactor is provided. The single-use bioreactor may include a bioprocess container, a shell, at least one agitator, at least one sparger, at least one gas filter inlet port for the sparger(s) and headspace overlay, at least one fill port, at least one harvest port, at least one sample port, and at least one probe.

[0070] According to the disclosure, a single-use bioreactor system is provided. The single-use bioreactor system may include a bioprocess container, a shell, at least one agitator, at least one sparger, at least one gas filter inlet ports for the sparger(s) and headspace overlay, at least one fill port, at least one harvest port, at least one sample port, and at least one probe

[0071] According to the disclosure, a single-use bioreactor system for the cultivation of mammalian cells is provided. The system may include a first single-use bioreactor, connected to at least one other bioreactor. The at least one other bioreactor may have a greater volume than said first single-use bioreactor and connected with any

number of single-use bioreactors. Each additional single-use bioreactors may have an increased volumes as compared to the prior single-use bioreactor. The multiple bioreactors may maintain a homogeneous environment with respect to pH, dissolved oxygen tension (DOT) and temperature, thus allowing for a well-mixed cell suspension and a blending of nutrient feeds within the bioreactor.

[0072] According to the disclosure, a method to cultivate and propagate mammalian cells is provided. The method may include cultivating under suitable conditions and in a suitable culture medium in a first single-use bioreactor, transferring the medium containing the cells obtained by propagation from the at least one mammalian cell is into a second single-use bioreactor, transferring the medium containing the cells obtained by propagation from the at least one mammalian cell is into a third single-use bioreactor, and cultivating the cells in the third bioreactor.

[0073] According to the disclosure, a single use bioreactor (SUB) system is provided. The system may include a flexible bioreactor bioprocess container that is for single use and disposable, a SUB shell configured to hold the flexible bioreactor bioprocess container, an agitator, a sparger, a plurality of ports, and at least one controller configured to control a plurality of parameters associated with the SUB system such that the SUB system produces biomaterial corresponding to biomaterial capable of being produced in a similarly sized stainless steel bioreactor.

[0074] In still another embodiment of the present disclosure, a bioreactor is disclosed that comprises a bioprocess container made from a liquid impermeable and flexible shape-conforming material, such as a flexible film. The bioprocess chamber defines a hollow enclosure for receiving a culture media. A mixing device comprising a rotatable shaft coupled to at least one agitator extends into the hollow enclosure of the bioprocess container. In accordance with the present disclosure, the rotatable shaft can be coupled to a top impeller and to a bottom impeller. Both the top impeller and the bottom impeller can be made from a polymer material. For instance, in one embodiment, the impellers may be 3-D printed. The top impeller and the bottom impeller can both define a hydrophilic surface. For instance, the polymer material used to form the impellers can comprise a hydrophilic polymer or can comprise a polymer that has been surface modified so as to render the surface hydrophilic.