perfusion process is distinguished from the fed-batch process by its lower capital cost and higher throughput.

[0005] In the fed-batch process, a culture is seeded in a bioreactor. The gradual addition of a fresh volume of selected nutrients during the growth cycle is used to improve productivity and growth. The product, typically a monoclonal antibody or a recombinant protein, is recovered after the culture is harvested. Separating the cells, cell debris and other waste products from the desired product is currently performed using various types of filters for separation, such as diatomaceous earth (DE) filters and membrane filters. Such filters are expensive and become clogged and non-functional as the bioreactor material is processed. A fed-batch bioreactor also has high start-up costs, and generally requires a large volume to obtain a cost-effective amount of product at the end of the growth cycle, and such processes include large amounts of non-productive downtime.

[0006] A perfusion bioreactor processes a continuous supply of fresh media that is fed into the bioreactor while growth-inhibiting byproducts are constantly removed. The nonproductive downtime can be reduced or eliminated with a perfusion bioreactor process. The cell densities achieved in perfusion culture (30–100 million cells/mL) are typically higher than for fed-batch modes (5–25 million cells/mL). However, a perfusion bioreactor requires a cell retention device to prevent escape of the culture when byproducts are being removed. These cell retention systems add a level of complexity to the perfusion process, requiring management, control, and maintenance for successful operation. Operational issues such as malfunction or failure of the cell retention equipment has previously been a problem with perfusion bioreactors. This has limited their attractiveness in the past.

[0007] It would be desirable to provide means that can reduce the cost and effort of using bioreactors and separating the desired products from the cells that make them.

~~BRIEF DESCRIPTION~~SUMMARY

[0008] The present disclosure relates, in various embodiments, to systems for producing biomolecules such as recombinant proteins or monoclonal antibodies, and to processes for separating these desirable products from a cell culture in a disposable

such as a 0x0 mode (i.e. a piston mode) to a 1x1, 2x2, 1x3, 3x1, 3x3, and other higher order modes and then cycle back through the lower modes of the crystal (not necessarily in straight order). This switching or dithering of the crystal between modes allows for various multidimensional wave shapes, along with a single piston mode shape to be generated over a designated time.

[0011] ~~Disclosed in various embodiments are~~In one aspect, there is provided a disposable bioreactor system~~s~~, comprising: a bag having a first end, a second end, and a port at the first end; at least one~~an~~ acoustophoresis device disposed about the first end of the bag, the at least one acoustophoresis device being separable from the bag; and an actuating mechanism operably connected to the second end of the bag, the actuating mechanism being configured to move the second end of the bag towards the at least one acoustophoresis device at the first end of the bag.

[0012] The bag may comprise multiple layers of differentially functioning polymers. The bag can be corrugated, thereby allowing the bag to collapse on itself.

[0013] The bag may further include a neck portion disposed at the first end of the bag, the neck portion including the port. In such embodiments, the acoustophoresis device can be disposed on the neck portion upstream of the port. The acoustophoresis device can be configured to generate a multi-dimensional standing wave upstream of the port.

[0014] The system may further include an impeller disposed within the bag. The side of the bag may include a liquid-tight access point through which an impeller can be inserted and removed from the interior volume of the bag. Alternatively, the side of the bag may include a jack for connecting to an impeller that is permanently sealed within the interior volume of the bag, the jack being used to provide power to the impeller.

[0015] In some embodiments, the actuating mechanism includes: a pan member disposed around the exterior of the second end of the bag; and a screw member operably connected to the pan member. Upon rotation of the screw member, the pan member is configured to move the second end of the bag towards the acoustophoresis device, thereby reducing the volume of the bag.

[0016] In other embodiments, the actuating mechanism includes: at least one reel disposed at the first end of the bag; and at least one cable operably connected to the second end of the bag and winding about the at least one reel. Upon movement of the

at least one cable about the at least one reel, the at least one cable moves the second end of the bag towards the acoustophoresis device, thereby reducing the volume of the bag.

[0016a] In another aspect, there is provided a method of of separated desired biomolecules from a mixture of solid waste and a liquid permeate, the method comprising: receiving a bag filled with the desired biomolecules, the solid waste, and the liquid permeate; actuating an actuating mechanism operably connected to a second end of the bag; collapsing the bag so that the solid waste and the liquid permeate flow towards a port or ports on a first end of the bag; and generating a multi-dimensional acoustic standing wave with at least one acoustophoresis device disposed upstream of the port or ports, thus reducing the amount of solid waste passing through the port or ports while permitting the biomolecules and the liquid permeate to pass through the port or ports.

[0017] Also disclosed are methods of separated desired biomolecules from a mixture of solid waste and a liquid permeate, comprising: receiving a bag filled with the desired biomolecules, the solid waste, and the liquid permeate; actuating an actuating mechanism operably connected to a second end of the bag; collapsing the bag so that the solid waste and the liquid permeate flow towards a port on a first end of the bag; and generating a multi-dimensional standing wave with an acoustophoresis device disposed upstream of the port, thus reducing the amount of solid waste passing through the port while permitting the biomolecules and the liquid permeate to pass through the port.

[0018] These and other non-limiting characteristics are more particularly described below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The following is a brief description of the drawings, which are presented for the purposes of illustrating the exemplary embodiments disclosed herein and not for the purposes of limiting the same.

[0020] **Figure 1** illustrates a first embodiment of a bioreactor system in an unactuated condition.

[0021] **Figure 2** illustrates the bioreactor system of Figure 1 in an actuated condition.

[0022] **Figure 3** illustrates a second embodiment of a bioreactor system in an unactuated condition.

[0023] **Figure 4** illustrates the bioreactor system of Figure 3 in an actuated condition.

[0024] **Figure 5** is a cross-sectional diagram of a conventional ultrasonic transducer.

[0025] **Figure 6** is a cross-sectional diagram of an ultrasonic transducer of the present disclosure. An air gap is present within the transducer, and no backing layer or wear plate is present.