

SPECIFICATION

TitlePNEUMATIC BIOREACTORField of Invention

5 The field of the invention pertains to apparatus for mixing solutions. More particularly, the invention relates to pneumatically operated mixers for use in closed, sterile environments.

Background of the Invention

10 Bioreactors have been used for cultivation of microbial organisms for production of various biological or chemical products in the pharmaceutical, beverage and biotechnological industry. A production bioreactor contains culture medium in a sterile environment that provides various nutrients required to support growth of the biological agents of interest. Conventional bioreactors use mechanically driven impellers to mix the liquid medium during cultivation. The
15 bioreactors can be reused for the next batch of biological agents after cleaning and sterilization of the vessel. The procedure of cleaning and sterilization requires a significant amount of time and resources, especially, to monitor and to validate each cleaning step prior to reuse for production of biopharmaceutical products. Due to the high cost of construction, maintenance and operation of the
20 conventional bioreactors, single use bioreactor systems made of disposable plastic material have become an attractive alternative.

 While several mixing methods of liquid in disposable bioreactors have been proposed in recent years, none of them provides efficient mixing for large scale (greater than 1000 liters) without expensive operating machinery. For this
25 reason, a number of non-invasive and/or disposable mixing systems that do not require an external mechanical operation have been developed. Many of these systems work well within certain size ranges, however, problems sometimes arise as larger mixing systems are attempted.

It is an objective of the present invention to provide a pneumatic bioreactor that is capable of efficiently and thoroughly mixing solutions without contamination. It is a further objective to such a reactor that can be scaled to relatively large sizes using the same technology. It is a still further objective of the invention to a bioreactor that can be produced in a disposable form. It is yet a further objective of the invention to provide a bioreactor that can be accurately controlled by internal pneumatic force, as to speed and mixing force applied to the solution without creating a foaming problem. Finally, it is an objective to provide a bioreactor that is simple and inexpensive to produce and to operate while fulfilling all of the described performance criteria.

Summary of the Invention

Embodiments of the present invention are directed to a pneumatic bioreactor which has mixing apparatus in a containment vessel. The mixing apparatus employs a gas supply and a buoyancy-driven mixing device actuated by the gas supply. The mixing apparatus substantially spans the cross-section of the containment vessel.

One aspect of the present invention provides a pneumatic bioreactor comprising a containment vessel; mixing apparatus in the containment vessel including a gas supply having at least one orifice in the vessel and at least one buoyancy-driven mixing device above the at least one orifice, respectively, the mixing apparatus substantially spanning the cross section of the containment vessel, wherein the buoyancy-driven mixing device includes a cylindrical chamber rotatably mounted relative to the containment vessel about a substantially horizontal axis and having an inner surface, an outer surface, at least one mixing plate extending axially with respect to the horizontal axis within the inner surface, and a plurality of gas holding members about the outer surface of the cylindrical chamber sized and shaped to entrap gas bubbles from the gas supply.

A further aspect of the present invention provides a method of mixing the biological contents of a bioreactor, the method comprising the steps of providing (a) a containment vessel for holding the biological contents and (b) a buoyancy-

driven mixing device located within the containment vessel and having a substantially horizontal axis, wherein the buoyancy-driven mixing device includes a cylindrical chamber rotatably mounted relative to the containment vessel about the substantially horizontal axis and having an inner surface, an outer surface, at least one mixing plate extending axially with respect to the horizontal axis within the inner surface, and a plurality of gas holding members about the outer surface of the cylindrical chamber sized and shaped to entrap gas bubbles from the gas supply; and rotating the mixing device in a substantially vertical plane about said axis to mix the contents of the containment vessel by introducing gas into the containment vessel at a location below the mixing device such that at least a portion of the gas is captured by the mixing device at a given point in time to provide buoyancy to cause such rotation.

In an embodiment of the present invention, the mixing device includes one or more floating mixers with a gas-holding chamber and a plurality of mixing elements about that chamber. The mixing elements are shaped to cause the mixer to agitate as the mixer rises in the containment vessel. A gas-venting valve permits gas to escape from the central chamber upon the chamber reaching the fluid surface.

In an embodiment of the present invention, the buoyancy driven mixing device includes at least one floating plunger having a central, gas-holding chamber and at least one disk disposed about the central chamber. Again, a gas venting valve permits escape of the gas as the central chamber reaches a fluid surface. A mixing partition is also disposed in the containment vessel adjacent the floating plunger with at least one aperture to augment mixing.

In an embodiment of the present invention, the buoyancy-driven mixing device includes a rotatably mounted cylindrical chamber which has an

inner surface, an outer surface, at least one mixing plate within the inner surface and a plurality of gas holding members about the outer surface.

Accordingly, embodiments of the present invention seek to provide an improved pneumatic bioreactor.

Description of the Drawings

Figure 1 is a perspective view of a first embodiment of the invention illustrating floating impellers and their control mechanisms;

Figure 2 is a top view of the Figure 1 embodiment illustrating the floating chamber affixed to the constraining member with the impeller blades rotating upon the chamber;

Figure 2A is a top view of the Figure 1 embodiment illustrating the floating chamber rotating within the constraining member with the impeller blades fixed to the chamber;

Figure 3 is a side elevational view of the Figure 1 embodiment;

Figure 4 is a side elevational view of the Figure 2A embodiment of the floating impeller;

Figure 4A is a side elevational view of the Figure 2 embodiment of the floating impeller;

Figure 5 is a perspective view of a second embodiment of the invention illustrating floating plungers and their control mechanisms;

Figure 6 is a top view of the Figure 5 embodiment illustrating the floating plungers;

Figure 7 is a perspective view of the gas supply and magnetic assent control mechanism;

Figure 8 is a cross-sectional side elevation of the floating chamber illustrating the vent cap in a closed position;

Figure 9 is a cross-sectional side elevation of the floating chamber illustrating the vent cap in an open position;

Figure 10 is a perspective view of a third embodiment of the invention illustrating a rotating drum mixer with gas supply;

Figure 11 is an end view of the Figure 10 embodiment illustrating a single gas supply;

Figure 12 is an end view of the Figure 10 embodiment illustrating a pair of gas supplies;

5 Figure 13 is a side elevational view of the Figure 10 embodiment illustrating a containment vessel;

Figure 14 is a perspective view of the Figure 5 embodiment illustrating a closable top and sterile filters; and

10 Figure 15 is a perspective view of the Figure 5 embodiment illustrating a temperature control jacket surrounding the vessel.

Detailed Description of the Preferred Embodiment

A pneumatic bioreactor 10, as illustrated in the embodiment of Figures 1-3, providing all of the desired features can be constructed from the following components. A containment vessel 15 is provided. The vessel 15 has a top 20, a closed bottom 25, a surrounding wall 30 and is of sufficient size to contain a fluid 35 to be mixed and a mixing apparatus 40. The mixing apparatus 40 includes at least one gas supply 45. The supply 45 terminates at an orifice 50 adjacent the bottom 25 of the vessel 15. At least one buoyancy-driven mixing device 55 is provided. The mixing device 55 moves in the fluid 35 as gas 60 from the supply 45 is introduced into and vented from the mixing device 55. When gas 60 is introduced into the gas supply 45 the gas 60 will enter the mixing device 55 and cause the device to mix the fluid 35.

The buoyancy-driven mixing device 55 further includes at least one floating mixer 65. The mixer 65 has a central, gas-holding chamber 70 and a plurality of mixing elements 75 located about the central chamber 70. The mixing elements 75 are shaped to cause the mixer 65 to agitate the fluid 35 as the mixer 65 rises in the fluid 35 in the containment vessel 15. The central chamber 70, as illustrated in Figures 8 and 9, has a gas-venting valve 80. The valve 80 permits escape of gas 60 as the central chamber 70 reaches a surface 85 of the fluid 35. A constraining member 90 is provided. The constraining member 90 limits horizontal movement of the floating mixer 65 as it rises or sinks in the fluid 35. When gas 60 is introduced into the gas supply 45, the gas 60 will enter the gas

holding chamber 70 and cause the floating mixer 65 to rise by buoyancy in the fluid 35 while agitating the fluid 35. When the gas venting valve 80 of the central chamber 70 reaches the surface 85 of the fluid 35, the gas 60 will be released and the floating mixer 65 will sink toward the bottom 25 of the containment vessel 15 where the central chamber 70 will again be filled with gas 60, causing the floating mixer 65 to rise.

Means 95, as illustrated in the embodiment of Figure 7, are provided for controlling a rate of ascent of the floating mixer 65. The means 95 for controlling the rate of ascent of the floating mixer 65 includes a ferromagnetic substance 100 attached to either of the floating mixer 65 or the constraining member 90 and a controllable electromagnet 105 located adjacent the bottom 25 of the containment vessel 15.

As illustrated in the embodiment of Figures 8 and 9, the central, gas-holding chamber 70 includes an opening 110. The opening 110 is located at an upper end 115 of the chamber 70. A vent cap 117 is provided. The vent cap 117 is sized and shaped to seal the opening 110 when moved upwardly against it by buoyancy from gas 60 from the supply 45. A support bracket 120 is provided. The support bracket 120 is located within the chamber 70 to support the vent cap 115 when it is lowered after release of gas 60 from the chamber 70. When the chamber 70 rises to the surface 85 of the fluid 35 the vent cap 115 will descend from its weight and the opening 110 will permit the gas 60 to escape, the chamber 70 will then sink in the fluid 35 and the vent cap 115 will again rise due to buoyancy from a small amount of gas 60 permanently enclosed in the vent cap 115, thereby sealing the opening 110.

As illustrated in the embodiment of Figures 1-3, a second floating mixer 125 is provided. A second constraining member 130 is provided, limiting horizontal movement of the second mixer 125 as it rises in the fluid 35. Additional gas supply 135 is provided. The additional supply 135 terminates at an orifice 143 adjacent the bottom 25 of the vessel 15. At least one pulley 140 is provided. The pulley 140 is attached to the bottom 25 of the containment vessel 15. A flexible member 145 is provided. The flexible member 145 attaches the chamber 70 of the floating mixer 65 to a chamber 150 of the second floating mixer 125. The

flexible member 145 is of a length permitting the gas venting valve 80 of the chamber 70 of the floating mixer 65 to reach the surface 85 of the fluid 35 while the chamber 70 of the second floating mixer 125 is spaced from the bottom 25 of the containment vessel 15. When the floating mixer 65 is propelled upwardly by buoyancy from the gas 60 from the supply 45 the second floating mixer 125 is pulled downwardly by the flexible member 145 until the gas 60 is released from the chamber 70 of the floating mixer 65 as its gas venting valve 80 reaches the surface 85 of the fluid 35. The chamber 70 will then sink in the fluid 35 as the second floating mixer 125 rises by buoyancy from gas 60 introduced from the additional supply 135. As can be seen in these Figures, the mixing apparatus substantially spans the cross section of the containment vessel.

The containment vessel 15 may be formed of resilient material 155, the material is sterilizable by gamma irradiation methods.

As illustrated in the embodiment of Figures 5 and 6, the buoyancy-driven mixing device 10 includes at least one floating plunger 160. The plunger 160 has a central, gas-holding chamber 70 and at least one disk 165 located about the central chamber 70. The disk 165 is shaped to cause the plunger 160 to agitate the fluid 35 as the plunger 160 rises in the fluid 35 in the containment vessel 15. The central chamber 70 has a gas-venting valve 80. The valve 80 permits escape of gas 60 as the central chamber 70 reaches a surface 85 of the fluid 35. A mixing partition 170 is provided. The partition 170 is located in the containment vessel 15 adjacent the floating plunger 160 and has at least one aperture 175 to augment a mixing action of the floating plunger 160. A constraining member 180 is provided. The constraining member 180 limits horizontal movement of the plunger 160 as it rises or sinks in the fluid 35. When gas 60 is introduced into the gas supply 45 the gas 60 will enter the gas holding chamber 70 and cause the floating plunger 160 to rise by buoyancy in the fluid 35 while agitating the fluid 35. When the gas venting valve 80 of the central chamber 70 reaches the surface 85 of the fluid 35, the gas 60 will be released and the floating plunger 160 will sink toward the bottom 25 of the containment vessel 15 where the central chamber 70 will again be filled with gas 60, causing the floating plunger 160 to rise.

A second floating plunger 185 is provided. A second constraining member 190 is provided, limiting horizontal movement of the second plunger 185 as it rises in the fluid 35. Additional gas supply 135 is provided. The additional supply 135 terminates at an orifice 143 adjacent the bottom 25 of the vessel 15. At least one
5 pulley 140 is provided. The pulley 140 is attached to the bottom 25 of the containment vessel 15. A flexible member 145 is provided. The flexible member 145 attaches the chamber 70 of the floating plunger 160 to a chamber of the second floating plunger 185. The flexible member 145 is of a length permitting the gas venting valve 80 of the chamber 70 of the floating plunger 160 to reach the
10 surface 85 of the fluid 35 while the chamber 70 of the second floating plunger 185 is spaced from the bottom 25 of the containment vessel 15. The mixing partition 170 is located between the floating plunger 160 and the second floating plunger 185. When the floating plunger 160 is propelled upwardly by buoyancy from the gas 60 from the supply 45 the second floating plunger 185 is pulled downwardly
15 by the flexible member 145 until the gas 60 is released from the chamber 70 of the floating plunger 160 as its gas venting valve 80 reaches the surface 85 of the fluid 30. The floating plunger 160 will then sink in the fluid 35 as the second floating plunger 185 rises by buoyancy from gas 60 introduced from the additional supply 135. As seen in these Figures, the mixing apparatus substantially spans
20 the cross section of the containment vessel.

As illustrated in the embodiment of Figures 10-13, the pneumatic bioreactor 10 includes a cylindrical chamber 195. The chamber 195 has an inner surface 200, an outer surface 205, a first end 210, a second end 215 and a central axis 220. At least one mixing plate 225 is provided. The mixing plate 225 is attached
25 to the inner surface 200 of the chamber 195. First 230 and second 235 flanges are provided. The flanges 230, 235 are mounted to the cylindrical chamber 195 at the first 210 and second ends 215, respectively. First 240 and second 245 pivot points are provided. The pivot points 240, 245 are attached to the first 230 and second 235 flanges, respectively and to the containment vessel 15, thereby
30 permitting the cylindrical chamber 195 to rotate about the central axis 220. A plurality of gas holding members 250 are provided. The members 250 extend from the first flange 230 to the second flange 235 along the outer surface 205 of

the cylindrical chamber 195 and are sized and shaped to entrap gas bubbles 255 from the gas supply 45. The gas supply 45 terminates adjacent the cylindrical chamber 195 on a first side 260 of the chamber 195 below the gas holding members 250. When gas 60 is introduced into the containment vessel 15 through the supply 45 it will rise in the fluid 35 and gas bubbles 255 will be entrapped by the gas holding members 250. This will cause the cylindrical chamber 195 to rotate on the pivot points 240, 245 in a first direction 262 and the at least one mixing plate 225 to agitate the fluid 35. As seen in these Figures, the mixing apparatus substantially spans the cross section of the containment vessel.

The rate of rotation of the cylindrical chamber 195 may be controlled by varying a rate of introduction of gas 60 into the gas supply 45.

As illustrated in Figure 12, an additional gas supply 135 may be provided. The additional supply 135 terminates adjacent the cylindrical chamber 195 on a second, opposite side 265 of the chamber 195 below the gas holding members 250. Gas 60 from the additional supply 135 causes the cylindrical chamber 195 to rotate on the pivot points 240, 245 in a second, opposite direction 270.

As illustrated in Figures 10 and 13, the at least one mixing plate 225 has at least one aperture 275 to augment mixing of the fluid 35 in the containment vessel 15.

As illustrated in Figure 14, the containment vessel 15 further includes a closable top 280. The top has a vent 285, permitting the escape of gas 60 from the gas supply 45 through a sterile filter 290.

As illustrated in Figure 15, a temperature control jacket 295 is provided. The jacket 295 surrounds the containment vessel 15.

As illustrated in Figures 1-3, a pneumatic bioreactor 10 includes a containment vessel 15. The vessel 15 has a top 20, a closed bottom 25, a surrounding wall 30 and is of sufficient size to contain a fluid 35 to be mixed and a mixing apparatus 40. The mixing apparatus 40 includes at least one gas supply 45. The supply 45 terminates at an orifice 50 at the bottom 25 of the vessel 15. At least one floating impeller 300 is provided. The impeller 300 has a central, gas-containing chamber 70 and a plurality of impeller blades 305 arcuately located about the central chamber 70. The impeller blades 305 are shaped to cause the

impeller 300 to revolve about a vertical axis 310 as the impeller 300 rises in fluid 35 in the containment vessel 15.

The central chamber 70 has a gas-venting valve 80. The valve 80 permits escape of gas 60 as the central chamber 70 reaches a surface 85 of the fluid 35.

5 An outside housing 315 is provided. The housing 315 is ring-shaped and surrounds the floating impeller 300 and constrains its lateral movement. At least one supporting pole 320 is provided. The pole 320 extends from the bottom 25 upwardly toward the top 20. The outside housing 315 is slidably attached to the supporting pole 320. The floating impeller 300 is rotatably attached to the outside

10 housing 315. When gas 60 is introduced into the gas supply 45 the gas 60 will enter the gas containing chamber 70 and cause the floating impeller 300 to rise in the fluid 35 while rotating and mixing the fluid 35. When the gas venting valve 80 of the central chamber 70 reaches the surface 85 of the fluid 35, the gas 60 will be released and the floating impeller 300 will sink toward the bottom 25 of the

15 containment vessel 15 where the central chamber 70 will again be filled with gas 60, causing the floating impeller 300 to rise.

As illustrated in Figures 2 and 4A, the impeller blades 305 are rotatably mounted to the central chamber 70 and the central chamber 70 is fixedly attached to the outside housing 315.

20 As illustrated in Figures 2A and 4, the impeller blades 305 are fixedly mounted to the central chamber 70 and rotatably mounted to the outside housing 315.

The outside housing 315 further includes a horizontal interior groove 322 located on an inner surface 325 of the housing 315. The impeller blades 305

25 include a projection 330, sized and shaped to fit slidably within the groove 322.

As illustrated in Figure 7, means 95 are provided for controlling a rate of ascent of the floating impeller 300.

The means 95 for controlling a rate of ascent of the floating impeller 300 includes a ferromagnetic substance 100 attached to either the floating impeller

30 300 or the outside housing 315 and a controllable electromagnet 105 located adjacent the bottom 25 of the containment vessel 15.

As illustrated in Figures 8 and 9, the central, gas-containing chamber 70 further includes an opening 110 located at an upper end 115 of the chamber 70. A vent cap 115 is provided. The vent cap 115 is sized and shaped to seal the opening 110 when moved upwardly against it by pressure from gas 60 from the supply 45. A support bracket 120 is provided. The support bracket 120 is located within the chamber 70 to support the vent cap 115 when it is lowered after release of gas 60 from the chamber 70. When the chamber 70 rises to the surface of the fluid 35 the vent cap 115 will descend from its weight and the opening 110 will permit the gas 60 to escape. The floating impeller 300 will then sink in the fluid 35 and the vent cap 115 will again rise due to pressure from gas 60 introduced into the chamber 70 from the gas 45, thereby sealing the opening 110.

The vent cap 115 further includes an enclosed gas cell 310. The cell 310 causes the cap 115 to float in the fluid 35 and thereby to reseal the opening 110 after the gas 60 has been released when the chamber 70 reached the surface 85 of the fluid 35.

As illustrated in Figures 1 and 3, the pneumatic bioreactor 10 further includes a second floating impeller 317. A second outside housing 324 surrounding the second floating impeller 317 is provided. At least one additional supporting pole 326 is provided. Additional gas supply 135 is provided. The additional supply 135 terminates at an orifice 143 at the bottom 25 of the vessel 15. The second outside housing 324 is slidably attached to the additional supporting pole 325. The second floating impeller 317 is rotatably attached to the second outside housing 324. At least one pulley 140 is provided. The pulley 140 is attached to the bottom 25 of the containment vessel 15.

A flexible member 145 is provided. The flexible member 145 attaches the chamber 70 of the floating impeller 300 to a chamber 70 of the second floating impeller 317. The flexible member 145 is of a length to permit the gas venting valve 80 of the chamber 70 of the floating impeller 300 to reach the surface 85 of the fluid 35 while the chamber 70 of the second floating impeller 317 is spaced from the bottom 25 of the containment vessel 15. When the floating impeller 300 is propelled upwardly by pressure from the gas 60 from the supply 45 the second floating impeller 315 will be pulled downwardly by the flexible member 145 until

the gas 60 is released from the chamber 70 of the floating impeller 300 as its gas venting valve 80 reaches the surface 85 of the fluid 35, the floating impeller 300 will then sink in the fluid 35 as the second floating impeller 315 rises under pressure from gas 60 introduced from the additional supply 135.

- 5 An appreciation of the other aims and objectives of the present invention and an understanding of it may be achieved by referring to the accompanying drawings and the detailed description of a preferred embodiment.

CLAIMS:

1. A pneumatic bioreactor comprising
a containment vessel;
mixing apparatus in the containment vessel including a gas supply having
at least one orifice in the vessel and at least one buoyancy-driven mixing device above the at least one orifice, respectively, the mixing apparatus substantially spanning the cross section of the containment vessel, wherein
the buoyancy-driven mixing device includes a cylindrical chamber rotatably mounted relative to the containment vessel about a substantially horizontal axis and having an inner surface, an outer surface, at least one mixing plate extending axially with respect to the horizontal axis within the inner surface, and a plurality of gas holding members about the outer surface of the cylindrical chamber sized and shaped to entrap gas bubbles from the gas supply.

2. The pneumatic bioreactor of claim 1, the gas supply having a second orifice, the first and second orifices being to either side of the horizontal axis below said gas holding members, gas from the two orifices causing the cylindrical chamber to rotate in opposite directions.

3. The pneumatic bioreactor of claim 1, the at least one plate has at least one aperture to augment mixing of the fluid in said containment vessel.

4. The pneumatic bioreactor of claim 1, wherein the containment vessel is disposable.

5. A method of mixing the biological contents of a bioreactor, the method comprising the steps of

providing (a) a containment vessel for holding the biological contents and (b) a buoyancy-driven mixing device located within the containment vessel and having a substantially horizontal axis, wherein the buoyancy-driven mixing device includes a cylindrical chamber rotatably mounted relative to the containment vessel about the substantially horizontal axis and having an inner surface, an outer surface, at least one mixing plate extending axially with respect to the horizontal axis within the inner surface, and a plurality of gas holding members about the outer surface of the cylindrical chamber sized and shaped to entrap gas bubbles from the gas supply; and

rotating the mixing device in a substantially vertical plane about said axis to mix the contents of the containment vessel by introducing gas into the containment vessel at a location below the mixing device such that at least a portion of the gas is captured by the mixing device at a given point in time to provide buoyancy to cause such rotation.