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(54) **STIRRED-TANK REACTOR SYSTEM**

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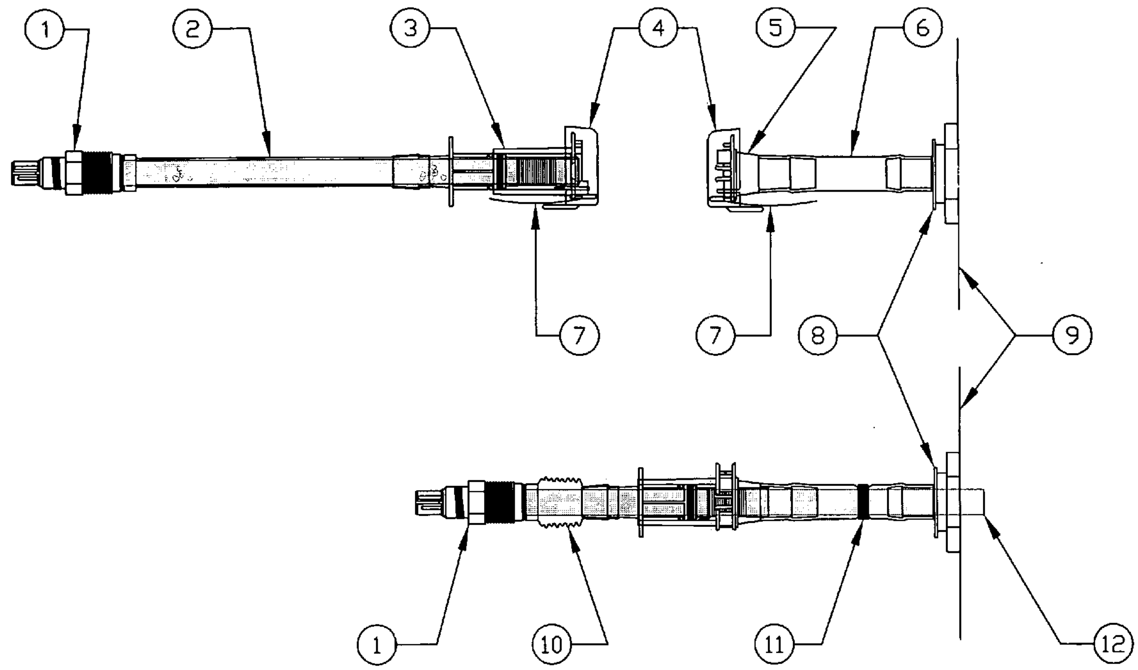
(57) **ABSTRACT**

The present invention relates to a stirred-tank reactor system and methods of preparing such systems. The present invention further encompasses the use of the stirred-tank reactor system as a disposable bioreactor and in kits with disposable elements.



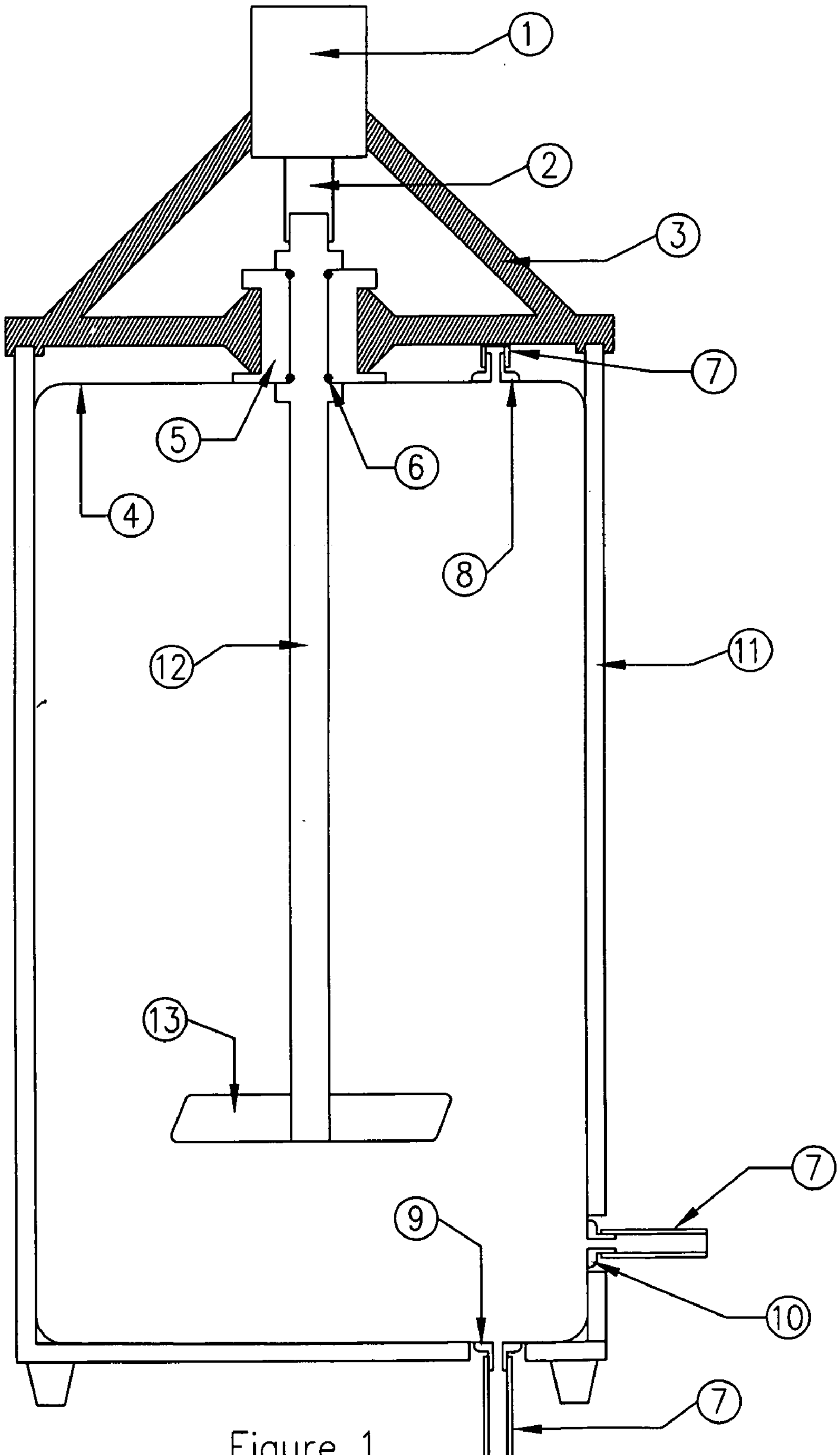


Figure 1

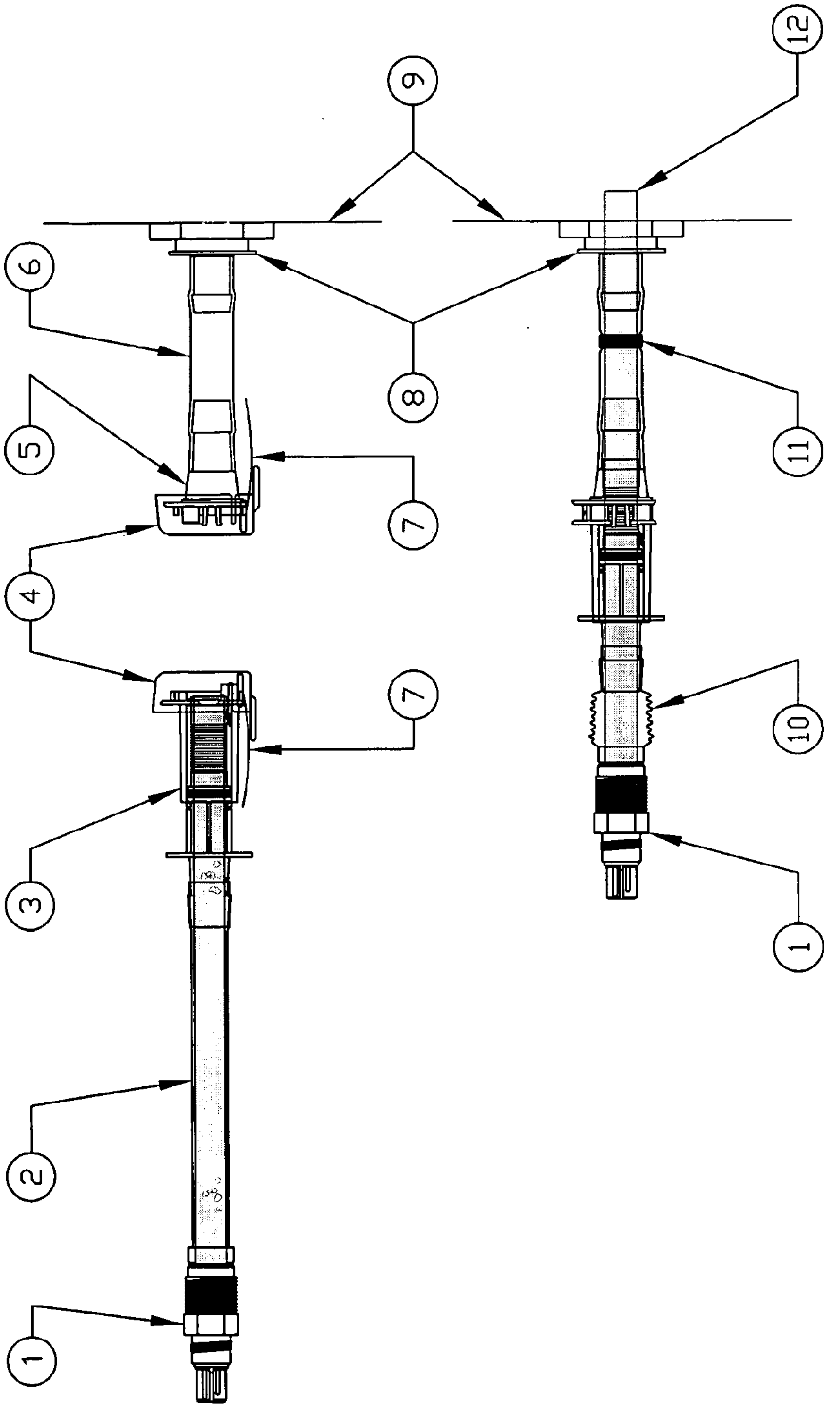


Figure 2

STIRRED-TANK REACTOR SYSTEM

FIELD OF THE INVENTION

[0001] The present invention relates to a stirred-tank reactor system and methods of preparing such systems. The present invention further encompasses the use of the stirred-tank reactor system as a disposable bioreactor and in kits with disposable elements.

BACKGROUND OF THE INVENTION

[0002] A bioreactor or fermenter is a container used for fermentation, enzymatic reactions, cell culture, biologicals, chemicals, biopharmaceuticals, tissue engineering, microorganisms, plant metabolites, food production and the like. Bioreactors vary in size from benchtop fermenters to stand-alone units of various sizes. The stringent asepsis requirements for sterile production in bioreactors usually requires elaborate systems to achieve the desired product volumes. Consequently, the production of products in aseptic bioreactors is costly which provides the motivation for pursuing improved systems.

[0003] Conventional bioreactors perfuse nutrient media through a single type of hollow fiber. The various disadvantages of such bioreactors include heterogeneous cell mass, difficult procurement of representative cell growth samples, poor performance due to inefficient oxygenation and an inability to control oxygen levels, and problems with contamination of cell cultures. Moreover, micro-environmental factors such as pH cannot be effectively controlled and a mixed culture or co-culture of cells is not possible. An improvement to such prior art bioreactors is the hollow fiber reactor, as covered in U.S. Pat. No. 5,622,857. This reactor comprises a reaction container, through which a central strand of porous hollow fibers extends, through which a nutrient solution is pumped. This central strand of hollow fibers is concentrically surrounded by a plurality of strands of hollow fibers, through which a gaseous medium is conveyed. The hollow fibers of these strands are also constituted in such a manner that the gaseous medium—for example oxygen or carbon dioxide—can at least partly emerge from these strands or enter into these strands respectively. This type of bioreactor achieves a somewhat enhanced nutrient media oxygenation as compared to prior art devices. However, occasional contamination of cell cultures and an inability to control pH levels effectively remain consistent problems.

[0004] The expense of producing cells, biopharmaceuticals, biologicals and the like in aseptic bioreactors is exacerbated by the required cleaning, sterilization and validation of the standard bioreactors (i.e., stainless steel or glass reactors). Attempts have been made to solve this problem with the development of pre-sterilized disposable bioreactor systems that need not be cleaned, sterilized or validated by end users. The use of such disposable bioreactor systems could provide significant savings. Furthermore, plastics are lightweight, easy to transport, and require less room than stainless steel or glass reactors. An example for the use of disposable elements in bioreactors is provided in U.S. Pat. No. 6,245,555 B1 which describes a reactor chamber with a support housing. The interior chamber of the support housing is lined with a disposable liner and sealed with a head plate attached to the liner to form a sealed chamber. Since the liner is open at the top, it must be used in a vertically oriented bioreactor to prevent the contamination of the head plate. Although this system provides a disposable liner, the head plate and the interior chamber still require cleaning and sterilization.

[0005] Another solution has been to develop flexible, disposable plastic vessels that do not require cleaning or sterilization and require only minimal validation efforts. For example, U.S. Pat. No. 5,523,228 describes a flexible, disposable, and gas permeable cell culture chamber that is horizontally rotated. The cell culture chamber is made of two sheets of plastic fused together. In addition, the culture chamber is made of gas permeable material and is mounted on a horizontally rotating disk drive that supports the flexible culture chamber without blocking airflow over the membrane surfaces. The chamber is placed in an incubator and oxygen transfer is controlled by controlling the gas pressure in the incubator according to the permeability coefficient of the bag. The rotation of the bag assists in mixing the contents of the bag. However, the cell culture chamber is limited to use within a controlled gas environment. Particularly, the cell culture chamber has no support apparatus and is thus limited to small volumes. Furthermore, the chamber does not provide an inlet and an outlet for media to be constantly pumped into and out of the chamber during rotation.

[0006] Some companies have developed a range of pre-sterile, disposable bioreactors that do not require cleaning or sterilizing (e.g., Wave Biotech, Bridgewater, N.J.). Such reactors are made of sheets of flexible, gas impermeable material to form a bag. The bag is partially filled with media and then inflated with air that continually passes through the bag's headspace. The media is mixed and aerated by rocking the bags to increase the air-liquid interface. However, since there is no solid housing that support the bags, the bags become cumbersome and difficult to handle as they increase in size. Furthermore, the wave action within the rocking bag creates damaging turbulent forces. Certain cell cultures, particularly human cell cultures, develop better under more gentle conditions.

[0007] Thus, there is a continuing need in the art to develop flexible, pre-sterilized, disposable bioreactors that are easy to handle and require little training to operate, yet provide the necessary gas transfer and nutrient mixing required for successful cell and tissue cultures. Such disposable bioreactors would be equally useful for the production of chemicals, biopharmaceuticals, biologicals, cells, microorganisms, plant metabolites, foods and the like.

BRIEF SUMMARY OF THE INVENTION

[0008] The present invention provides a stirred-tank reactor system with disposable elements, such as a flexible plastic bag with an attached bearing, shaft, and impeller assembly. The instant invention further relates to the use of this novel stirred-tank reactor system as a disposable bioreactor and in kits with disposable elements. The advantages of the present invention are numerous. Particularly, the stirred-tank reactor system may be pre-sterilized and does not require a steam-in-place (SIP) or clean-in-place (CIP) environment for changing from batch to batch or product to product in a culture or production system. As such, the system requires less regulatory control by assuring zero batch-to-batch contamination and can, thus, be operated at a considerable cost-advantage and with minimal or no preparation prior to use. In addition, the system is a true stirred-tank reactor system unlike other disposable reactors systems. This provides the added advantage that the instant invention offers a hydrodynamic environment that can be scaled to various sizes similar to conventional non-disposable reactor systems. Since the system does not require cleaning or sterilizing it combines a flexible, easy-to-use, true stirred-tank reactor environment with zero cross-contamination during the cell culture or production process.

[0009] One aspect of the present invention provides a stirred-tank reactor system, comprising a flexible bag with at least one opening, wherein the bag functions as a sterile container for a fluidic medium; a shaft situated within the bag; an impeller attachable to the shaft, wherein the impeller is used to agitate the fluidic medium to provide a hydrodynamic environment; and a bearing attached to the shaft and to the opening of the bag. The bag may be affixed to the shaft and the bearing through at least one seal or o-ring such that the inside of the bag remains sterile. The seals or o-rings can be affixed to the bag. The system may be disposable and pre-sterilized. The bag may further include a pH sensor and a dissolved-oxygen sensor, wherein the sensors are incorporated into the bag. In addition, the system may include at least one internal pouch sealed to the bag, wherein the pouch has one end that can be opened to the outside of the bag such that a probe (i.e., a temperature probe, a pH probe, a dissolved gas sensor, an oxygen sensor, a carbon dioxide (CO_2) sensor, a cell mass sensor, a nutrient sensor, an osmometer, and the like) can be inserted into the reactor. The system may also include at least one port in the bag allowing for the connection of a device such as a tube, a filter, a sampler, a probe, or a connection device to the port. A port allows for sampling; gas flow in and out of the bag; liquid or media flow in and out of the bag; inoculation; titration; adding of chemostat reagents; sparging; and the like.

[0010] Another aspect of the present invention provides a stirred-tank reactor system, comprising a flexible bag with at least one opening, wherein the bag functions as a sterile container for a fluidic medium; a shaft situated within the bag; an impeller attachable to the shaft, wherein the impeller is used to agitate the fluidic medium to provide a hydrodynamic environment; and a bearing attached to the shaft and to the opening of the bag. The system may further include a housing, such as a reactor housing, on the outside of the bag, wherein the housing includes at least one support that holds the bearing and a motor, and wherein the bag is contained within the housing. The housing may further include a plurality of baffles such that the bag folds around the baffles. Optionally, the system further encompasses a heater (e.g., a heating pad, a steam jacket, a circulating fluid or water heater, etc.) that can be located between the bag and the housing. Alternatively, the heater may be incorporated into the housing (e.g., a permanent reactor housing with incorporated heating system).

[0011] In another aspect of the invention, the stirred-tank reactor system includes a permanent housing with a product loop with flow past a pH sensor and a dissolved-oxygen sensor, wherein the sensors are incorporated into the housing. The permanent housing includes, but is not limited to, a metal barrel, a plastic barrel, a wood barrel, a glass barrel, and the like.

[0012] The invention also contemplates a method for preparing a stirred-tank reactor system, comprising providing a flexible bag with at least one opening, wherein the bag functions as a sterile container for a fluidic medium; inserting a shaft with an impeller attachable to the shaft into the bag, wherein the impeller is used to agitate the fluidic medium to provide a hydrodynamic environment; attaching a bearing to the shaft and to the opening of the bag; and sealing the bag to the shaft and the bearing such that the inside of the bag remains sterile. The stirred-tank reactor system prepared by this method includes at least one disposable element including, but not limited to, the bag, the shaft, the impeller, and the bearing.

[0013] The invention further encompasses a kit comprising a stirred-tank reactor system and instructions for use. The kit includes a disposable stirred-tank reactor system. The kit may also include a stirred-tank reactor system with at least one disposable element such as the bag, the shaft, the impeller, or the bearing. The bag may be affixed to the shaft and the bearing through at least one seal or o-ring such that the inside of the bag remains sterile. Furthermore, the bag may include a pH sensor and a dissolved-oxygen sensor, wherein the sensors are incorporated into the bag. The kit may also include at least one internal pouch sealed to the bag, wherein the pouch includes one end that can be opened to the outside of the bag such that a probe can be inserted into the reactor. In addition, the system may include at least one port in the bag allowing for the connection of a device to the port, wherein the device includes, but is not limited to, a tube, a filter, a sampler, and the like.

[0014] Another aspect of the invention provides a bag for use in a stirred-tank reactor system. The bag may be a disposable, flexible, plastic bag. The bag may also include at least one disposable element including, but not limited to, a seal, an o-ring, a port, a pouch, a tube, a filter, a sampler, a probe, a sensor, a connection device, or the like.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The present invention is best understood when read in conjunction with the accompanying figures which serve to illustrate the preferred embodiments. It is understood, however, that the invention is not limited to the specific embodiments disclosed in the figures.

[0016] **FIG. 1** depicts a longitudinal cross-section of one embodiment of the stirred-tank reactor system, wherein the stirred-tank reactor system is placed into a permanent housing.

[0017] **FIG. 2** depicts a probe connection in order to illustrate that a probe can be attached to the stirred-tank reactor system via a sterile or aseptic connection.

DETAILED DESCRIPTION OF THE INVENTION

[0018] a) Definitions and General Parameters

[0019] The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein.

[0020] The term "flexible bag" refers to a container that holds a fluidic medium. The bag may include one or more layer(s) of flexible or semi-flexible waterproof material depending on size, strength and volume requirements. The inside surface of the bag is preferably smooth and provides a sterile environment (e.g., for culturing cells or other organism, for food production, etc.). The bag may include one or more openings, pouches (e.g., for inserting one or more probes, devices, etc.), ports (e.g., for the connection of one or more probes, devices, etc.) or the like. Furthermore, the bag provides a disposable alternative to a solid vessel in a conventional stirred-tank bioreactor. The flexible bag may further include a shaft, an impeller, a bearing and seals or o-rings, and may be entirely disposable.

[**0021**] The term "fluidic medium" means, for the purpose of the specification and claims, any biological fluid, cell culture medium, tissue culture medium, culture of microorganisms, culture of plant metabolites, food production, chemical production, biopharmaceutical production, and the like. The fluidic medium is not limited to any particular consistency and its viscosity may vary from high to medium to low. When the fluidic medium is a cell culture medium the system may be operated in batch mode, semi-batch mode, fed-batch mode, or continuous mode.

[**0022**] The term "impeller" refers to a device that is used for agitating or mixing the contents of a stirred-tank reactor system (e.g., bioreactor). The impeller may agitate the fluidic medium by stirring or other mechanical motion. The impeller of the instant invention includes, but is not limited to, a Rushton, a marine, a hydrofoil, a pitched blade, and any other commercially available impeller.

[**0023**] A "hydrodynamic" environment of the instant invention refers to an environment that is influenced by the motion of fluids and the forces acting on solid bodies immersed in these fluids within the stirred-tank reactor system.

[**0024**] b) The Stirred-Tank Reactor System

[**0025**] The stirred-tank reactor system of the present invention provides a flexible and disposable bag for a variety of purposes, including culturing cells, microorganisms, or plant metabolites as well as processing foods, chemicals, biopharmaceutical and biologicals. The disposable bag may include disposable elements such as a shaft, impeller and bearing and is designed to fit into a permanent housing such as a reactor housing. The bag may further include one or more openings, pouches, ports or the like. The stirred-tank reactor system allows a user to operate the culture or production with relative ease and little training. In particular, the disposable system does not require cleaning or sterilizing. Furthermore, the system does not need continuous validation between production runs. Thus, it combines a flexible, easy-to-use, true stirred-tank reactor environment with zero cross-contamination during the production process.

[**0026**] Referring to the drawings, **FIG. 1** depicts a flexible bag **(4)** with at least one opening and an agitation shaft **(12)** with an attachable impeller **(13)**. As shown, the agitation shaft **(12)** and attached impeller **(13)** are situated within the bag **(4)**. Further, the agitation shaft **(12)** is connectable to a bearing **(5)**, wherein the bearing can be sealed to the bag through seal(s) or o-ring(s) **(6)**. The bag **(4)**, agitation shaft **(12)**, impeller **(13)**, and bearing **(5)**, including seals or o-rings **(6)** are optionally disposable. The disposable bag can be a flexible, plastic bag. The bag **(4)** can be affixed to the agitation shaft **(12)** and the bearing **(5)** through at least one seal or o-ring **(6)** such that the inside of the bag remains sterile. The seals or o-rings can be further affixed to the bag as is shown in **FIG. 1**. Additionally, the disposable stirred-tank reactor system may be connected to a support or one or more bracket(s) **(3)** that hold the bearing **(5)** and motor **(1)**. In one embodiment (as shown in **FIG. 1**), the support **(3)** is a motor and bearing support **(3)**, wherein the upper end of the agitation shaft **(12)** is further connected to a motor coupling **(2)**. The motor coupling **(2)** is connected to the motor **(1)** which drives the stirring motion of the agitation shaft **(12)** and impeller **(13)** leading to a hydrodynamic environment within the bag **(4)**. The bag **(4)** is designed to fit into a housing **(11)** such as a barrel or chamber. The housing may be a metal barrel, a plastic barrel, a wood barrel, a glass barrel, or any other barrel or chamber made from a solid material. In one embodiment of the instant invention, the housing further includes a plurality of baffles, wherein the bag folds around the baffles. In another embodiment, the flexible bag **(4)** further includes a top port (single or multiple) **(8)**, a bottom port (single or multiple) **(9)**, and a side port (single or multiple) **(10)**, wherein flexible tubing **(7)** can be connected to one or more of these ports.

[**0027**] The stirred-tank reactor system may optionally include a heater such as a heating pad, a steam jacket, or a circulating fluid or water heater. In one embodiment, the heater is located between the bag **(4)** and the housing **(11)**. In another embodiment, the heater is incorporated into the housing **(11)** (e.g., into a double wall between the reactor housing and the bag). In yet another embodiment, the stirred-tank reactor system is placed inside an incubator. The heater allows for heating or warming of a specific culture or production. This is particularly important for cell cultures which are often grown at 37° C.

[**0028**] In one embodiment of the instant invention, the bag **(4)**, the bearing **(5)**, the seal(s) or o-ring(s) **(6)**, the tubing **(7)**, the top port(s) **(8)**, the bottom port(s) **(9)**, the side port(s) **(10)**, the shaft **(12)**, and the impeller **(13)** are disposable. The motor **(1)**, the motor coupling **(2)**, the bracket(s) or motor and bearing support **(3)**, and the housing **(11)** are permanent.

[**0029**] c) Devices and Ports

[**0030**] The stirred-tank reactor system may also include sensors and other devices. In one embodiment, the bag includes a pH sensor and a dissolved-oxygen sensor, wherein the sensors are incorporated into the bag. As such, the sensors are disposable with the bag. In another embodiment, the sensors are attachable to the bag and are separate units. Such sensors may optionally be reusable after sterilization. In another embodiment, the system includes a product loop with flow past a pH sensor and dissolved-oxygen sensor, wherein the sensors are incorporated into the reactor housing. The system is flexible and provides alternative ways of supplying optional equipment of various kinds (e.g., sensors, probes, devices, pouches, ports, etc.). The system may also include one or more internal pouches that are sealed to the bag. In one preferred embodiment, the pouch has at least one end that can be opened to the outside of the bag to insert a probe into the reactor (i.e., the bag) while remaining on the exterior of the bag. The probe may be, for example, a temperature probe, a pH probe, a dissolved gas sensor, an oxygen sensor, a carbon dioxide sensor, a cell mass sensor, a nutrient sensor, an osmometer or any other probe that allows for testing or checking the culture or production. In another preferred embodiment, the system includes at least one port in the bag allowing for the connection of a device to the port. Such a device includes, but is not limited to, a tube, a filter, a connector, a probe, and a sampler. The incorporation of various ports into the bag allows for gas flow in and out of the bag as well as liquid flow in and out of the bag. Such ports also allow for sampling or testing the media or culture inside the bag. Tubing, filters, connectors, probes, samplers or other devices can be connected to the ports by using any desirable tubing connection technology. Pouches and ports that are sealed or affixed to the bag are disposable with the bag. The bag may also include a sparger (i.e., the component of a reactor that sprays air into the medium) sealed to the bag which can be disposed off with the bag.

[0031] Particularly, ports may be incorporated at any place on the flexible bag to accommodate the following:

[0032] Headspace gas in

[0033] Headspace gas out

[0034] Sparge gas in

[0035] Temperature probe

[0036] pH probe

[0037] Dissolved oxygen probe

[0038] Other desired probes

[0039] Sample apparatus

[0040] Media in

[0041] Titrant in

[0042] Inoculum in

[0043] Nutrient feeds in

[0044] Harvest out

[0045] Each port may have flexible tubing attached to the port, to which media bags, sample devices, filters, gas lines, or harvest pumps may be attached with sterile or aseptic connections. In one embodiment, the ports are sealed onto the flexible bag during bag manufacture, and are sterilized with the bag assembly.

[0046] Devices that may be used to make aseptic connections to the flexible tubing are the following:

[0047] WAVE sterile tube fuser

[0048] TERUMO sterile tubing welder

[0049] PALL KLEENPAK connector

[0050] Connection made under a laminar flow hood, using aseptic techniques

[0051] BAXTER Hayward proprietary "HEAT-TO-HEAT" connection using metal tubing and an induction heater

[0052] In another embodiment, flexible tubing that is attached to an appropriate stainless-steel valve assembly may be sterilized separately (e.g., via autoclave), and then used as a way to connect the disposable bioreactor to traditional reactors or process piping. The valve assembly is used to make a traditional steam-in-place (SIP) connection to a traditional reactor or other process, and the flexible tubing is used to make a sterile or aseptic connection to a port on the disposable reactor.

[0053] Referring to the drawings, **FIG. 2** depicts a probe connection that can be employed with the stirred-tank reactor system of the instant invention. In one embodiment (as shown in **FIG. 2**), the probe **(1)** is connected to a flexible sleeve **(2)** or bag which extends to one half of a PALL connector **(3)**. The PALL connector **(3)** can be connected to the other half of the PALL connector **(5)** to provide for a sterile connection between the probe and the stirred-tank reactor system. The PALL connectors **(3)**, **(5)** include covers **(4)** and filters **(7)** to keep the connection site sterile. Sterile tubing **(6)** extends from the other half of the PALL connector **(5)** to a reactor port **(8)** of the reactor vessel **(9)** of the stirred-tank reactor system. In order to attach the probe, the PALL connection is made by removing the covers **(4)**, mating the connectors **(3, 5)**, removing the filters **(7)**, and sliding the movable part of the connector into position. The probe sensor tip **(12)** is then pushed into the reactor as the flexible sleeve or bag bunches or compresses **(10)**. The probe senor tip **(12)** is then in direct contact with the inside of the reactor vessel **(9)**. A clamp **(11)** is placed around the probe and tubing to seal the reactor contents from the PALL connection assembly. Thus, when a sterile connection is made between the two halves of the PALL connectors **(3, 5)**, the flexible sleeve **(2)** or bag becomes compressed **(10)** and the probe is in contact with the culture or production media.

[0054] In one embodiment, the probes may be sterilized separately (e.g., via autoclave) then attached to the reactor via a sterile or aseptic connection. For example, a probe assembly may be made by inserting a probe **(1)** into one half of a PALL KLEENPAK connector **(3)** and sealing the probe to the connector using a flexible sleeve or bag **(2)** as described above and shown in **FIG. 2**. The sleeve extends from the outside end of the probe to the barb of the PALL connector. This assembly is sterilized separately. The other half of the PALL connector **(5)** is connected to a port **(8)** on the reactor **(9)** via flexible tubing **(6)** that will accommodate the probe. This assembly is sterilized as part of the reactor. The PALL connector is described in detail in U.S. Pat. No. 6,655,655 and incorporated herein by reference in its entirety.

[0055] d) Cultures

[0056] The stirred-tank reactor system is designed to hold a fluidic medium such as a biological fluid, a cell culture medium, a culture of microorganisms, a food production, or the like. When the fluidic medium is a cell culture the system can be operated in batch-mode, semi-batch mode, fed-batch mode, or continuous mode. A batch culture is a large scale cell culture in which a cell inoculum is cultured to a maximum density in a tank or fermenter, and harvested and processed as a batch. A fed-batch culture is a batch culture which is supplied with either fresh nutrients (e.g., growth-limiting substrates) or additives (e.g., precursors to products). A continuous culture is a suspension culture that is continuously supplied with nutrients by the inflow of fresh medium, wherein the culture volume is usually constant. Similarly, continuous fermentation refers to a process in which cells or micro-organisms are maintained in culture in the exponential growth phase by the continuous addition of fresh medium that is exactly balanced by the removal of cell suspension from the bioreactor. Furthermore, the stirred-tank reactor system can be used for suspension, perfusion or microcarrier cultures. Generally, the stirred-tank reactor system can be operated as any conventional stirred-tank reactor with any type of agitator such as a Rushton, hydrofoil, pitched blade, or marine. The agitation shaft **(12)** can be mounted at any angle or position relative to the housing **(11)**, such as upright centered, upright offset, or 15° offset. The control of the stirred-tank reactor system can be by conventional means without the need for steam-in-place (SIP) or clean-in-place (CIP) control. In fact, the system of the instant invention is not limited to sterile bioreactor operation, but can be used in any operation in which a clean product is to be mixed using a stirred tank, for example, food production or any clean-room mixing without the need for a clean-room.

[0057] e) The Kit

[0058] The invention encompasses a kit that includes a stirred-tank reactor system and instructions for use. In a preferred embodiment, the kit includes a disposable stirred-tank reactor system. Accordingly, the kit includes at least one disposable element such as the bag, the shaft, the impeller, or the bearing. Preferably, the kit is entirely disposable. The flexible, disposable bag may be affixed to the shaft and the bearing through at least one seal or o-ring such that the inside of the bag remains sterile. In addition, the bag may include a pH sensor and a dissolved-oxygen sensor, wherein the sensors are incorporated into the bag and are disposable with the bag. The kit may also include one or more internal pouches that are sealed to the bag. The pouch has one end that can be opened to the outside of the bag such that a probe can be inserted into the reactor. The probe may be a temperature probe, a pH probe, a dissolved gas sensor, an oxygen sensor, a carbon dioxide (CO_2) sensor, a cell mass sensor, a nutrient sensor, an osmometer, and the like. Furthermore, the system may include at least one port in the bag allowing for the connection of a device to the port, wherein the device includes, but is not limited to, a tube, a filter, a sampler, a probe, a connector, and the like. The port allows for sampling, titration, adding of chemostat reagents, sparging, and the like. The advantage of this kit is that it is optionally entirely disposable and easy-to-use by following the attached instructions. This kit comes in different sizes depending on the preferred culture volume and can be employed with any desired reaction chamber or barrel. This kit is pre-sterilized and requires no validation or cleaning. The kit can be used for cell culture, culture of microorganisms, culture of plant metabolites, food production, chemical production, biopharmaceutical production, and others.

[0059] In another embodiment the kit includes a housing or barrel that holds the disposable bag. Such a housing or barrel can be supplied with the bag or provided separately.

[0060] f) Examples

[0061] The following specific examples are intended to illustrate the invention and should not be construed as limiting the scope of the claims.

[0062] (i) A Disposable Bioreactor

[0063] One example of a stirred-tank reactor system of the instant invention is a disposable bioreactor. The bioreactor is similar to a 600 liter media bag with built-in agitation and attachable sensors (e.g., pH sensors, temperature sensors, dissolved oxygen (dO2) sensors, etc.). The reactor is operated via conventional controllers. The agitator (e.g., agitation shaft and impeller) and bearing are disposable and built into the bag. The motor attaches to a support (e.g., motor and bearing support) or bracket(s) on the 600 liter barrel that holds the bag. In size, shape, and operation, this bioreactor appears similar to a stainless steel reactor with a sterile liner, however, the bioreactor of this invention provides a multitude of advantages compared to a conventional stainless steel reactor. Most importantly, the need for cleaning and steam sterilization is eliminated. The bag is pre-sterilized by irradiation and, thus, ready for use. In fact, no cleaning, sterilization, validation or testing is required at culture start-up or between culture runs. Consequently, the bioreactor provides a culture environment of zero cross-contamination between runs. In conventional systems, the majority of costs are related to clean-in-progress (CIP) and steam-in-progress (SIP) as well as the design of a skid and control system to oversee these functions. These costs are eliminated in the disposable bioreactor and multiple products may be cultured or manufactured simultaneously and with much greater ease.

[0064] The disposable bioreactor can be easily scaled-up by using larger culture bags and larger barrels to hold the bags. Multiple bioreactors can be operated at the same time without any need for extensive engineering or cleaning. The bioreactor is a true stirred tank with well characterized mixing. As such, the bioreactor has the added advantage that it can be scaled and its contents transferred to a stainless steel reactor if desired. Notably, the bioreactor combines ease of use with low cost and flexibility and provides, thus, a new technical platform for cell culture.

[0065] (ii) Cell Culture

[0066] The disposable bioreactor of the instant invention can be used for a batch culture in which cells are inoculated into fresh media. As the cells grow, they consume the nutrients in the media and waste products accumulate. For a secreted product, when the culture has run its course, cells are separated from the product by a filtration or centrifugation step. For viral-vector production, cells are infected with a virus during the growth phase of the culture, allowing expression of the vector followed by harvest. Since there is zero cross-contamination in the bioreactor it works well with batch cultures.

[0067] The bioreactor can also be used for perfusion cultures, wherein product and/or waste media is continuously removed and the volume removed is replaced with fresh media. The constant addition of fresh media, while eliminating waste products, provides the cells with the nutrients they require to achieve higher cell concentrations. Unlike the constantly changing conditions of a batch culture, the perfusion method offers the means to achieve and maintain a culture in a state of equilibrium in which cell concentration and productivity may be maintained in a steady-state condition. This can be accomplished in the disposable bag as easily as in any conventional stainless steel reactor. For viral-vector production, the perfusion process allows for an increase in the cell concentration and, thereby the post-infection virus titer. For a secreted product, perfusion in the bioreactor offers the user the opportunity to increase the productivity by simply increasing the size of the culture bag. Most importantly, there is no need for sterilization, validation, or cleaning because the system experiences zero cross-contamination during the production process.

[0068] Various modifications and variations of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the art are intended to be within the scope of the claims.

What is claimed is:

1. A stirred-tank reactor system, comprising:

(i) a flexible bag with at least one opening, wherein the bag functions as a sterile container for a fluidic medium;

(ii) a shaft situated within the bag;

(iii) an impeller attachable to said shaft, wherein said impeller is used to agitate the fluidic medium to provide a hydrodynamic environment; and

(iv) a bearing attached to said shaft and to said opening of the bag.

2. The stirred-tank system of claim 1, wherein said system is disposable.

3. The stirred-tank system of claim 1, wherein said system is pre-sterilized.

4. The stirred-tank reactor system of claim 1, wherein said system further comprises a housing on the outside of the bag, wherein said housing includes at least one support that holds the bearing and a motor, and wherein said bag is contained within the housing.

5. The stirred-tank reactor system of claim 4, wherein said system further includes a heater selected from the group consisting of a heating pad, a steam jacket, and a circulating fluid or water heater.

6. The stirred-tank reactor system of claim 5, wherein said heater is located between the bag and the housing.

7. The stirred-tank reactor system of claim 5, wherein said heater is incorporated into the housing.

8. The stirred-tank reactor system of claim 4, wherein the housing further includes a plurality of baffles and the bag folds around the baffles.

9. The stirred-tank reactor system of claim 4, wherein said system further includes a product loop with flow past a pH sensor and a dissolved-oxygen sensor, wherein said sensors are incorporated into the housing.

10. The stirred-tank reactor system of claim 4, wherein said housing is selected from the group consisting of a metal barrel, a plastic barrel, wood barrel, and a glass barrel.

11. The stirred-tank reactor system of claim 1, wherein said bag is a disposable, plastic bag.

12. The stirred-tank reactor system of claim 1, wherein said fluidic medium is selected from the group consisting of a biological fluid, a cell culture medium, a culture of microorganisms, and a food production.

13. The stirred-tank reactor system of claim 12, wherein the fluidic medium is a cell culture medium and the system is operated in batch mode, semi-batch mode, fed-batch mode, or continuous mode.

14. The stirred-tank reactor system of claim 1, wherein said impeller agitates the fluidic medium by stirring.

15. The stirred-tank reactor system of claim 14, wherein said impeller is selected from the group consisting of Rushton, hydrofoil, pitched blade, and marine.

16. The stirred-tank reactor system of claim 1, wherein said bag is affixed to the shaft and the bearing through at least one seal or o-ring such that the inside of the bag remains sterile.

17. The stirred-tank reactor system of claim 16, wherein said seals or o-rings are affixed to the bag.

18. The stirred-tank reactor system of claim 16, wherein said bag further includes a pH sensor and a dissolved-oxygen sensor, wherein said sensors are incorporated into the bag.

19. The stirred-tank reactor system of claim 16, wherein said system further includes at least one internal pouch sealed to the bag, wherein the pouch includes one end that can be opened to the outside of the bag such that a probe can be inserted into the reactor.

20. The stirred-tank reactor system of claim 19, wherein said probe is selected from the group consisting of a temperature probe, a pH probe, a dissolved gas sensor, an oxygen sensor, a carbon dioxide sensor, a cell mass sensor, a nutrient sensor, and an osmometer.

21. The stirred-tank reactor system of claim 16, wherein said system further includes a least one port in the bag allowing for the connection of a device to the port, wherein said device is selected from the group consisting of a tube, a filter, a sampler, a probe and a connector.

22. The stirred-tank reactor system of claim 21, wherein the port allows for gas flow in and out of the bag.

23. The stirred-tank reactor system of claim 21, wherein the port allows for media flow in and out of the bag.

24. The stirred-tank reactor system of claim 21, wherein the port allows for sampling, inoculation, titration, adding of chemostat reagents, and sparging.

25. A method for preparing a stirred-tank reactor system, comprising:

(i) providing a flexible bag with at least one opening, wherein the bag functions as a sterile container for a fluidic medium;

(ii) inserting a shaft with an impeller attachable to said shaft into the bag, wherein said impeller is used to agitate the fluidic medium to provide a hydrodynamic environment;

(iii) attaching a bearing to said shaft and to said opening of the bag; and

(iv) sealing the bag to the shaft and the bearing such that the inside of the bag remains sterile.

26. The method of claim 25, wherein said stirred-tank reactor system is pre-sterilized.

27. The method of claim 25, wherein said stirred-tank reactor system is disposable.

28. The method of claim 25, wherein said stirred-tank reactor system includes at least one disposable element selected from the group consisting of the bag, the shaft, the impeller, and the bearing.

29. A kit comprising:

(i) the stirred-tank reactor system of claim 16; and

(ii) instructions for use.

30. The kit of claim 29, wherein said stirred-tank reactor system is disposable.

31. The kit of claim 29, wherein said stirred-tank reactor system includes at least one disposable element selected from the group consisting of the bag, the shaft, the impeller, and the bearing.

32. The kit of claim 29, wherein said bag is affixed to the shaft and the bearing through at least one seal or o-ring such that the inside of the bag remains sterile.

33. The kit of claim 29, wherein said bag further includes a pH sensor and a dissolved-oxygen sensor, wherein said sensors are incorporated into the bag.

34. The kit of claim 29, wherein said system further includes at least one internal pouch sealed to the bag, wherein the pouch includes one end that can be opened to the outside of the bag such that a probe can be inserted into the reactor.

35. The kit of claim 34, wherein said probe is selected from the group consisting of a temperature probe, a pH probe, a dissolved gas sensor, an oxygen sensor, a carbon dioxide sensor, a cell mass sensor, a nutrient sensor and an osmometer.

36. The kit of claim 29, wherein said system further includes a least one port in the bag allowing for the connection of a device to the port, wherein said device is selected from the group consisting of a tube, a filter, a sampler, a probe and a connector.

37. The kit of claim 36, wherein the port allows for gas flow in and out of the bag.

38. The kit of claim 36, wherein the port allows for media flow in and out of the bag.

39. The kit of claim 36, wherein the port allows for sampling, inoculation, titration, adding of chemostat reagents, and sparging.

40. A bag for use in the stirred-tank reactor system of claim 1.

41. The bag of claim 40, wherein said bag is a disposable plastic bag.

42. The bag of claim 40, wherein said bag further includes at least one disposable element selected from the group consisting of a seal, an o-ring, a port, a pouch, a tube, a filter, a sampler, a probe, a sensor and a connector.

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