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DESCRIPTION

Field of the Invention

[0001] The present invention relates to stirred tank containers, and related methods.

Background of the Invention

[0002] The general process for the manufacture of biomolecules, such as proteins, particularly recombinant proteins, typically involves two main steps: (1) the expression of the protein in a host cell, followed by (2) the purification of the protein. The first step involves growing the desired host cell in a bioreactor to effect the expression of the protein. Some examples of cell lines used for this purpose include Chinese hamster ovary (CHO) cells, myeloma (NSO) bacterial cells such as e-coli and insect cells. Once the protein is expressed at the desired levels, the protein is removed from the host cell and harvested. Suspended particulates, such as cells, cell fragments, lipids and other insoluble matter are typically removed from the protein-containing fluid by filtration or centrifugation, resulting in a clarified fluid containing the protein of interest in solution as well as other soluble impurities.

[0003] The second step involves the purification of the harvested protein to remove impurities which are inherent to the process. Examples of impurities include host cell proteins (HCP, proteins other than the desired or targeted protein), nucleic acids, endotoxins, viruses, protein variants and protein aggregates. This purification typically involves several chromatography steps, which can include affinity chromatography, ion exchange, hydrophobic interaction, etc. on solid matrices such as porous agarose, polymeric or glass or by membrane based adsorbers.

[0004] One example of a chromatography process train for the purification of proteins involves protein-A affinity, followed by cation exchange, followed by anion exchange. The protein-A column captures the protein of interest or target protein by an affinity mechanism while the bulk of the impurities pass through the column to be discarded. The protein then is recovered by elution from the column. Since most of the proteins of interest have isoelectric points (PI) in the basic range (8-9) and therefore being positively charged under normal processing conditions (pH below the PI of the protein), they are bound to the cation exchange resin in the second column. Other positively charged impurities are also bound to this resin. The protein of interest is then recovered by elution from this column under conditions (pH, salt concentration) in which the protein elutes while the impurities remain bound to the resin. The anion exchange column is typically operated in a flow through mode, such that any negatively charged impurities are bound to the resin while the positively charged protein of interest is recovered in the flow through stream. This process results in a highly purified and concentrated protein solution.

[0005] Other alternative methods for purifying proteins have been investigated in recent years. One such method involves a flocculation technique. In this technique, a soluble polyelectrolyte is added to an unclarified cell culture broth to capture the suspended particulates and a portion of the soluble impurities thereby forming a flocculant, which is subsequently removed from the protein solution by filtration or centrifugation.

[0006] Alternatively, a soluble polyelectrolyte is added to clarified cell culture broth to capture the biomolecules of interest, thereby forming a flocculant, which is allowed to settle and can be subsequently isolated from the rest of the solution. The flocculant is typically washed to remove loosely adhering impurities. Afterwards, an increase in the solution's ionic strength brings about the dissociation of the target protein from the polyelectrolyte, subsequently resulting in the resolubilization of the polyelectrolyte into the protein-containing solution.

[0007] In co-pending application U.S. Serial No. 12/004,314 filed December 20, 2007, a polymer, soluble under certain conditions, such as temperature, pH, salt, light or combinations thereof, is used to bind impurities while in its soluble state and is then precipitated out upon a change in condition (pH or temperature, etc.) removing the impurities with it. The biomolecule of interest is then further treated using traditional chromatography or membrane adsorbers and the like.

[0008] All of the protein purification technologies discussed above share a common theme, namely, to first remove suspended particulates in a first distinct step and then in a second step separate the biomolecules of interest from soluble impurities which are inherent to the process.

[0009] In situ product recovery with derivatized magnetic particles is one example of a protein purification technique where the biomolecules of interest can be purified directly from an un-clarified cell culture broth. In this technique, a polymer shell encapsulating a magnetic bead is functionalized with an affinity ligand that seeks out and binds the target protein. A magnetic field is then applied to collect the bead-protein complexes, leaving behind the soluble impurities and insoluble particulates.

[0010] The main drawback of this technique is that it requires appreciable capital investments in design, construction and validation of high-gradient magnetic separators. Also, the technique does not lend itself to disposable applications, which are poised to become the norm for protein purification in the Bioprocess industry.

[0011] In co-pending application filed December 16, 2008 under Attorney Docket No. MCA-1046, entitled "Purification of Proteins" by Moya, Wilson, et al., there is disclosed a polymer such as a soluble polymer capable of substantially irreversibly binding to insoluble particulates and a subset of soluble impurities and also capable of reversibly binding to one or more desired biomolecules in an unclarified biological material containing stream and the methods of using such a material to purify one or more desired biomolecules from such a stream without the need for prior clarification. More specifically, this co-pending application discloses a stimuli responsive polymer such as a selectively soluble polymer capable of selectively and reversibly binding to one or more desired biomolecules in an unclarified biological material containing stream and the methods of using such a polymer to purify one or more desired biomolecules from such a complex mixture of materials including the biomolecule(s) of interest and various impurities such as other proteins (host cell proteins), DNA, virus, whole cells, cellular debris and the like without the need for prior clarification of the stream.

[0012] The polymer is soluble under a certain set of process conditions such as one or more of pH, salt concentration, temperature, light, or electrical field, and is able to interact and complex with insoluble impurities (cells, debris, etc.) and a fraction of the soluble impurities, and is rendered insoluble and precipitates out of solution upon a change in conditions (temperature, salt concentration, light, electrical field, or pH), e.g. a stimuli responsive polymer. Only when precipitated out of solution, the polymer is capable of reversibly binding to one or more desired biomolecules within the stream (protein, polypeptide, etc.) in an unclarified cell broth. The precipitate can then be removed from the stream, such as by being filtered out from the remainder of the stream and the desired biomolecule is recovered such as by selective elution from the precipitate.

[0013] The removal of the precipitate, however, can be problematic, as it is typically in the form a large mass of sludge.

[0014] WO 96/37600 A1 describes a filtering device for processing and culturing a sample, wherein said device comprises a single filtration membrane.

[0015] US 2008/255027 A1 describes a process for purifying a biomolecule by precipitating the biomolecule with a polymer and subsequently recovering the biomolecule using a filtration step. It would be desirable to provide an apparatus and method for the efficient purification of samples, particularly those containing biomolecules, preferably within a single, integral, apparatus that reduces or eliminates one or more process steps that can result in contamination or material loss.

Summary of the Invention

[0016] In one aspect, the invention provides an assembly for culturing or processing a fluid sample comprising a first container (22) having an interior space, a first base (100) sealingly affixed to said first container (22) and at least one membrane (110) sealed to said base (100) for filtering said fluid sample; an outlet (32b) in said base (100) for the filtered fluid sample, a second container (22') in fluid communication with the outlet (32b) of said first base (100), wherein said second container (22') is sealingly affixed to a second base (100'), said second base (100') comprising a membrane (110') and a supporting surface comprising grooves for fluid flow. In certain embodiments, said second container (22') supports at least one membrane (110'). In certain embodiments, said first container (22) is a bioreactor. In certain embodiments, the assembly further comprises an agitator within the body of said first container (22) to agitate said sample. In certain embodiments, said first base (100) comprises a support surface (101) formed with grooves (102) for the flow of fluid, said grooves (102) being in fluid communication with an aperture (103) in said base, said aperture being in fluid communication with a port (32b) for draining fluid from the base (100). In certain embodiments, said at least one membrane (110) sealed to said first base (100) is a coarse membrane, and wherein said membrane (110') of said second base (100') is a 0.2 micron sterilizing grade membrane.

[0017] In another aspect, the invention provides a method for purifying a biomolecule selected from the group consisting of proteins and antibodies from a mixture containing impurities comprising:

1. (a) providing an assembly comprising providing a first container (22) having an interior space, a first base (100) adapted to be sealingly affixed to said first container (22) and supporting at least one membrane (110) sealed to said first base (100) for filtering said mixture; an outlet (32b) in said base (100), a second container (22') in fluid communication with the outlet (32b) of said first base (100), and a second base (100') sealingly affixed to said second container (22'), said second base (100') comprising a second membrane (110') and a supporting surface comprising grooves for fluid flow,
2. (b) providing the mixture at a set of conditions,
3. (c) adding one or more polymers, soluble in said mixture under the set of conditions and capable of reversibly and selectively binding to the biomolecule, wherein said one or more polymers are selected from the group consisting of poly(N-vinyl caprolactam), poly(N-acryloylpiperidine), poly(N-vinyl-isobutyramide), poly(N-isopropylacrylamide), poly (N,N'-diethylacrylamide), poly(N-acryloyl-N-alkylpiperazine), hydroxyalkylcellulose, copolymers of acrylic acid and methacrylic acid, and polymers and copolymers of 2- or 4-vinylpyridine and chitosan with either a ligand or functional group attached to it,
4. (d) mixing the one or more solubilised polymers throughout the mixture;
5. (e) precipitating the one or more polymers and bound biomolecule out of solution by changing the set of conditions in the mixture;
6. (f) washing said precipitate by contacting said precipitate with a wash solution and filtering the supernatant through said first membrane,
7. (g) recovering the bound biomolecule from the polymer and filtering the biomolecule through said second membrane.

[0018] In certain embodiments, said filtering of said supernatant and said filtering of said biomolecule are carried out in the same apparatus. In certain embodiments, the biomolecule is an antibody selected from the group consisting of a recombinant antibody, a recombinant monoclonal antibody, a polyclonal antibody, a humanized antibody and an antibody fragment. In certain embodiments, said biomolecule is a protein.

<u>Brief Description of the Drawings</u>

[0019]

FIG. 1 is a perspective view of a bioreactor as disclosed herein;

FIG. 2 is a cross-sectional view of a portion of the bioreactor of Figure 1;

FIG 3 is a perspective view of a bioreactor base as disclosed herein;

FIG. 4 is a perspective view of the base of FIG. 3, including a membrane sealed thereon;

FIG. 5 is a perspective view of a bioreactor assembly, including a housing, a bioreactor base, and a filtration base;

FIG. 6 is a perspective view of a filtration base in accordance with certain embodiments; and

FIG. 7 is a perspective view of an agitator in accordance with certain embodiments.

[0020] Only figures 6 and 7 correspond to embodiments and examples of the invention.

Detailed Description

[0021] Suitable containers or housings useful herein are not particularly limited. For purposes of illustration, reactors, and in particular, bioreactors, will be discussed in detail, which include disposable as well as reusable bioreactors. For example, solvent-resistance bioreactors having a borosilicate glass cylinder and PTFE components, such as those commercially available from Millipore Corporation, can be used. Similarly, disposable bioreactors that utilize bags, or that are formed of semi-rigid or rigid molded plastic, can be used. Such disposable bioreactors are generally pre-sterilized. Means for agitation within the bioreactor is also not particularly limited, and includes impeller-based agitation, magnetic stirrers, as well as wave-induced agitation and agitation induced by gas bubbles. Agitation is important in preventing solids from settling and plugging the one or more membranes used for purification.

[0022] The following description is in reference to a bioreactor. Those skilled in the art will appreciate that it is for illustrative purposes only, and that the embodiments disclosed herein are applicable to any container containing a liquid sample having, or ultimately forming, a sample having a relatively high solids content.

[0023] Turning now to FIGS. 1 and 2, bioreactor 2 is shown held in a stand 4, which is comprised of several legs 6 (in this example 3 legs although one continuous leg or 2 large legs or more than 3 legs can also be used) and a support rim 8. As shown the legs 6 may have an optional support piece 10 at or near the bottom to keep the legs 6 from spreading when the bioreactor 2 is filled and in the stand 4.

[0024] Depending upon the type of circulation or agitation system used, the stand 4 may also support the drive mechanism 12 (as shown) for the circulation mechanism, which typically is a stirrer or paddle assembly 14. In this particular example, the drive mechanism 12 is a motor and is mounted to the top of the centered above the top 16 of the bioreactor 2 by several arms 18 (although 3 are shown alternative numbers may be used). Other features such as mounting blocks (not shown) and the like may be formed on the top 16 or support rim 8 to support the drive mechanism 12. As shown, the drive mechanism 12 has a shaft 20 that can be attached to the stirrer as explained later herein. Other stands can be used in lieu of the design described above and will work equally well.

[0025] The bioreactor body 22 (only partially shown in FIG. 1) has an interior space into which the fluids, cells, probes and other devices of the bioreactor are at least partially contained. The body 22 is sealably attached to the top 16. This may be by a mechanical seal such as a rubber gasket and clips 24 (as shown) or by a clamp, such as a band clamp or Ladish or TriClover clamp, mated threads on the top 16 and body 22 and the like. Alternatively, they may be sealed by adhesives or heat sealing of the top 16 to the body 22 or formed together in one piece such as in a rotomolding apparatus.

[0026] The body 22 has one or more sidewalls 26 that extend downwardly from the top 16. As shown, there is one sidewall 26 of a circular or cylindrical design. Alternatively, there can be 3, 4, or more sidewalls if desired (not shown).

[0027] Preferably, the body 22 is made of a single piece of molded plastic or glass. Alternatively it may be made of two or more pieces of plastic or glass that are sealed together such as by heat, glue, or gaskets (not shown). Suitable polymers which can be used to form the top and body include but are not limited to polycarbonates, polyesters, nylons, PTFE resins and other fluoropolymers, acrylic and methacrylic resins and copolymers, polysulphones, polyethersulphones, polyarylsulphones, polystyrenes, polyetherimides, nylons, polyesters, polyethylene terephthalates (PET), polyvinyl chlorides, chlorinated polyvinyl chlorides, ABS and its alloys and blends, polyolefins, preferably polyethylenes such as linear low density polyethylene, low density polyethylene, high density polyethylene, and ultrahigh molecular weight polyethylene and copolymers thereof, polypropylene and copolymers thereof and metallocene generated polyolefins. Preferred polymers are polyolefins, in particular polyethylenes and their copolymers; polystyrenes; and polycarbonates. The top and body may be made of the same polymer or different polymers as desired. In reusable embodiments, the body can be made of glass, acrylic, or other materials not deleterious to the process. The body 22 also can be a disposable plastic bag, as is known in the art.

[0028] Also formed in the bioreactor 2 of this example are one or more ports 30 (in this example there are three types 30a-c (for a total of 5 ports) formed in the top 16 and one or more ports 32 in the body 22 (in this example there are at least two different types 32 a-b for a total of seven ports overall) . The top 16 and body 22 may have multiple ports of similar and/or of different styles to provide one with the number of ports, of the desired type, in the desired locations throughout the bioreactor 2. These ports 30, 32 or at least a portion of them are formed as part of the top 16 and/or body 22. They may be formed with threads that mate to sealable covers such as closed caps, gasketed caps with a throughbore within the gasket, or various Luer fittings. Alternatively, one or more of the ports can be made in the plastic top 16 and/or body 22 by drilling or burning a hole and then mounting (such as by heat bonding or adhesives) a port in place through or around the hole. Many different port styles and sizes can be accommodated.

[0029] Ports 30a may be used for liquid or gas entrance or exit or for probes such as pH probes, thermometers or thermocouples or the like. Ports 30b may be used for similar purposes. Port 30c is for the stirrer shaft described in further detail herein. Alternatively, if the bioreactor is an airlift design and doesn't use a stirrer rod, the port 30c may be used to house the airline to the sparger at or near the bottom of the body or for any other desired purpose. Ports 32a may be used for sampling of the liquid or for probes such as pH, temperature, dissolved oxygen, lactose level, etc. as are common on such bioreactors. Ports 32a while shown as being formed on the sidewall 26 may also be formed in the bottom if desired as shown in Figure 2. Port 32b is valved port which can be used to supply gas to the body 22 and/or as a drain or outlet from the body. It may serve both functions by attaching a 3 position valve or Y-shaped tube with valves such as pinch valves on each arm of the Y to control flow (not shown). One suitable system for the valve of port 32b is a LYNX® connector available from Millipore Corporation of Billerica, Massachusetts and as shown in US Patent Publication No. 2005/0016620.

[0030] Preferably, one or more ports 32 of the body are formed in a location that is below the normal liquid/gas interface level of the bioreactor.

[0031] If desired, one or more of the ports 32a or b in FIG. 1 may be used to provide gases to the body's interior. A plastic frit such as a POREX® porous material, a microporous membrane or ceramic stone or sintered metal filter may be attached to the inside of the port within the body to provide the sized gas bubbles desired. Alternatively, a port 30a in the top 16 may be used to hold a tube that extends down into the body to provide the gas supply. Again it may use a frit or ceramic stone or sintered metal filter or a membrane to provide the desired bubble size. Alternatively, gases can be provided to the interior of the body through the porous filter/membrane 110 within the stirred cell assembly and the supply of gas can be provided through port 32b.

[0032] FIG. 2 shows a bioreactor 2 with top 16 and body 22 sealed to each other and a suitable stirring mechanism 14 in place. The stirring mechanism shown is formed of a shaft 40 and one or more paddles, circular disk, impellers, vanes or the like 42. The shaft 40 extends through port 30c and is connected to the shaft 20 of the drive mechanism 12 (not shown). Preferably one or more o-rings in the port 30c allow for movement of the shaft 40 without compromising the integrity of the seal within the body 22. Alternatively, the "agitation" to avoid plugging can be effected by ultrasonic waves or vibration directed at the membrane or filter surface to prevent the solids from collecting on the surface. Another method to prevent plugging the filter/membrane is to cause the solids to float to the top of the liquid phase by introducing gas bubbles which adhere to the solids.

[0033] In accordance with certain examples, the bioreactor 22 is a cylindrical tube, and is removably and sealingly affixed to a base in order to provide a stirred cell assembly. For example, in the embodiment shown, shaft 40 is extended below paddle 42 via a short shaft portion 40', and an additional paddle or the like 42' is added (FIG. 7). The paddle 42' is preferably positioned just above the membrane 110 (discussed below) in the base in order to avoid contact with the membrane which could damage it. So positioned, it agitates the fluid just above the membrane and prevents solids (e.g., affinity beads, precipitate or floc) from settling on the membrane, which tend to clog or plug the pores of the membrane. Preferably the paddle is sufficiently wide such that it substantially corresponds to the width of the effective diameter of the membrane, or is slightly smaller than such width, in order to provide uniform fluid agitation over the effective filtration area of the membrane. In certain embodiments, the paddle 42' can be constructed of a suitable material, such as rubber or a sponge-like material, so that contact with the surface of the membrane during agitation does not damage the membrane, and is acceptable, in order to further ensure that solids do not settle on the membrane surface. Those skilled in the art will appreciate that suitable means other than a paddle, such as a circular disk or wave agitation, to sufficiently agitate the fluid in the interior space of the body 22, are within the scope of the embodiments disclosed herein.

[0034] Turning to FIG.3, a bioreactor base 100 is shown, which includes a supporting surface 101 formed with grooves 102 or the like for the flow of fluid. The configuration of grooves 102 is not particularly limited, although the preferred configuration is concentric circles as illustrated. The grooves 102 are in fluid communication with an aperture 103, which in turn is in fluid communication with port 32b, for draining fluid from the base 100.

[0035] The surface 101 of the base 100 supports one or more membranes 110 (FIG. 4). Preferably one of the one or more membranes is a relatively coarse filter or membrane, particularly when the solids content of the broth is high, such as about 20-35% solids by volume. Use of a coarse filter or membrane as an initial filtration step helps protect and prolong the service life of subsequent downstream filtration through tighter, generally more expensive membranes, such as a 0.2 micron sterilizing grade membrane (discussed in greater detail below). Suitable membranes include, but are not limited to, polymers such as but not limited to olefins such as polyethylene including ultrahigh molecular weight polyethylene, polypropylene, EVA copolymers and alpha olefins, metallocene olefinic polymers, PFA, MFA, PTFE, polycarbonates, vinyl copolymers such as PVC, polyamides such as nylon, polyesters, cellulose, cellulose acetate, regenerated cellulose, cellulose composites, polysulfone, polyethersulfone, polyarylsulfone, polyphenylsulfone, polyacrylonitrile, polyvinylidene fluoride (PVDF), and blends thereof. The membrane selected depends upon the application, desired filtration characteristics, particle type and size to be filtered and the flow desired. Preferred membrane based filters include DURAPORE® PVDF membranes available from Millipore Corporation of Billerica Massachusetts, MILLIPORE EXPRESS® and MILLIPORE EXPRESS® PLUS or SH PES membranes available from Millipore Corporation of Billerica Massachusetts. Prefilters, depth filters and the like can also be used in these embodiments such as Polygard®

prefilters (Polygard CE prefilters) and depth filters (Polygard CR depth filters) available from Millipore Corporation of Billerica Massachusetts.

[0036] Depending on the mixture, polymer and the nature of biomolecule, the filter may be hydrophilic or hydrophobic. Preferred filters are hydrophilic and are low in protein binding.

[0037] The filter, be it membrane or otherwise, may be symmetric in pore size throughout its depth such as DURAPORE® PVDF membranes available from Millipore Corporation of Billerica Massachusetts, or it may be asymmetric in pore size through its thickness as with MILLIPORE EXPRESS® and MILLIPORE EXPRESS® PLUS or SH PES membranes available from Millipore Corporation of Billerica Massachusetts. It may contain a prefilter layer if desired, either as a separate upstream layer or as an integral upstream portion of the membrane itself.

[0038] Depending on the size of the particles generated, there may be instances in which the membrane is an ultrafiltration membrane. For example, in cases in which the particle size is small compared to the pore size of a microporous membrane, then a membrane with smaller pores (in the UF range) would be more appropriate to avoid plugging. Suitable ultrafiltration membranes include regenerated cellulose and polyethersulfone membranes, including those with a pore size larger than 0.2 microns, e.g., generally those with pore sizes of 0.45, 0.65, 1.0, 2.0 microns or larger. Optionally a porous support (not shown) can be placed between the surface 101 of the base and the membrane(s) 110. The membrane(s) (and support if present) are sealed against the base such as with an O-ring 106, which in turn can be held in place by a support ring 107, such as an acrylic ring. Where more than one membrane 110 is used, they can be assembled in a stacked relationship. Where more than one membrane is used, each membrane need not be of the same performance characteristics (e.g, pore size, flux, capacity, surface chemistry, etc). For example, the upper membrane against the paddle 42' may be of a larger pore size than the lower membrane(s) and/or it may be of a different material than the lower membrane(s).

[0039] The bioreactor body 22, such as a cylindrical tube, is placed in sealing relationship with the base 100, as shown in FIG. 5. A plurality of legs 6' can be provided, which extend downwardly from the base 100 to support the same.

[0040] In certain embodiments, where additional purification is desired, a further filter base can be added to the assembly, as shown in FIGS. 5 and 6. Thus, a base 100', similar to base 100, is provided, again with a supporting surface having suitable grooves, and one or more membranes sealingly supported thereon, such as with a suitable O-ring and support ring. For example, a sterilizing membrane, such as a 0.2 micron membrane, can be used (optionally along with a suitable porous support). Sealed to the filter base 100' is a housing 22', which provides a cavity or interior space between the bioreactor base 100 and the filter base 100'. The housing 22' can be a cylindrical tube, preferably having the same diameter as the bioreactor housing 22, and made of the same material. It should have a height sufficient to accommodate at least a portion of the volume of fluid to be purified that is received directly from the bioreactor. The top edge of the housing 22' preferably protrudes radially inwardly, and preferably includes an O-ring 106' so that the housing 22' and base 100 can be affixed in sealing relation. A plurality of legs 6" can be provided, extending downwardly from the base 100' to support the assembly. Although it is preferred that the filter base 100' be integral to the bioreactor assembly to form a one-piece reactor assembly for sample processing and direct purification, in certain embodiments this subsequent purification step could be carried out with a filter that is physically separate from (although optionally in fluid communication with) the bioreactor body 22.

[0041] The housing 22' includes an inlet port 50 that can be placed in fluid communication with the outlet 32b of the base 100, such as with suitable tubing 51 (FIG. 5). The filter base 100' includes an outlet port 32b' in fluid communication with the drain (not shown) in the base, for directing the biomolecule of interest to a suitable point of use, such as a further purification step (e.g, a chromatography process train).

[0042] An alternative embodiment is to have the outlet of the second housing 22' in fluid communication with the outlet 32 of the base 100 but to have the second housing contain no filter or membrane. Instead the outlet port 32' is in fluid communication via a tube or other conduit (not shown) with a self contained filter device (not shown) such as a Millex® filter or an Optiscale® or Opticap® filter that then sterile filters the biomolecule of interest. The outlet of this filter device is then connected to a suitable point of use, such as a further purification step (e.g., a chromatography process train).

[0043] Suitable valving and sensing equipment can be associated with one or more of the various inlets and outlets to detect or measure and control flow or any other characteristic, such as the presence of the biomolecule or the presence of impurities, as appropriate or desired. For example, during the cell culture phase, the outlet 32b of the base 100 is closed so that the fluid remains in the body 22 when the gas is applied through port 32a or 30a.

[0044] In certain embodiments where a polymer is added to a cell culture broth to selectively and releasably bind a biomolecule of interest, suitable polymers include poly(N-vinyl caprolactam), poly(N-acryloylpiperidine), poly(N-vinylisobutyramide), poly(N-substituted acrylamide) including [poly(N-isopropylacrylamide), poly(N,N'-diethylacrylamide), and poly(N-acryloyl-N-alkylpiperazine)], Hydroxyalkylcellulose, copolymers of acrylic acid and methacrylic acid, polymers and copolymers of 2 or 4-vinylpyridine and chitosan with either a ligand or functional group attached to it.

[0045] Suitable biomolecules of interest include proteins and antibodies. Suitable antibodies include antibody selected from the group consisting of a recombinant antibody, a recombinant monoclonal antibody, a polyclonal antibody, a humanized antibody and an antibody fragment.

[0046] In operation, the sterile device is placed within the stand and the various connections for air, liquid, probes, sampling, etc. are attached to the device at the appropriate ports. The device is filled with media to a desired level forming a liquid/air interface somewhere below where the top 16 is attached to the body 22 to leave a head space of gas as is common in such devices. At least one port 32 is below the level of the interface.

[0047] The media is then seeded with the organism to be grown, be it plant, animal cell (CHO or NSO cells for instance) virus, yeast, mold or bacteria (such as E. coli) and the liquid is circulated or agitated and air/gases and liquids moved into or out of the device in a manner to effectively grow the culture inside.

[0048] A polymer soluble under a certain set of process conditions is added, and is rendered insoluble and precipitates out of solution upon a change in conditions (e.g., temperature, salt concentration, light, electrical field, or pH). Alternatively, affinity or ion exchange beads or beads having any ligand or functionality capable of purifying the biomolecule can be added to bind to the biomolecule of interest or to the soluble impurities. Agitation is continued to inhibit the solids from settling, and the solid, which in this embodiment includes the precipitate that contains the polymer, impurities such as cells and cell debris, host cell proteins, DNA and the like and the desired biomolecule, can be washed one or more times (such as with a suitable buffer) to ensure that any impurities in the liquid or entrapped in or on the polymer have been removed. The wash step(s) can be carried out by filtration through the one or more membranes in the base 100, with supernatant being sent to waste via port 32b.

[0049] The biomolecule of interest then can be recovered, such as by selective elution of the target biomolecule from the precipitate (or beads) such as by altering the ionic strength and/or pH conditions of the solution while the impurities, including soluble and insoluble material, remain complexed with the precipitated polymer. Recovery is carried out preferably along with a sterilizing filtration step, by causing the filtration base 100' to be in fluid communication with the base 100, such as by connecting the outlet of the base 100 to the inlet 50 of the body 22'. Accordingly, permeate from the outlet of the base 100 enters the body 22', wets the membrane 110', and filtration through the membrane 110' proceeds. The purified biomolecule of interest is then recovered in the elution pool via the outlet port 32b' of base 100'. The precipitated polymer-impurity complex (or the affinity beads) may be discarded. The driving force for filtration may be pressure or vacuum.

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- US20050016620A [0029]

Patentkrav

1. Indretning til kultivering eller forarbejdning af en fluidprøve, omfattende en første beholder (22) med et indvendigt rum, en første bund (100), som er tætnende fastgjort til den første beholder (22), og mindst én membran (110), som er forseglet til bunden (100) til filtrering af fluidprøven; en udgang (32b) i bunden (100) til den filtrerede fluidprøve, en anden beholder (22') i fluidforbindelse med udgangen (32b) af den første bund (100), hvor den anden beholder (22') er tætnende fastgjort til en anden bund (100'), hvor den anden bund (100') omfatter en membran (110') og en understøttende flade, som omfatter riller til fluidstrømmen.

2. Indretning ifølge krav 1, hvor den anden beholder (22') bærer mindst én membran (110').

3. Indretning ifølge krav 1, hvor den første beholder (22) er en bioreaktor.

4. Indretning ifølge krav 1, yderligere omfattende en agitator indvendigt i legemet af den første beholder (22) til omrøring af prøven.

5. Indretning ifølge krav 1, hvor den første bund (100) omfatter en understøtningsflade (101), som er udformet med riller (102) til strømmen af fluid, hvor rillerne (102) er i fluidforbindelse med en åbning (103) i bunden, hvor åbningen er i fluidforbindelse med en udledning (32b) for at bortlede fluid fra bunden (100).

6. Indretning ifølge krav 1, hvor den mindst én membran (110), som er forseglet til den første bund (100), er en grov membran, og hvor membranen (110') af den anden bund (100') er en sterilfiltermembran med 0,2 micron.

7. Fremgangsmåde til rensning af et biomolekyle, der er udvalgt fra gruppen bestående af proteiner og antistoffer fra en blanding, der indeholder urenheder, omfattende:

(a) tilvejebringelse af en indretning, omfattende tilvejebringelse af en første beholder (22) med et indvendigt rum, en første bund (100), som er tætnende fastgjort til den første beholder (22) og understøtter mindst én membran (110), som er forseglet til den første bund (100) til filtrering af blandingen; en udgang (32b) i bunden (100), en anden beholder (22') i fluidforbindelse med udgangen (32b) af den første bund (100), og en anden bund (100') er tætnende fastgjort til den anden beholder (22'), hvor den anden bund (100') omfatter en anden membran (110') og en understøttende flade, som omfatter riller til fluidstrømmen,

(b) tilvejebringelse af blandingen på et sæt betingelser,

(c) tilsætning af en eller flere polymerer, som kan opløses i blandingen på sættet af betinger og kan bindes reversibelt og selektivt til biomolekylet, hvor den ene eller flere polymerer er udvalgt fra gruppen bestående af poly(N-vinyl caprolactam), poly(N-acryloylpiperidin), poly(N-vinylisobutyramid), poly-(N-isopropylacrylamid), poly(N,N'-diethylacrylamid), poly(N-acryloyl-N-alkylpiperazin), hydroxyalkylcellulose, copolymerer af acrylsyre og methacrylsyre, og polymerer og copolymerer af 2- eller 4-vinylpyridin og chitosan med enten en ligand eller funktionel gruppe fastgjort dertil,

(d) blanding af en eller flere opløste polymerer i den samlede blanding;

(e) bundfældning af en eller flere polymerer og det bundne biomolekyle ud af opløsningen ved at ændre sættet af betingelser i blandingen;

(f) vask af præcipitatet ved bringe præcipitatet i kontakt med en vaskeopløsning og filtrering af supernatanten gennem den første membran,

(g) genvinding af det bundne biomolekyle fra polymeren og filtrering af biomolekylet gennem den anden membran.

8. Fremgangsmåde ifølge krav 7, hvor filtreringen af supernatanten og filtreringen af biomolekylet udføres i den samme indretning.

9. Fremgangsmåde ifølge krav 7, hvor biomolekylet er et antistof, der er udvalgt fra gruppen bestående af et rekombinant antistof, et rekombinant, monoklonalt antistof, et polyklonalt antistof, et humaniseret antistof og et antistoffragment.

10. Fremgangsmåde ifølge krav 7, hvor biomolekylet er et protein.

DRAWINGS

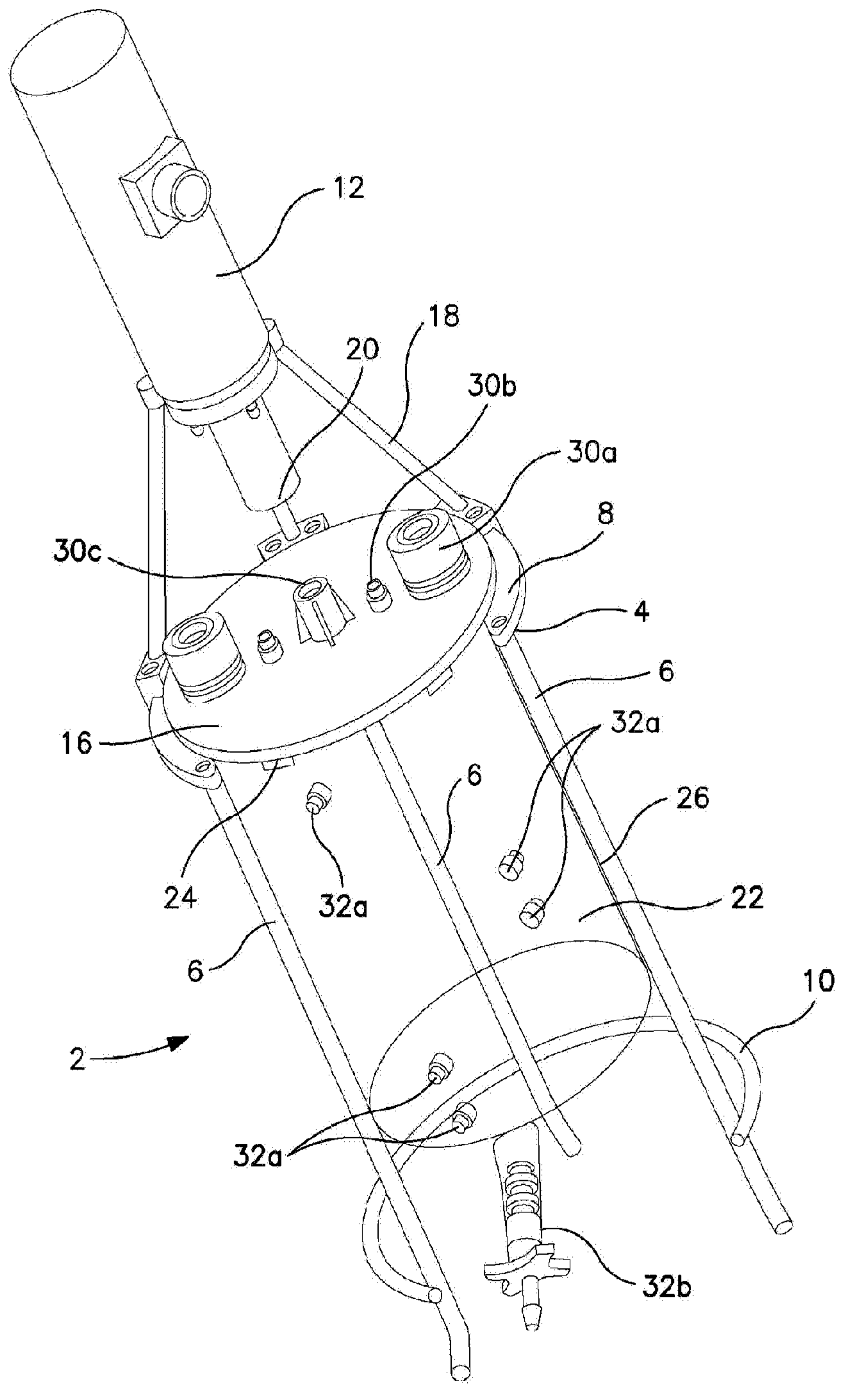

FIG. 1

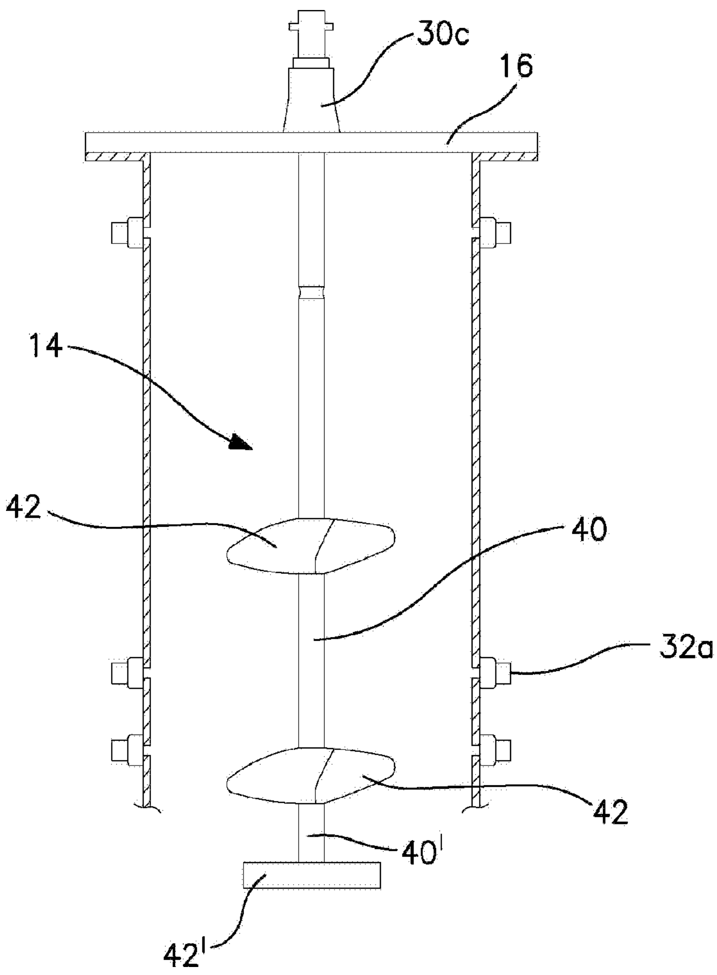

FIG. 2

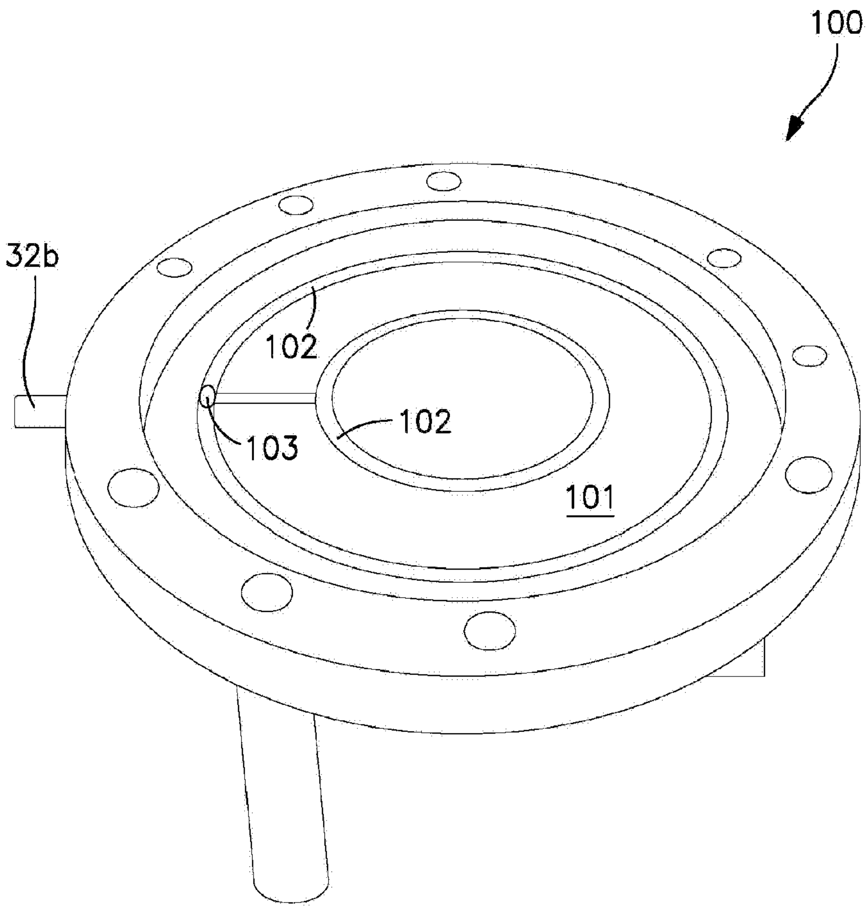

FIG. 3

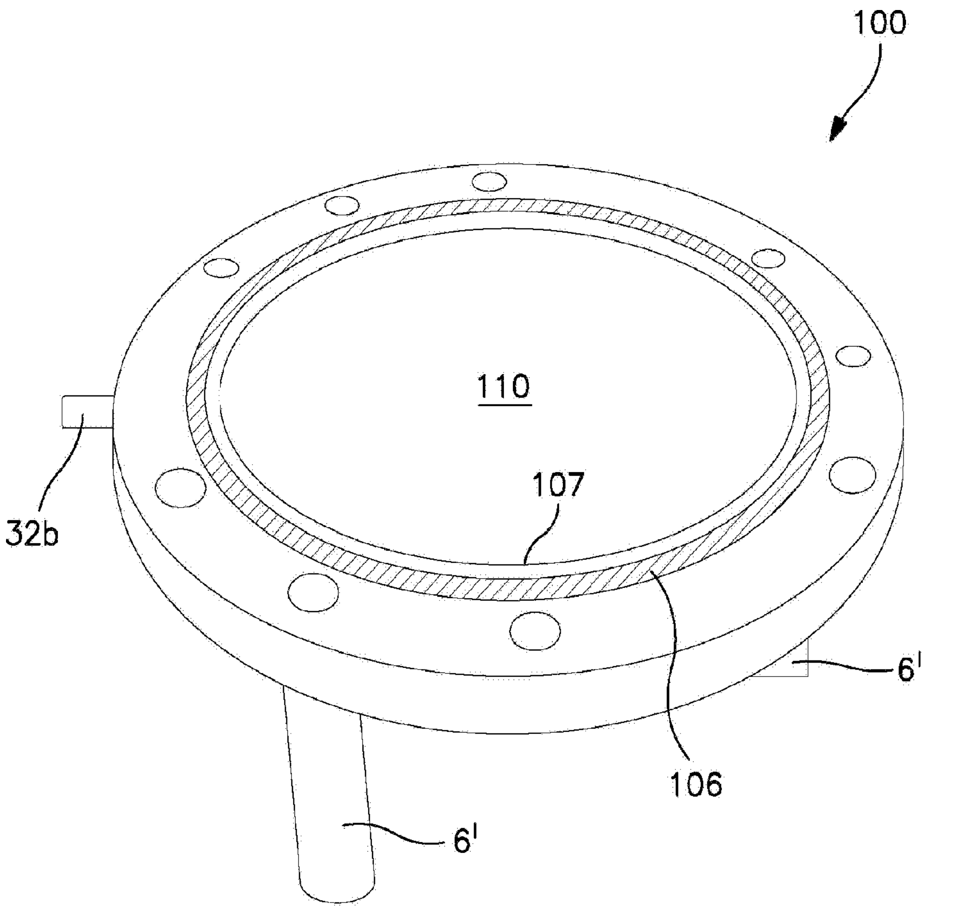

FIG. 4

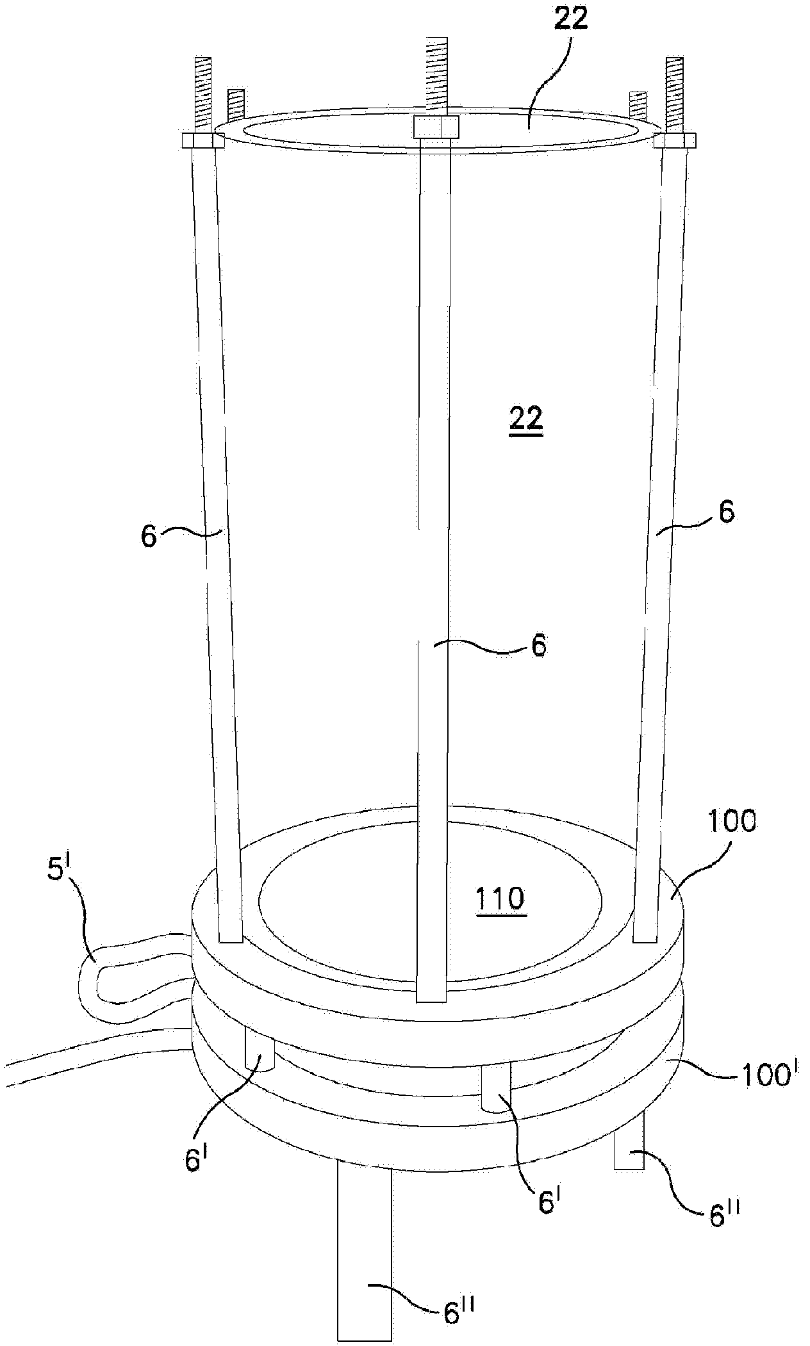

FIG. 5

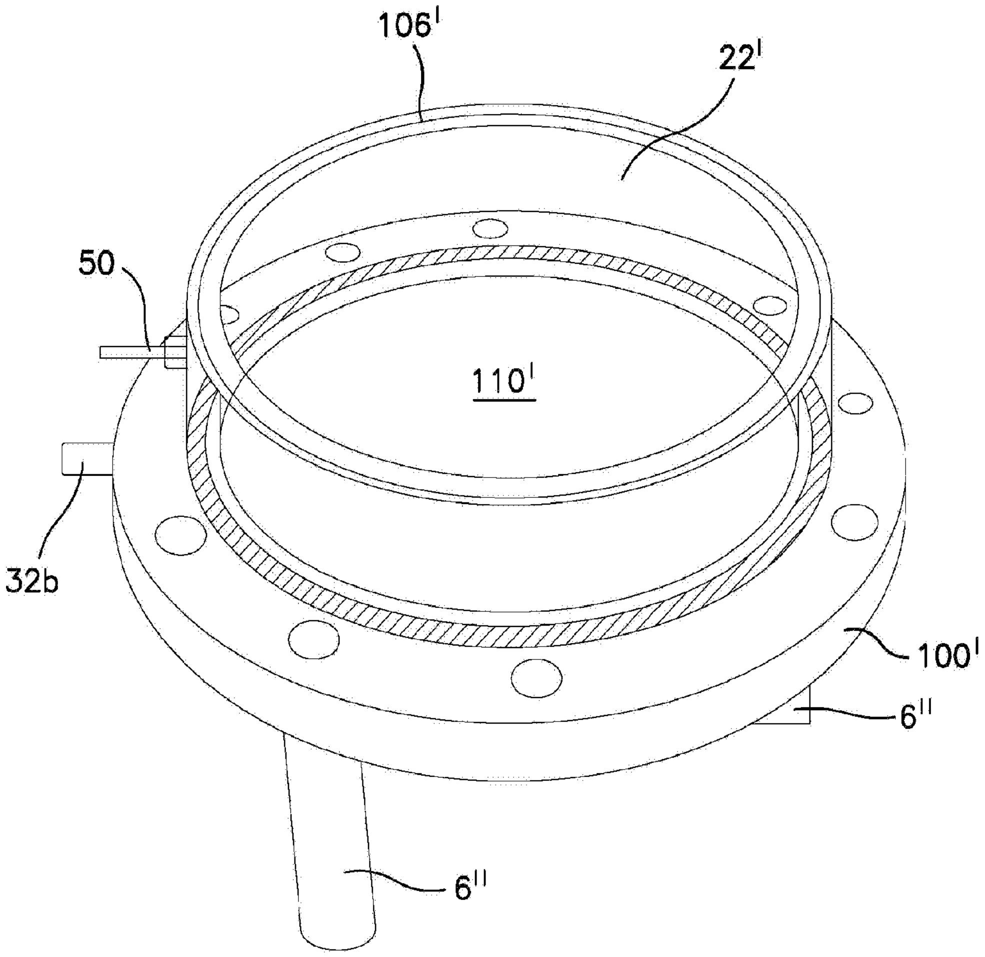

FIG. 6

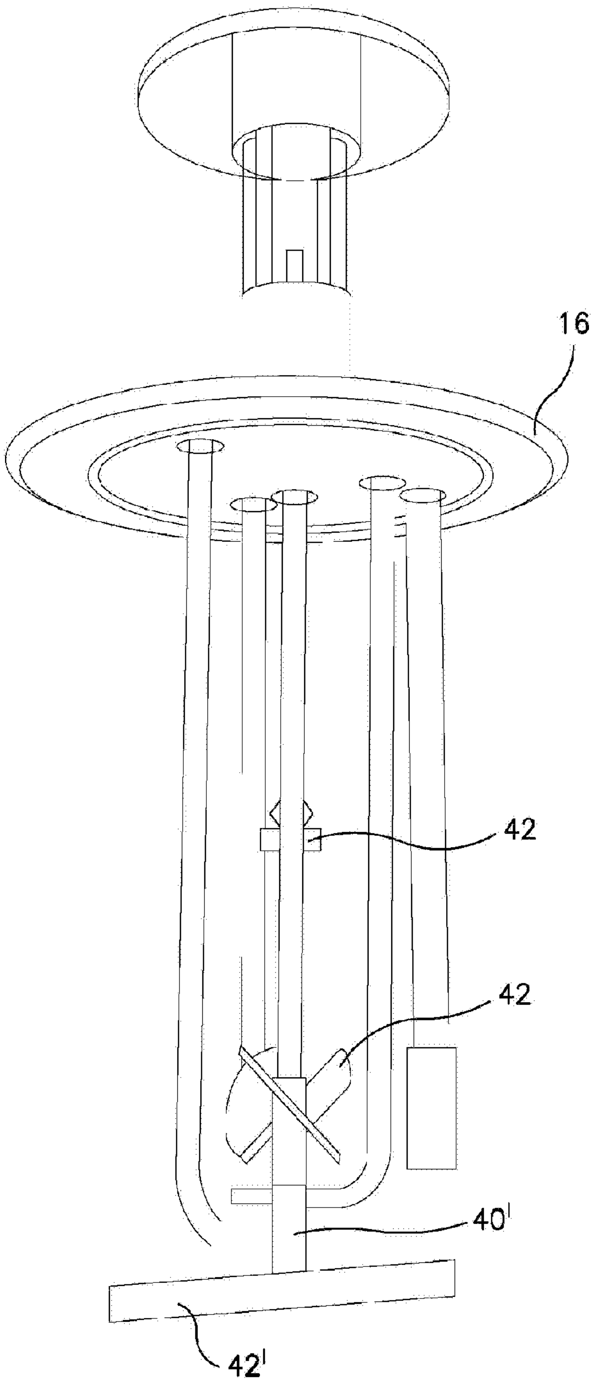

FIG. 7