

BACKGROUND OF THE INVENTION

[0001] Membranes having isotropic (symmetric) and anisotropic (asymmetric) structures, e.g., multiple layer and composite membranes, are known in the art. However, conventional membranes have not been suitable for some applications, e.g., they have not reliably provided one or more of the following: desired throughput, sterility grade filtration, and robustness. The present disclosure provides for ameliorating at least some of the disadvantages of the prior art. These and other advantages of the present disclosure will be apparent from the description as set forth below.

BRIEF SUMMARY OF THE INVENTION

[0002] In an aspect, the disclosure provides a porous membrane comprising an upstream porous asymmetric layer having an asymmetric layer thickness, and a porous structure reducing the passage of bacteria therethrough; a downstream porous isometric polymeric layer contacting the porous asymmetric layer, the porous isometric layer having an isometric layer thickness and a porous structure reducing the passage of bacteria therethrough; wherein the porous membrane has a total membrane thickness; and the isometric layer thickness is from 45% to 80% (e.g., from 48% to 80%) of the asymmetric layer thickness.

[0003] In another aspect, the disclosure provides a method of processing a fluid, the method comprising passing the fluid through a porous membrane described herein, in the direction from the asymmetric layer toward the isometric layer.

[0004] In a further aspect, the present disclosure provides a method of making a porous membrane described herein, the method comprising preparing a first solution comprising a first polymer and a solvent for the first polymer and a second solution comprising a second polymer and a solvent for the second polymer. In accordance with embodiments of the present disclosure, the method includes casting the first solution onto a support with a first speed and casting the second solution on the first solution and forming a pre-membrane. In embodiments, the method further includes quenching the pre-membrane in a fluid at a second speed, leaching the pre-membrane, and drying the membrane.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0005] FIGs. 1A and 1B depict representative scanning electron microscope (SEM) images of the asymmetric layer (FIG. 1A) and the isometric layer (FIG. 1B). FIG. 1A further illustrates the four zones of the asymmetric layer.

[0006] FIGs. 2A and 2B illustrate results of a bacterial challenge study on isometric membranes, in accordance with the isometric layer of the porous membranes described herein. FIG. 2A is a scatter plot graph showing bubble point (K_L) in psi along the x-axis and recovery of bacteria from filtrate in CFU along the y-axis for the isometric membrane samples. FIG. 2B is a scatter plot graph showing K_L in psi along the x-axis and the log reduction value (LRV) along the y-axis of the isometric membrane samples. A total of 44 samples were tested and were grouped according to thickness in mil as shown in the legends. The line labeled “Cliff” indicates the bacterial challenge (BC) cliff, and the line labeled “LSL” indicates the lower specification limit. Data points labeled assignable cause were identified as having deformations causing damage to the test samples and preventing proper testing conditions.

[0007] FIG. 3 illustrates results of a bacterial challenge study on asymmetric membranes, in accordance with the asymmetric layer of the porous membranes described herein. FIG. 3 is a scatter plot graph showing water bubble point (WBP) in psi along the x-axis and the log reduction value (LRV) along the y-axis of the asymmetric membrane samples. A total of 69 samples were tested, although 17 were excluded as not representative due to deformations or damaged samples preventing proper testing conditions. The vertical dotted line labeled “38 psi” indicates the BC cliff, and the horizontal line labeled “3.5 LRV” indicates the minimal LRV value used to determine the BC cliff.

[0008] FIGs. 4A, and 4B depict, in exploded views, an illustrative filter assembly (filter capsule) that can be used with the membranes described herein. FIG. 4A illustrates from a superior view a filter capsule with a hollow cylindrical filter element comprising a pleated membrane according to an aspect of the disclosure and a structure arranged between the lower end of the filter element and the outlet portion of the housing to reduce hold up (dead) volume, and FIG. 4B illustrates the same from an inferior view.

[0009] FIGs. 5A and 5B depict, in exploded view, an illustrative filter assembly (filter capsule) that can be used with the membranes described herein. FIG. 5A illustrates from a superior view a filter capsule including a cassette with at least one planar filter element comprising a membrane according to an aspect of the invention and a protrusion of the

cassette and membrane within to reduce hold up volume, and FIG. 5B illustrates the same from an inferior view.

DETAILED DESCRIPTION OF THE INVENTION

[0010] An aspect of the present disclosure provides a porous membrane comprising an upstream porous asymmetric layer and a downstream isometric layer that both can reduce the passage of bacteria therethrough, wherein the membrane has a total thickness, and wherein the isometric layer has a thickness from 45% to 80% of the asymmetric layer thickness. The asymmetric layer can act as a bacteria pre-filter reducing the amount of bacteria that enter the isometric layer. Advantageously, this can allow the isometric layer to have a reduced thickness, while unexpectedly still maintaining the capability of sterile filtration. This can allow for the membrane to have a reduced total thickness compared to conventional dual layer membranes. Advantageously, in accordance with embodiments of the present disclosure, the thinness of the membrane thickness can result in, *inter alia*, higher throughput during filtration and/or improved filtration since more of the membrane (when pleated, for example) can be loaded into a filter device, e.g., as a filter element, cartridge, or cassette.

[0011] In an aspect, the disclosure provides a porous membrane comprising an upstream porous asymmetric layer having an asymmetric layer thickness, and a porous structure reducing the passage of bacteria therethrough; a downstream porous isometric polymeric layer contacting the porous asymmetric layer, the porous isometric layer having an isometric layer thickness and a porous structure reducing the passage of bacteria therethrough; wherein the porous membrane has a total membrane thickness; and the isometric layer thickness is from 45% to 80% of the asymmetric layer thickness.

[0012] In accordance with aspects of the disclosure, layers of the membrane are formed from the same polymer and solvent, however, varying the viscosity, additives, and treatment, or different polymers, additives, and/or viscosities can be used for different layers.

[0013] In some embodiments, the isometric layer thickness is from 45% to 80% of the asymmetric layer thickness. For example, in accordance with embodiments of the present disclosure, the isometric layer thickness relative to the asymmetric layer thickness is from 50% to 80%, e.g., from 55% to 80%, from 60% to 80%, from 65% to 80%, from 70% to 80%, from 75% to 80%, from 45% to 75%, from 50% to 75%, from 55% to 75%, from 60% to 75%, from 65% to 75%, from 70% to 75%, from 45% to 70%, from 50% to 70%, from 55% to 70%, from 60% to 70%, from 65% to 70%, from 45% to 65%, from 50% to 65%,

from 55% to 65%, from 60% to 65%, from 45% to 60%, from 50% to 60%, from 55% to 60%, from 45% to 55%, from 50% to 55%, or from 45% to 50%, etc.

[0014] The porous membrane described herein can have any appropriate mean flow pore size (MFP). As used herein, the term “mean flow pore size” (also referred to as “MFP size”) refers to the average or mean pore size as calculated by the relationship between pore size and pressure described in the Washburn equation, $P = -2\gamma \times \cos \theta / r$ (where P is pressure, γ is surface tension, θ is contact angle, and r is pore size).

[0015] As described herein, MFP size is measured by either mercury intrusion or capillary flow porometry methods. Capillary flow porometry is useful to characterize symmetric membrane pore size and pore size range and is also useful to characterize membranes that will be deformed under high pressure, as this method can be performed at relatively low pressure (<35 psi). Alternatively, mercury intrusion, can provide useful pore size characterization information for asymmetric membranes, as this method measures all diameters of open and blind pore sizes. In some embodiments, the MFP size of the isometric layer is from 310 nm to 400 nm. For example, in accordance with embodiments of the present disclosure, the MFP size of the isometric layer from 320 nm to 400 nm, e.g., from 330 nm to 400 nm, from 340 nm to 400 nm, from 350 nm to 400 nm, from 350 nm to 400 nm, from 360 nm to 400 nm, from 370 nm to 400 nm, from 380 nm to 400 nm, or from 390 nm to 400 nm, etc. In some embodiments the MFP of the asymmetric layer is from 390 nm to 420 nm, e.g., from 400 nm to 420 nm, or from 410 nm to 420 nm, etc.

[0016] The porous membrane described herein can have any appropriate quantitative bubble point (K_L). As used herein, the term “quantitative bubble point” (also referred to as “ K_L ,” “water bubble point,” and/or “WBP”) refers to when a membrane is wetted and air is pushed through the membrane at a given rate until a pressure is reached when the air flow rate is dramatically increased, which can be visually observed by the appearance of bubbles. In some embodiments, the K_L of the isometric layer from 45 psi to 75 psi. For example, in accordance with embodiments of the present disclosure, the K_L of the isometric layer is from 48 psi to 75 psi, e.g., from 48 psi to 70 psi, from 48 psi to 65 psi, from 48 psi to 60 psi, from 48 psi to 55 psi, from 55 psi to 75 psi, from 55 psi to 70 psi, from 55 psi to 65 psi, from 65 psi to 75 psi, or from 70 psi to 75 psi, etc. In some embodiments, the K_L of the asymmetric layer is from 25 psi to 55 psi. For example, in accordance with embodiments of the present disclosure, the K_L of the asymmetric layer is from 30 psi to 55 psi, e.g., from 30 psi to 50 psi, from 30 psi to 45 psi, from 30 psi to 40 psi, from 35 psi to 55 psi, from 35 psi to 50 psi, from

35 psi to 40 psi, from 40 psi to 55 psi, from 40 psi to 50 psi, from 40 psi to 45 psi, from 45 psi to 55 psi, or from 50 psi to 55 psi, etc.

[0017] The porous membrane described herein can have any appropriate log reduction level. As used herein, the term “log reduction level” or “LRV” refers to the reduction in bacteria after being passed through a membrane, with each unit representing a reduction by a power of 10. LRV is calculated using the equation $LRV = \text{Log}10 \left(\frac{\text{Total Challenge}}{\text{Total Recovery}} \right)$. Where the total challenge is the amount of bacteria that were loaded into the membrane, and the total recovery is the amount of bacteria that passed through the membrane, both are usually measured as colony forming units (CFUs).

[0018] As described herein, LRV is measured using a bacterial challenge test with a challenge load from 10^7 CFU/cm² to 10^8 CFU/cm² *Brevundimonas diminuta* bacteria. As used herein the term, “reducing the passage of bacteria” refers to a membrane or a layer of a membrane that has an LRV of greater than 1. As used herein, the term “substantially reducing the passage of bacteria” refers to a membrane or layer of a membrane with an LRV of 7 or greater. In some embodiments, the LRV of the isometric layer is from 7 to 10. In some embodiments, the LRV of the asymmetric layer is from 2.5 to 7.5. For example, in accordance with embodiments of the present disclosure, the LRV of the asymmetric layer is from 3 to 7.5, e.g., from 4 to 7.5, from 5 to 7.5, or from 6 to 7.5, etc.

[0019] The advantages of the porous membrane as described herein can be seen across various layer densities. For example, in some embodiments, the isometric layer thickness is from 55 μm to 125 μm. In accordance with embodiments of the present disclosure, the isometric layer thickness is from 60 μm to 125 μm, e.g., from 65 μm to 125 μm, from 70 μm to 125 μm, from 75 μm to 125 μm, from 80 μm to 125 μm, from 85 μm to 125 μm, from 90 μm to 125 μm, from 95 μm to 125 μm, from 100 μm to 125 μm, from 105 μm to 125 μm, from 110 μm to 125 μm, from 115 μm to 125 μm, from 120 μm to 125 μm, etc. In some embodiments, the asymmetric layer thickness is from 75 μm to 120 μm. For example, in accordance with embodiments of the present disclosure, the asymmetric layer thickness is from 80 μm to 120 μm, from 85 μm to 120 μm, from 90 μm to 120 μm, from 95 μm to 120 μm, from 100 μm to 120 μm, from 105 μm to 120 μm, from 110 μm to 120 μm, from 115 μm to 120 μm, etc. In some embodiments, the total membrane thickness is from 130 μm to 245 μm. For example, in accordance with embodiments of the present disclosure, the total membrane thickness is from 135 μm to 245 μm, from 140 μm to 245 μm, from 145 μm to 245 μm, from 150 μm to 245 μm, from 155 μm to 245 μm, from 160 μm to 245 μm, from

165 μm to 245 μm , from 170 μm to 245 μm , from 175 μm to 245 μm , from 180 μm to 245 μm , from 185 μm to 245 μm , from 190 μm to 245 μm , from 195 μm to 245 μm , from 200 μm to 245 μm , from 205 μm to 245 μm , from 210 μm to 245 μm , from 215 μm to 245 μm , from 220 μm to 245 μm , from 225 μm to 245 μm , from 230 μm to 245 μm , from 235 μm to 245 μm , from 240 μm to 245 μm , etc.

[0020] In some embodiments the asymmetric layer comprises four zones each having a thickness and porosity; (a) a first zone having a first zone thickness from 20 μm to 40 μm and a porosity from 40% to 55%, (b) a second zone having a second zone thickness from 10 μm to 30 μm and a porosity from 30% to 50%, (c) a third zone having a third zone thickness from 10 μm to 30 μm and a porosity from 20% to 50%, and (d) a fourth zone having a fourth zone thickness from 5 μm to 15 μm and a porosity from 10% to 50%. FIG. 1A shows a representative scanning electron microscope (SEM) image of the asymmetric layer, where the four zones can be seen with zone 1 on the top, followed by zone 2, zone 3, and zone 4 on the bottom of the image.

[0021] The porous membrane described herein can have any appropriate porosity. In this respect, the term “porosity,” as used herein, refers to the percentage of a membrane that is composed of pores. As described herein, porosity is measured by either using representative SEM images or mercury porosimetry. Representative SEM images are used to measure porosity, such as FIG. 1A and 1B, which are SEM images of the asymmetric layer (including its four zones) and the isometric layer, respectively. Porosity is measured by using representative SEM images and using software, particularly Image J (developed by the National Institutes of Health), to calculate the percent of the membrane composed of pores. Alternatively, porosity is measured by mercury porosimetry. Using mercury porosimetry, mercury will be pushed through a membrane and the amount of mercury, which occupies the pores, is used to calculate the percentage of the membrane composed of pores, as well as pore size and distribution.

[0022] In some embodiments wherein the asymmetric layer comprises four zones (i) the first zone has a mean pore size from 1300 nm to 1800 nm, (ii) the second zone has a mean pore size from 700 nm to 1200 nm, (iii) the third zone has a mean pore size from 400 nm to 800 nm, and (iv) the fourth zone has a mean pore size from 400 nm to 900 nm.

[0023] As used herein, the term “mean pore size” refers to the average or mean size of pores as calculated by taking representative SEM images of a membrane and using software, such as Image J, to measure the pore sizes.

[0024] The surface characteristics of the layers can be modified (e.g., to affect the CWST, to include a surface charge, e.g., a positive or negative charge, and/or to alter the polarity or hydrophilicity of the surface) by wet or dry oxidation, by coating or depositing a polymer on the surface, or by a grafting reaction. Modifications include, e.g., irradiation, a polar or charged monomer, coating and/or curing the surface with a charged polymer, and carrying out chemical modification to attach functional groups on the surface. Grafting reactions may be activated by exposure to an energy source such as gas plasma, vapor plasma, corona discharge, heat, a Van der Graff generator, ultraviolet light, electron beam, or to various other forms of radiation, or by surface etching or deposition using a plasma treatment. Illustrative modifications include, for example, those described in U.S. Patent 9,469,737, and U.S. Patent 6,083,393 which are each incorporated by reference.

[0025] In some embodiments the membrane further comprises an interface between the asymmetric layer and the isometric layer, the interface layer having a first portion contacting the asymmetric layer and a second portion contacting the isometric layer; and (a) the asymmetric layer has a region contacting the first portion of the interface layer, the region including cells having a first diameter; (b) the isometric layer has a region contacting the first portion of the interface layer, the region including cells having a second diameter; and the first portion of the interface layer comprises cells having the first diameter, and the second portion of the interface layer comprises cells having the second diameter, wherein each of the portions of the interface layer has a mixture of the cells having a first diameter and the cells having a second diameter.

[0026] In some embodiments, the interface membrane includes a first polymer from the asymmetric layer and a second, different, polymer from the isometric layer.

[0027] In aspects, the disclosure provides a method of processing a fluid, comprising passing the fluid through, or contacting a fluid with a membrane described herein, in the direction from the asymmetric layer toward the isometric layer. Membranes according to aspects of the disclosure can be used in a variety of applications, including, for example, sterile filtration applications, filtering fluids for the electronics industry, filtering fluids for the pharmaceutical and biopharmaceutical industries at all process stages, such as, but not limited to, filtering antibody - (especially monoclonal antibody) and/or protein-containing fluids, final drug formulations, such as nanosuspensions including liposomes, emulsions, exosomes and nanospheres; recombinant proteins including monoclonal antibodies, antibody fragments, bispecific antibodies and antibody-drug conjugates; blood products including IVIG and Human Serum Albumin; small molecule drugs, for example, Doxorubicin; viruses,

filtering fluids for the food and beverage industry, clarification, and filtering cell culture fluids.

[0028] In aspects, the disclosure provides a method of making a porous membrane described herein, the membrane having an upstream porous asymmetric layer and a downstream porous isometric polymeric layer contacting the porous asymmetric layer, comprising: preparing a first solution comprising a first polymer and a solvent for the first polymer; preparing a second solution comprising a second polymer and a solvent for the second polymer; casting the first solution onto a support with a first speed; casting the second solution on the first solution and forming a pre-membrane; quenching the pre-membrane in a water at a second speed; leaching the pre-membrane; and drying the membrane.

[0029] In aspects, the disclosure provides suitable solutions including polymers, which can include polymers such as, for example, polyaromatics; sulfones (e.g., polysulfones, including aromatic polysulfones such as, for example, polyethersulfone (PES), bisphenol A polysulfone, polyarylsulfone, and polyphenylsulfone), polyamides, polyimides, polyvinylidene halides (including polyvinylidene fluoride (PVDF)), polyolefins, such as polypropylene and polymethylpentene, polyesters, polystyrenes, polycarbonates, polyacrylonitriles (including polyalkylacrylonitriles), cellulosic polymers (such as cellulose acetates and cellulose nitrates), fluoropolymers, and PEEK. Solutions comprising polymers can include a mixture of polymers, e.g., a hydrophobic polymer (e.g., a sulfone polymer) and a hydrophilic polymer (e.g., polyvinylpyrrolidone).

[0030] In some embodiments, in addition to one or more polymers, solutions comprise at least one solvent, and may further comprise at least one non-solvent. Suitable solvents include, for example, dimethyl formamide (DMF); N,N dimethylacetamide (DMAC); N methyl pyrrolidone (NMP); tetramethylurea; dioxane; diethyl succinate; dimethylsulfoxide; chloroform; and tetrachloroethane; and mixtures thereof. Suitable nonsolvents include, for example, water; various polyethylene glycols (PEGs; e.g., PEG 400, PEG 1000); various alcohols, e.g., methanol, ethanol, isopropyl alcohol (IPA), amyl alcohols, hexanols, heptanols, and octanols; alkanes, such as hexane, propane, nitropropane, heptanes, and octane; and ketone, ethers and esters such as acetone, butyl ether, ethyl acetate, and amyl acetate; and various salts, such as calcium chloride, magnesium chloride, and lithium chloride; and mixtures thereof.

[0031] In some embodiments, a solution comprising a polymer can further comprise, for example, one or more polymerization initiators (e.g., any one or more of peroxides, ammonium persulfate, aliphatic azo compounds (e.g., 2,2'-azobis(2-amidinopropane)

dihydrochloride (V50)), and combinations thereof), and/or minor ingredients such as surfactants and/or release agents.

[0032] In some embodiments, the solutions comprising the polymers are cast into thin films, one on top of the other, exposed to a gaseous environment for a predetermined period of time, followed by quenching in a nonsolvent for the polymers. In some embodiments, the first solution is spread in a layer (bottom layer) onto a support (such as a nonporous support), and the second solution is spread in a layer (upper layer) onto the first solution, and the membrane is later separated from the support after quenching. However, in some embodiments, the support (porous or non-porous) is incorporated into the final structure if desired. In some embodiments the support is a belt.

[0033] A variety of devices known in the art are used for casting. Suitable devices include, for example, mechanical spreaders, that comprise spreading knives, doctor blades, or spray/pressurized systems. One example of a spreading device is an extrusion die or slot coater, comprising a casting chamber into which the casting formulation (solution comprising a polymer) are introduced and forced out under pressure through a narrow slot. A variety of air gaps are suitable for use in some embodiments of the disclosure, and the air gaps are the same for the knives/doctor blades, or different. A variety of casting speeds are suitable as is known in the art. In some embodiments, the casting speed is from 6 to 8 feet/minute. In accordance with some embodiments of the present disclosure, the casting speed is 7 feet/minute. In some aspects, the casting is performed at a temperature. In some embodiments, the casting temperature is from 20 °C to 30 °C. In accordance with some embodiments of the present disclosure, the casting temperature is 26 °C. In some aspects, the casting is performed at a humidity. In some embodiments, the humidity is from 60% to 100%. In accordance with some embodiments of the present disclosure, the humidity is 75%. In some embodiments, cast solutions are exposed to air after casting but before quenching. In some embodiments the air, e.g., humid air, is circulated (for example, using one or more fans) to enhance contact with the cast solutions. In some embodiments, the air is not circulated. In some embodiments the support with the cast solutions thereon is immersed in a quenching bath to effect phase separation of the polymer solutions in a continuously layered sequence to form an integral multilayer (i.e., layers bonded together such that the membrane behaves as a single structure that does not delaminate or separate under normal use conditions) microporous polymeric membrane. In some embodiments, the quenching fluid is water. In some embodiments, the quenching fluid has a temperature. In some embodiments the quenching fluid temperature is from 20 °C to 60 °C, from 45 °C to 55 °C, or from 45 °C to 50

°C, etc. In some embodiments, after formation, the membrane is washed to remove residual solvent, dried, and wound onto a core. In some embodiments, the washing solution is deionized water or ethanol.

[0034] In some embodiments, there is a time interval between casting the first solution, and casting the second solution on the first solution. In some embodiments, the time interval is from 1 second to 60 seconds. In some embodiments, the time interval is 2 seconds to 30 seconds.

[0035] In some embodiments, the membrane comprises a pleated membrane.

[0036] Aspects of the membrane can be used in a variety of cassettes, cartridges and/or filter assemblies (filter capsules). In some embodiments the membrane is used in a cassette or cartridge and/or filter assembly (filter capsule), for example, as described in commonly assigned, copending U.S. Patent Application 63/539,405, filed September 20, 2023, incorporated herein by reference.

[0037] Briefly, in an embodiment, the cassette or cartridge can be arranged in a filter assembly comprising a first housing and a second housing. In some embodiments, the first housing includes an inlet, a first cavity, and at least two first guide pins, and the second housing includes an outlet, a second cavity, and at least two second guide pins. Further, the cassette can include a first planar side and a second planar side, with at least two first guide pin openings and at least two second guide pin openings, along with a cassette outlet. However, guide pins and guide pin openings are optional for any embodiment of the invention. While the illustrated embodiment shows two first guide pins, two second guide pins, two first guide pin openings and two second guide pin openings, embodiments of the invention can include at least one first guide pin and at least one first guide pin opening and/or at least one second guide pin and at least one second guide pin opening; or an embodiment of the invention is without a first guide pin and a first guide pin opening and/or without a second guide pin and a second guide pin opening. Illustratively, an embodiment without guide pins and guide pin openings can be produced by, for example, using an external guide on either side of the cassette to guide the cassette into place, or, for example, inserting a pin temporarily through the cassette to guide the cassette into the housing, followed by removing the pin. Each planar side (e.g., the first and second planar sides) includes an inner and outer surface. The inner surfaces of the planar sides delimits a cassette cavity.

[0038] With respect to the first and second housings, in some embodiments, the housings are configured to be joined such that the cassette is arranged within the first cavity and the

second cavity such that the optional first guide pin or pins each penetrate one of the optional first guide pin opening or openings, and the optional second guide pin or pins each penetrate one of the optional second guide pin opening or openings. The inlet is in fluid communication with the outlet through the cassette cavity and the cassette outlet.

[0039] In an embodiment, the filter cassette is configured for arrangement in a pressurized filter capsule. The cassette can comprise a first planar side and a second planar side. The first and second planar sides delimit a first outer surface and a second outer surface of the cassette, respectively. The second outer surface of the cassette is opposite the first outer surface. In addition, in accordance with some embodiments, the cassette further comprises a first inner surface and a second inner surface which further delimit a cassette cavity, where at least one of the first outer surface and the second outer surface includes a grid of openings in fluid communication with the cassette cavity. In embodiments, the cassette comprises an outlet protruding from the first planar side and the second planar side and in fluid communication with the cassette cavity and a membrane arranged on the grid of openings upstream of the cassette cavity.

[0040] An illustrative embodiment of a filter assembly (filter capsule) that can be used with the porous membranes described herein is shown in FIGs. 4A and 4B. FIG. 4A depicts a superior view of an exploded capsule filter capsule assembly according to an embodiment of the present disclosure. In particular, an upper housing and internal components thereof are illustrated, as well as a filter cartridge with a pleated membrane that is configured for arrangement within the filter capsule assembly, and partially within and between the upper housing and lower housing. The illustrated filter cartridge comprise a hollow cylindrical filter element comprising a pleated membrane according to an aspect of the disclosure, and the filter capsule also includes a structure arranged between the lower end of the filter element and the outlet portion of the housing to reduce hold up (dead) volume. This structure helps reduce hold-up volume by filling and thereby reducing open space within the filter capsule. Reducing the hold-up volume in such a manner advantageously allows for increasing the recovery of filtered fluid. FIG. 4B depicts an inferior view of the same exploded filter capsule of FIG. 4A.

[0041] Another illustrative embodiment of a filter assembly (filter capsule) that can be used with the porous membranes described herein is shown in FIGs. 5A and 5B. FIG. 5A illustrates an exploded view of a filter capsule assembly according to an embodiment of the present disclosure. In particular, an upper housing and internal components thereof are illustrated, as well as a cassette that is configured for arrangement within the filter capsule

assembly, and partially within and between the upper housing and lower housing. The upper housing includes an upper housing cavity in which a plurality of ribs and, in some embodiments, one or more guiding pins, are arranged. The ribs help secure the cassette and/or membranes within the upper housing and guide the flow and flow distribution of a liquid received into the upper housing via its inlet, thereby ensuring predictable and balanced flow of the liquid to be filtered towards the outer surface or surfaces of the cassette, which provide for filtration of the liquid. The ribs also reduce the surface area of the cassette and/or membranes mounted thereon that is directly exposed to contact with the liquid to be filtered, thereby reducing an internal wetted area. The guide pin or pins, if present, are arranged to extend in a similar manner to the ribs and serve to help secure the cassette within the upper housing.

[0042] The upper housing cavity is sized and shaped to receive the cassette. The upper housing also includes circumferential supports and lateral supports that both provide structural rigidity for the upper housing as a whole and strengthen it for an increased pressure rating and allow for a reduction in the quantity of material required for forming the upper housing as a whole. The hemispherical shape of the upper housing further contributes to its rigidity and ability to form a pressure vessel that can withstand increased internal pressure. The upper housing includes an upper joining surface by which the upper housing may be joined to the lower housing.

[0043] The cassette includes a protruding outlet that extends away from the first planar side and that provides for a fluid connection between an outside of the cassette and an internal cavity of the cassette. As a result, only liquid that has been filtered (e.g., through one or more membranes) may exit the cassette via outlet. The protrusion of the membrane is configured to be secured to the outlet. The protrusion ensures coverage of hold-up openings, such as the grid openings along the protruding outlet of the cassette, by the membrane, thereby ensuring that hold-up volume of a filter capsule may be reduced and ensuring that even liquid that flows too far below the grid of a cassette can still be filtered and expelled from the filter capsule.

[0044] FIG. 5B illustrates the filter capsule assembly of FIG. 5A from an inferior view that more clearly shows features of the cassette and internal features of the lower housing. The cassette includes a grid formed by a plurality of openings that provide fluid communication between an internal cavity of the cassette and an external environment or, in an assembled state of the filter capsule, between an internal cavity of the cassette and the internal volume of the filter capsule configured to receive a liquid to be filtered. The

illustrated cassette further includes an O-ring configured to seal against an internal surface of the lower housing and thereby prevent liquid that is to be filtered from bypassing the cassette and exiting the filter capsule without first being filtered. However, an O-ring is not required. For example, the cassette can be welded into place, with the weld acting as a seal.

[0045] In similar fashion to the upper housing, the lower housing may include a plurality of ribs and, in some embodiments, one or more guide pins in a lower housing cavity. The upper housing cavity and the lower housing cavity are configured to be joined to form a larger unitary cavity within which the cassette is arranged. The lower housing also includes circumferential supports and lateral supports that provide structural rigidity for the lower housing as a whole and strengthen it for an increased pressure rating. The hemispherical shape of the lower housing further contributes to its rigidity and ability to form a pressure vessel that can withstand increased internal pressure. The lower housing includes a lower joining surface by which the lower housing may be joined to the upper housing.

[0046] Additional embodiments for the filter cassette will be apparent to one of ordinary skill in the art, including illustrative embodiments shown and described in U.S. Patent Application 63/539,405, incorporated herein by reference.

[0047] The disclosure is further illustrated by the following exemplary aspects. However, the disclosure is not limited by the following aspects.

[0048] (1) A porous membrane, or method of preparing a porous membrane, as described herein.

[0049] (2) A porous membrane comprising: (a.) an upstream porous asymmetric polymeric layer having an asymmetric layer thickness, and a porous structure reducing the passage of bacteria therethrough; (b.) a downstream porous isometric polymeric layer contacting the porous asymmetric layer, the porous isometric layer having an isometric layer thickness and a porous structure reducing the passage of bacteria therethrough; wherein the porous membrane has a total membrane thickness; and the isometric layer thickness is from 45% to 80% (e.g., from 48 to 80%) of the asymmetric layer thickness.

[0050] (3) The membrane of aspect 2, wherein the mean flow pore (MFP) size of the isometric layer is from 310 nm to 400 nm (e.g., from 311 nm to 393 nm).

[0051] (4) The membrane of aspect 2 or 3, wherein the MFP of the asymmetric layer is from 380 nm to 420 nm (e.g., from 384 nm to 412 nm).

[0052] (5) The membrane of any one of aspects 2-4, wherein the quantitative bubble point (K_L) of the isometric layer is from 45 psi to 75 psi (e.g., from 48 psi to 72 psi).

- [0053] (6) The membrane of any one of aspects 2-5, wherein the K_L of the asymmetric layer is from 25 psi to 55 psi (e.g., from 29 psi to 54 psi).
- [0054] (7) The membrane of any one of aspects 2-6, wherein the log reduction level (LRV) of the isometric layer is from 7 to 10 (e.g., from 7.2 to 9.8).
- [0055] (8) The membrane of any one of aspects 2-7, wherein the LRV of the asymmetric layer is from 2.5 to 7.5 (e.g., from 3 to 7.4).
- [0056] (9) The membrane of any one of aspects 2-8, wherein the isometric layer thickness is from 55 μm to 125 μm (e.g., from 60 μm to 124 μm).
- [0057] (10) The membrane of any one of aspects 2-9, wherein the asymmetric layer thickness is from 75 μm to 120 μm (e.g., from 78 μm to 118 μm).
- [0058] (11) The membrane of any one of aspects 2-10, wherein the total membrane thickness is from 130 μm to 245 μm .
- [0059] (12) The membrane of any one of aspects 2-11, wherein the asymmetric layer comprises four zones each having a thickness and porosity; (a.) a first zone having a first zone thickness from 20 μm to 40 μm and a porosity from 40% to 55% (e.g., from 41% to 54%), (b.) a second zone having a second zone thickness from 10 μm to 30 μm and a porosity from 30% to 50% (e.g., from 31% to 49%), (c.) a third zone having a third zone thickness from 10 μm to 30 μm and a porosity from 20% to 50% (e.g., from 23% to 46%), and (d.) a fourth zone having a fourth zone thickness from 5 μm to 15 μm and a porosity from 10% to 50% (e.g., from 12% to 46%).
- [0060] (13) The membrane of aspect 12, wherein (i.) the first zone that has a mean pore size from 1300 nm to 1800 nm, (ii.) the second zone that has a mean pore size from 700 nm to 1200 nm, (iii.) the third zone that has a mean pore size from 400 nm to 800 nm, and (iv.) the fourth zone that has a mean pore size from 400 nm to 900 nm.
- [0061] (14) The membrane of any one of aspects 2-13, wherein the isometric layer is capable of substantially reducing the passage of bacteria therethrough.
- [0062] (15) The membrane of any one of aspects 2-14, wherein the membrane further comprises an interface between the asymmetric layer and the isometric layer, the interface layer having a first portion contacting the asymmetric layer and a second portion contacting the isometric layer; and (a.) the asymmetric layer has a region contacting the first portion of the interface layer, the region including cells having a first diameter; (b.) the isometric layer has a region contacting the first portion of the interface layer, the region including cells having a second diameter; and the first portion of the interface layer comprises cells having the first diameter, and the second portion of the interface layer comprises cells having the

second diameter, wherein each of the portions of the interface layer has a mixture of the cells having a first diameter and the cells having a second diameter.

[0063] (16) A method of processing a fluid, comprising passing the fluid through the membrane of any one of aspects 2-15, in the direction from the asymmetric layer toward the isometric layer.

[0064] (17) A method of making the porous membrane of any of aspects 2-15, the membrane having an upstream porous asymmetric layer and a downstream porous isometric polymeric layer contacting the porous asymmetric layer, comprising: (a.) preparing a first solution comprising a first polymer and a solvent for the first polymer; (b.) preparing a second solution comprising a second polymer and a solvent for the second polymer; (c.) casting the first solution onto a support with a first speed; (d.) casting the second solution on the first solution and forming a pre-membrane; (e.) quenching the pre-membrane in a fluid at a second speed; (f.) leaching the pre-membrane; and (g.) drying the membrane.

[0065] It shall be noted that the preceding aspects are illustrative and not limiting. Other exemplary combinations are apparent from the entirety of the description herein. It will also be understood by one of ordinary skill in the art that various embodiments may be used in various combinations with the other embodiments provided herein.

[0066] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0067] This example demonstrates an illustrative protocol for the preparation of a porous membrane according to an embodiment of the disclosure.

[0068] A formulation was prepared having the following components and percentages by weight of the formulation: Polyethylene Glycol (PEG) included at approximately 60% to 70%; Poly (Ethylene Glycol 400 Dimethacrylate) (DM 400) included at approximately 0.1% to 1%; Poly (Ethylene Glycol Monomethacrylate) (MM) included at approximately 0.1% to 1%; 2-Hydroxyethyl Methacrylate (HEMA) included at approximately 0.01% to 0.5%; Tetracycline included at approximately 0.1% to 1%; WAKO 201 included at approximately 0.1% to 1%; 2,2'-Azobis(2-methylpropionamidine) dihydrochloride (V50) included at approximately 0.01% to 0.1%; H₂O included at approximately 1% to 5%; High Molecular Weight Polyethersulfone (HMW PES) included at approximately 8% to 15%;

dimethylformamide (DMF) included at approximately 8% to 15%; and N-methyl-2-pyrrolidone (NMP) included at approximately 1% to 10%.

[0069] The formulation was mixed at approximately 40 °C to 50 °C for approximately 40 to 50 h. and cast on a belt at speed of approximately 90 inch/minute (in/min) to 100 in/min at a belt temperature of approximately 20 °C to 30 °C and a humidity of approximately 70% to 80%. The cast membrane was leached with DI water for approximately 5 to 15 min, followed by 50% ethanol/50% DI water for approximately 5 to 15 min, and approximately 80 °C to 90 °C DI water for approximately 5 to 15 min. The membrane sample was passed through an oven and approximately 55 °C to 65 °C for approximately 5 to 15 min.

EXAMPLE 2

[0070] This example demonstrates an illustrative protocol for the preparation of a porous membrane according to an embodiment of the disclosure.

[0071] A formulation was prepared having the following components and percentages by weight of the formulation: Polyethylene Glycol (PEG) included at approximately 30% to 40%; trimethylolpropane trimethacrylate (TMPTMA) 400 included at approximately 0.05% to 0.5%; 2-Hydroxyethyl Methacrylate (HEMA) included at approximately 0.05% to 0.5%; poly(ethylene glycol) dimethacrylate (PEGDM) included at approximately 0.1% to 1%; ammonium persulfate (AMPS) included at approximately 0.01% to 0.1%; RO Water included at approximately 1% to 10%; Plasdane K90 included at approximately 1% to 5%; polyether sulfone (PES) included at approximately 5% to 15%; and N-methyl-2-pyrrolidone (NMP) included at approximately 40% to 50%.

[0072] The formulation was mixed at approximately 40 °C to 50 °C for approximately 40 to 50 h. and quenched with water at a speed of approximately 5 to 10 feet/min. with a water temperature of approximately 45 °C to 50 °C. The cast membrane was leached with DI water for approximately 5 to 15 min, followed by 50% ethanol/50% DI water for approximately 5 to 15 min, and approximately 80 °C to 90 °C DI water for approximately 5 to 15 min. The membrane sample was passed through an oven and approximately 55 °C to 65 °C for approximately 5 to 15 min.

EXAMPLE 3

[0073] This example demonstrates illustrative protocols for determining the thickness and the K_L of the porous membranes of Examples 1 and 2.

[0074] Membrane Thickness: The plunger was manually raised, the membrane was inserted into the federal thickness gauge, and the plunger was manually lowered. The thickness value was subsequently displayed and recorded.

[0075] Membrane K_L : The porous membrane was cut into squares that were wet with water. The wet membrane was loaded into the sample holder of a K_L stand. The K_L point was determined to be the point at which the air flow rate was dramatically increased at a given air pressure.

[0076] Tables 1 and 2 demonstrate the thickness and K_L of the porous membranes of Example 1 and Example 2, respectively.

Table 1. K_L and Thickness of the Porous Membranes of Example 1.

Membrane	Thickness (mil)	K_L (psi)
1	2.35	52.35
2	2.52	50.67
3	3.28	47.93
4	3.93	51.47
5	4.36	55.00
6	4.87	55.77
7	4.41	56.23
8	3.59	58.33
9	2.88	60.03
10	2.59	60.87
11	2.61	66.10
12	2.93	64.77
13	3.65	62.80
14	4.48	60.70
15	2.56	54.75
16	2.85	54.48
17	3.55	54.23
18	3.44	64.65
19	3.48	63.80
20	3.42	55.90

21	3.45	61.77
22	3.53	68.20
23	3.43	63.43
24	3.40	57.57
25	3.47	65.33
26	3.47	64.97
27	3.55	70.20

Table 2. K_L and Thickness of the Porous Membranes of Example 2.

Membrane	Thickness (μm)	K_L (psi)
1	95.3	40.8
2	98.4	29.1
3	78.3	37.1
4	98.5	42.2
5	90.8	30.2
6	100.0	40.7
7	101.8	38.6
8	105.0	32.8
9	103.8	47.6
10	97.4	54.2
11	88.3	38.8
12	78.1	33.0
13	94.7	37.9
14	85.8	45.2
15	83.9	46.0
16	98.5	43.5
17	117.8	49.4
18	113.0	38.6

[0077] As seen in Tables 1 and 2, the thickness and K_L were determined for 27 porous membranes of Example 1, which had a thickness of 2.35 mil to 4.87 mil and exhibited a K_L value of 47.93 psi to 70.20 psi. The thickness and K_L were also determined for 18 porous

membranes of Example 2, which had a thickness of 78.1 μm to 117.8 μm and exhibited a K_L value of 29.1 psi to 54.2 psi.

EXAMPLE 4

[0078] This example demonstrates an illustrative protocol for determining the bacterial challenge ratings / log reduction value of the porous membranes of Examples 1 and 2.

[0079] The porous membrane, required solutions, and equipment were autoclaved. A *B. Dim* bacterial challenge solution was prepared with a titer of approximately 10^5 CPU/mL to 10^{10} CPU/mL. The membrane was loaded into the bacterial challenge test stand, and a capture membrane (e.g., GN membrane) was attached onto the downstream of the membrane sample. The membrane was wet with sterile water and pre-challenged with sterile air at a pressure of approximately 75% to 85% of the membrane K_L . The *B. Dim* bacterial solution was flowed through the membrane samples. The membrane was post-challenged with sterile air at a pressure of approximately 75% to 85% of the membrane K_L . The capture membrane was cultured to form plaque at approximately 30°C to 40°C for approximately 1 to 3 days. The bacteria density was counted to determine the log reduction value, which was calculated as: $LRV = \log_{10} \left(\frac{\text{Total Challenge}}{\text{Total Recovery}} \right)$, where the total recovery was measured as CFU in filtrate.

[0080] The relationship between bacterial recovery and retention (LRV) to K_L was shown in FIGs. 2A and 2B for the membranes of Example 1. The bacterial challenge (BC) cliff value, which was defined as the threshold K_L value above which the LRV will be stable and the bacteria recovery will reach zero, was determined to be 56 psi. The lower specification limit, which was calculated as 10% above the BC cliff value, for the membranes of example 1 was therefore determined to be 62 psi. This graph demonstrates that for the isometric membranes of Example 1 the K_L value has a greater impact on bacterial retention than membrane thickness. This suggests that thinner isometric layers could be used in dual layer membranes without loss of bacterial retention ability.

[0081] The relationship between bacterial retention (LRV) to water bubble point (WBP) was shown in FIG. 3 for the membranes of Example 2. The bacterial challenge (BC) cliff value, which was defined as the threshold WBP value above which the LRV will be larger than 4.0 with a range of ± 0.5 , was determined to be 38 psi. This graph demonstrates that for the asymmetric membranes of Example 2 the K_L value has a greater impact on bacterial retention than membrane thickness. This suggests that thinner asymmetric layers could be used in dual layer membranes without loss of bacterial retention ability.

[0082] Tables 3 and 4 demonstrate the bacterial challenge rating and LRV of the porous membranes of Example 1 and Example 2, respectively.

Table 3. Bacterial Challenge Data for the Porous Membranes of Example 1.

Pre-Challenge (psi)	44.24 – 68.17
Post-Challenge (psi)	44.96 – 68.17
Total Challenge CFU Total	1.8E+09 – 6.0E+09
Total Recovery CFU Total	0.0E+00 – >200
Titer Reduction	2.7E+07 – 6.0E+09
CFU/cm ²	1.4E+07 – 4.8E+07
Log Reduction Value	7.2 – 9.8

Table 4. Bacterial Challenge Data for the Porous Membranes of Example 2.

Pre-Challenge (psi)	26.11 – 55.84
Post-Challenge (psi)	26.11 – 55.11
Total Challenge CFU Total	1.40E+09 – 8.85E+09
Total Recovery CFU Total	3.20E+02 – 1.30E+09
Titer Reduction	4.31 – 2.77E+07
CFU/cm ²	1.10E+07 – 6.97E+07
Log Reduction Value	0.63* – 7.44

[0083] As seen in Tables 3 and 4, the bacterial challenge ratings and LRV were determined for 27 porous membranes of Example 1. The bacterial challenge rating was determined to be 1.4E+07 .CFU/cm² – 4.8E+07 CFU/cm² and the log reduction value was 7.2 – 9.8. The bacterial challenge ratings and LRV were also determined for 18 membranes of Example 2. The bacterial challenge rating was determined to be 1.10E+07 .CFU/cm² – 6.97E+07 CFU/cm² and the log reduction value was 0.63 – 7.44. * Lower values for the LRVs of some of the membranes of Example 2 can be attributed to several variables. For example, the pressure may not have been obtainable for the pre- and post-challenge, which could be due to the presence of small defects. Alternatively, a lower thickness of the retentive portion could result in a low LRV. The retention is a function of both pore size and effective thickness, not the total thickness, of the retentive portion. Therefore, if the thickness is too low, the retention may be low as well.

EXAMPLE 5

[0084] This example demonstrates an illustrative protocol for determining the mean flow pore size of the porous membranes of Examples 1 and 2.

[0085] The porous membrane was cut into small discs of approximately 20 mm to 30 mm and wet with Porefil with 16 dynes/cm. The wet membrane sample was loaded into the sample holder of a Porolux™ 1000 commercially available from Porometer, headquartered in Belgium. The pressure range was set to approximately 95 psi to 105 psi. Approximately 45 to 55 steps were set and the porometer was initiated. The porometer reported the mean flow pore size based on the gas flow rate – gas pressure function.

[0086] Tables 5 and 6 demonstrate the mean flow pore size of the porous membranes of Example 1 and Example 2, respectively.

Table 5. Mean Flow Pore Size of Porous Membranes of Example 1.

Membrane	Mean Flow Pore Size (nm)
1	313
2	311
3	329
4	391
5	343
6	340

Table 6. Mean Flow Pore Size of Porous Membranes of Example 2.

Membrane	Mean Flow Pore Size (nm)
1	384
2	385
3	412

[0087] As seen in Tables 5 and 6, respectively, the porous membranes of Example 1 exhibited a mean flow pore size of 311 nm to 391 nm, and the porous membranes of Example 2 exhibited a mean flow pore size of 384 nm to 412 nm.

EXAMPLE 6

[0088] This example demonstrates illustrative methods used to determine the porosity of the porous membranes of Example 2.

[0089] Scanning Electron Microscope: The porous membrane was imaged using a Phenom™ XL G2 Desktop Scanning Electron Microscope, commercially available from Thermo Fisher Scientific, Inc. (Waltham, Massachusetts). Image J software was utilized to calculate the porosity of the porous membrane.

[0090] Porosimetry Instrument: The porous membrane sample was loaded into a Mercury Poremaster GT 33, commercially available from Quantachrome Instruments (Boynton Beach, Florida). The instrument pushed mercury through the membrane pores and recorded the amount of mercury that went into the membrane samples as a function of pressure. Based on this data, the instrument calculated the pore size and distribution.

[0091] Table 7 demonstrates the mean flow pore size of the porous membranes of Example 2.

Table 7. Porosity of the Membranes of Example 2.

	Membrane 1	Membrane 2	Membrane 3	Membrane 4	Membrane 5	Membrane 6
Zone 1	53%	54%	46%	47%	42%	41%
Zone 2	42%	42%	31%	34%	49%	49%
Zone 3	30%	30%	23%	26%	46%	46%
Zone 4	27%	25%	12%	16%	46%	44%

[0092] As seen in Table 7, the porous membranes of Example 2 had a Zone 1 porosity of approximately 41% to 54%, a Zone 2 porosity of approximately 31% to 40%, a Zone 3 porosity of approximately 23% to 46%, and a Zone 4 porosity of approximately 12% to 46%.

EXAMPLE 7

[0093] This example demonstrates an illustrative method used to determine the water flow rate of the porous membrane.

[0094] A water flow rate test stand was used. The porous membrane was cut into squares which were wet with water. Membrane samples were loaded into the sample holder and clamped through air activated devices. The instrument flowed water through the membrane at an approximate pressure of 5 psi to 15 psi. After the air bubbles were bled off and the

instrument was stabilized, the water flow rate value was recorded for an average of 5 to 30 seconds using a mass flow meter.

[0095] The membranes of Example 1 had a water flow rate of 20 to 60 mL.min⁻¹.cm⁻² at 10 psi and 29 °C water temperature. The membranes of Example 2 had a water flow rate of 40 to 120 mL.min⁻¹.cm⁻² at 10 psi and 29 °C water temperature.

EXAMPLE 8

[0096] This example demonstrates an illustrative method used to determine the burst stretch, burst strength, and burst elongation of the porous membranes.

[0097] An Instron[®] 5543 burst tester commercially available from Instron[®], headquartered in Norwood, Massachusetts, or an equivalent, is utilized. The porous membrane is cut into discs with a diameter of approximately 75 mm to 100 mm and inserted into the sample holder. The instrument applies force of approximately 0.1 to 5 lbs/second to the membrane until it breaks. The travel distance and or membrane elongation is simultaneously recorded. The maximum force at which the membrane is broken is defined as the membrane burst force. The maximum of the membrane elongation at which the membrane is broken, divided by the original membrane size, is defined as the membrane stretch percentage. The burst force divided by the membrane thickness is defined as the normalized burst force.

[0098] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0099] The use of the terms “a” and “an” and “the” and “at least one” and similar referents in the context of describing the disclosure (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term “at least one” followed by a list of one or more items (for example, “at least one of A and B”) is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a

shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the disclosure and does not pose a limitation on the scope of the disclosure unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the disclosure.

[0100] Preferred embodiments of this disclosure are described herein, including the best mode known to the inventors for carrying out the disclosure. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the disclosure to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the disclosure unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. A porous membrane comprising
 - a. an upstream porous asymmetric polymeric layer having an asymmetric layer thickness, and a porous structure reducing the passage of bacteria therethrough;
 - b. a downstream porous isometric polymeric layer contacting the porous asymmetric layer, the porous isometric layer having an isometric layer thickness and a porous structure reducing the passage of bacteria therethrough;wherein the porous membrane has a total membrane thickness; and the isometric layer thickness is from 45% to 80% (e.g., from 48 to 80%) of the asymmetric layer thickness.
2. The membrane of claim 1, wherein the mean flow pore (MFP) size of the isometric layer is from 310 nm to 400 nm (e.g., from 311 nm to 393 nm).
3. The membrane of claim 1 or 2, wherein the MFP of the asymmetric layer is from 380 nm to 420 nm (e.g., from 384 nm to 412 nm).
4. The membrane of any one of claims 1-3, wherein the quantitative bubble point (K_L) of the isometric layer is from 45 psi to 75 psi.
5. The membrane of any one of claims 1-4, wherein the K_L of the asymmetric layer is from 25 psi to 55 psi.
6. The membrane of any one of claims 1-5, wherein the log reduction level (LRV) of the isometric layer is from 7 to 10 (e.g., from 7.2 to 9.8).
7. The membrane of any one of claims 1-6, wherein the LRV of the asymmetric layer is from 2.5 to 7.5.
8. The membrane of any one of claims 1-7, wherein the isometric layer thickness is from 55 μm to 125 μm .
9. The membrane of any one of claims 1-8, wherein the asymmetric layer thickness is from 75 μm to 120 μm .
10. The membrane of any one of claims 1-9, wherein the total membrane thickness is from 130 μm to 245 μm .
11. The membrane of any one of claims 1-10, wherein the asymmetric layer comprises four zones each having a thickness and porosity:
 - a. a first zone having a first zone thickness from 20 μm to 40 μm and a porosity from 40% to 55% (e.g., from 41% to 54%);

- b. a second zone having a second zone thickness from 10 μm to 30 μm and a porosity from 30% to 50% (e.g., from 31% to 49%);
 - c. a third zone having a third zone thickness from 10 μm to 30 μm and a porosity from 20% to 50% (e.g., from 23% to 46%); and
 - d. a fourth zone having a fourth zone thickness from 5 μm to 15 μm and a porosity from 10% to 50% (e.g., from 12% to 46%).
12. The membrane of claim 11, wherein
- i. the first zone that has a mean pore size from 1300 nm to 1800 nm;
 - ii. the second zone that has a mean pore size from 700 nm to 1200 nm;
 - iii. the third zone that has a mean pore size from 400 nm to 800 nm; and
 - iv. the fourth zone that has a mean pore size from 400 nm to 900 nm.
13. The membrane of any one of claims 1-12, wherein the isometric layer is capable of substantially reducing the passage of bacteria therethrough.
14. A method of processing a fluid, comprising passing the fluid through the membrane of any one of claims 1-13, in the direction from the asymmetric layer toward the isometric layer.
15. A method of making the porous membrane of any of claims 1-13, the membrane having an upstream porous asymmetric layer and a downstream porous isometric polymeric layer contacting the porous asymmetric layer, comprising:
- a. preparing a first solution comprising a first polymer and a solvent for the first polymer;
 - b. preparing a second solution comprising a second polymer and a solvent for the second polymer;
 - c. casting the first solution onto a support with a first speed;
 - d. casting the second solution on the first solution and forming a pre-membrane;
 - e. quenching the pre-membrane in a fluid at a second speed;
 - f. leaching the pre-membrane; and
 - g. drying the membrane.