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(54) Benævnelse: **Systems for water extraction**

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(57) Sammendrag:

**The present invention relates to a water extraction system comprising a flow cell comprising a membrane; said membrane comprising an active layer comprising immobilized aquaporin water channels and a support layer, and said membrane having a feed side and a non-feed side; and an aqueous source solution in fluid communication with the feed side of the membrane.**

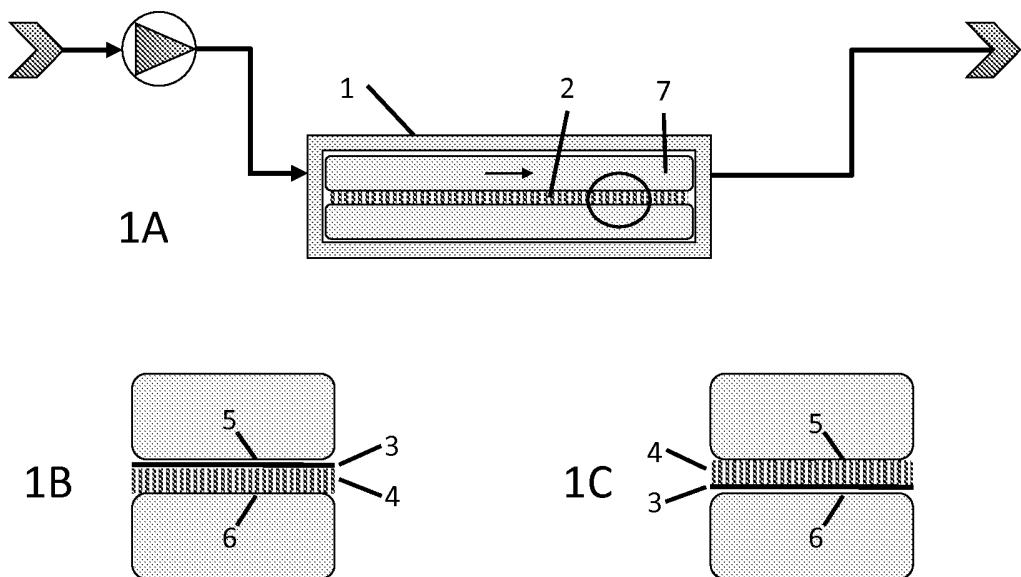


Fig. 1

## SYSTEMS FOR WATER EXTRACTION

### FIELD OF THE INVENTION

The present invention relates to a system for water extraction said system comprising

- 5 a flow cell housing a filter membrane, where said membrane has an active layer comprising immobilized aquaporin water channels, and a porous support layer, and where an aqueous source solution is in fluid communication with said membrane.
- In addition, the invention relates to systems for removal of contaminants from water sources and systems for generation of diluted nutrient solutions for irrigation purposes
- 10 using fertilizer drawn forward osmosis.

### BACKGROUND OF THE INVENTION

Water is the most essential component of life. However, with the growing scarcity of clean water, more and more interest is being paid to extraction of clean water from seawater and industrial water and to treatment of industrial process water and difficult

- 15 wastewater streams. There is also an interest in the possibility of gentle water extraction from valuable solutions - from food streams to solutions of proteins and peptides or valuable small organic compounds.

Among different water purification techniques, reverse osmosis, forward osmosis and nanofiltration have become popular for water extraction because of their effectiveness

- 20 in removing low molecular weight solutes, such as small organic compounds and ions. However, these water extraction techniques are still energy-intensive and not always sufficiently selective. Examples are contaminants, such as dissolved boron compounds naturally present in seawater and in contaminated groundwater, and which can pose a problem in desalinated water for irrigation and drinking water, and
- 25 arsenic compounds that are frequently present in natural surface and ground water sources, e.g. in alluvial plains and moraine deposits.

Kim et al. 2012 studied boron rejection in various FO and RO water filtration experiments and found a maximum boron retention of about 50 to 55 % in FO mode.

However, this low boron filtration efficiency may necessitate several filtration cycles

- 30 in order to obtain a desired low boron content in the resulting filtrate. Thus it is crucial to develop improved water extraction systems, such as systems that are able to remove water contaminants, such as boron or arsenic, and preferably in few or only

one filtration step(s). In addition, it is a purpose of the invention to provide a water extraction system adapted for fertilizer draw forward osmosis (FDFO), where seawater or any suitable saltwater source can be used as a feed solution and a concentrated inorganic plant nutrient solution is used as a draw solution resulting in a 5 final fertilizer solution having a sufficiently low osmolality or boron content as to allow it to be used as a liquid fertilizer, e.g. as irrigation water with added nutrients.

## SUMMARY OF THE INVENTION

It is an object of the present invention to provide a water extraction system utilizing 10 aquaporin water channels immobilized or filter membrane supported, such as in the form of a supported or immobilized liquid membrane formulation. With reference to Fig. 1 said system comprises a flow cell (1) comprising a membrane (2) where said membrane comprises an active layer (3) comprising aquaporin water channels and a support layer (4), and said membrane having a feed side (5) and a non-feed side (6); 15 and said system further comprising an aqueous source solution (7) in fluid communication with said feed side. The present invention provides a novel system for selective water extraction, wherein a filter membrane incorporating aquaporin water channels, such as the aquaporin Z water channels, provide to the system the unique and selective water transporting properties of said channels, i.e. highly efficient water 20 flux, high salt rejection, low energy consumption in forward osmosis operation mode, high rejection of small organic solutes, intrinsic low fouling propensity, and robust operation conditions, especially of the membranes used in the system.

Other objects of the invention will be apparent to the person skilled in the art from the following detailed description and examples.

## 25 BRIEF DESCRIPTION OF THE DRAWINGS

**Fig. 1A** shows a schematic diagram of the essential features of a water extraction system, wherein: (1) is the flow cell; (2) is the membrane; and (7) is the aqueous source solution. **Fig. 1B** shows the embodiment where the active layer (3) is on the feed side (5) of the membrane and the support layer (4) is on the non-feed side (6) of 30 the membrane. **Fig. 1C** shows the embodiment where the support layer (4) is on the feed side (5) of the membrane and the active layer (3) is on the non-feed side (6) of the membrane.

**Fig. 2** shows a schematic diagram of a Forward Osmosis (FO) system for water extraction from a feed stream, wherein: (10) is the feed stream; (1) is the flow cell with the membrane (2); (11) is the concentrated feed stream; (8) is the draw solution in fluid communication with the draw side of the membrane; and (9) is a draw solution concentration unit (9).

**Fig. 3** shows a schematic diagram of a Fertilizer Drawn Forward Osmosis (FDFO) desalination system, wherein: (10) is the feed stream, e.g. seawater; (13) is the concentrated fertilizer solution; (1) is the flow cell with the membrane; (12) is the partly diluted fertilizer solution which can be re-circulated to achieve higher degree of dilution; (14) is the additional freshwater tank for final adjustment of the degree of dilution of the fertilizer solution; (11) is the concentrated feed stream, e.g. up-concentrated seawater; (15) is the diluted fertilizer solution ready for use.

**Fig. 4** shows a schematic diagram of a Reverse Osmosis (RO) system, wherein: (18) is the feed tank; (16) is a pump; (17) is a valve; (19) is the permeate and (20) is the permeate tank. The flow from the pump through the flow cell and back to the valve is a pressurized flow.

**Fig. 5** shows a schematic diagram of a Forward Osmosis (FO) system for desalination with regeneration of the draw solution to extract the product water, wherein (21) is the feed stream, e.g. seawater; (1) is the flow cell with the membrane (2); (22) is the concentrated feed stream; (23) is the concentrated draw solution; (8) the draw solution in fluid communication with the flow cell; (24) the diluted draw solution; (9) the draw solution recovery system; and (25) the desalinated product water, free of draw solution solutes.

**Fig. 6** shows a schematic diagram of a pressure retarded osmosis (PRO) system, wherein: (1) is the flow cell with the membrane (2); (26) is the feed stream, e.g. fresh water or seawater having a lower osmolality than the draw stream; (16) is a pump; (27) is the feed water bleed; (28) is the draw stream, e.g. seawater or brine; (29) is a pump; (30) is the diluted and pressurized draw stream; (31) is a turbine to generate power; (32) and (34) are depressurized draw water; and (33) is a pressure exchanger to assist in pressurizing the incoming draw stream.

**Fig. 7** shows a schematic diagram of a FO concentrator, wherein (41) is the base unit containing a flow inlet and flow outlet to ensure an optimal draw solution flow profile beneath the membrane (45); (42) is the disposable top unit; the membrane (45) is

secured and sealed to the top unit together with an additional seal (43) to the base unit; (44) is an optional flow generator to stir the solution in the top unit; (46) is an inline monitoring system to monitor and continuously display the degree of concentration in the top unit feed solution, e.g. the volume and weight can be inspected visually; (47) is the feedback loop mechanism designed to stop the concentration process once the desired concentration is reached; (48) is a pump to recirculate the draw solution; and (49) is a disposable draw solution pouch containing customized draw solution for different concentration processes.

5 **Figs. 8** shows a schematic diagram of a modified FO concentrator, wherein (41) is the base unit containing a customized flow inlet and flow outlet to ensure an optimal draw solution flow profile beneath the membrane (45), the base unit contains a securing mechanism for the disposable top unit (42); (43) is an O-ring to secure and seal the flow cell; (44) is an optional flow generator to stir the solution in the top unit; (46) is an inline monitoring system to monitor and continuously display the degree of concentration in the top unit feed solution; (47) is the feedback loop mechanism designed to stop the concentration process once the desired concentration is reached; (48) is a pump to recirculate the draw solution; and (49) is a disposable draw solution pouch containing customized draw solution for different concentration processes; and (50) is an optional mesh support above the membrane (45) to provide stability.

10 15 **Figs. 9** shows a schematic diagram of the top unit of the FO concentrator in Fig. 8 seen from above, wherein (51) are means for the clamping together of the top and the base units.

20 **Fig. 10** shows a schematic diagram of the base unit of the FO concentrator in Fig. 8 seen from above.

## 25 DETAILED DESCRIPTION OF THE INVENTION

More specifically, the invention relates to systems for water extraction as detailed below.

### **Water extraction system with removal of contaminants**

30 The present invention relates to systems using RO and or FO for the removal of contaminants, such as trace contaminants including heavy metals and toxic inorganic compounds, from water sources. Examples include removal of boron contamination from fresh water sources to be used for various purposes where boron is unwanted, e.g. for human consumption. Boron is an especially troublesome contamination in sea

water sources when these are used for desalination to produce irrigation water and potable water. Existing technologies require two filtration passes in order to obtain sufficiently low boron concentration. The system of the invention offers removal of up to about 65 % of the dissolved boron in a fresh water source at about neutral pH 5 after only one RO pass and up to about 75 % removal during an FO process at neutral pH, cf. the Example 1 below. Another example is the removal of arsenic contamination where the system of the invention can remove about 100 % after both RO and FO filtration, cf. Example 2 below.

10 **Water extraction system for fertilizer drawn forward osmosis desalination (FDFO)**

Recently, there has been increasing interest in substituting diminishing freshwater sources with desalinated water for irrigation of crops, and further addition of diluted nutrient salt solutions to the irrigation water (FDFO). However, there are 15 disadvantages in connection with the use of available FO membranes, such as the membranes that may be obtained from the Hydro well filter modules (Hydration Technologies Inc.) the disadvantage being mainly the relatively large reverse salt flux (Js) of the nutrient salts, e.g. potassium chloride, where figures as high as 59.58 g/m<sup>2</sup>h have been mentioned in the literature (0.222 mmoles/m<sup>2</sup>s, Phuntsho et al. 2012) or 20 6.8 to 15.3 g/m<sup>2</sup> h (Achilli et al. 2010 using a flat-sheet cellulose triacetate (CTA) membrane from Hydration Technology Innovations, LLC, Scottsdale, AZ). It is desirable to have as low as possible a Js in order to minimize loss of the valuable nutrient ions. Herein we show that it is possible to obtain Js of less than 4 g/m<sup>2</sup> h in an 25 FO system using a TFC-AqpZ membranes with amphiphile P8061 as vesicle forming substance (prepared according to the experimental section below), a 2 M KCl solution as draw, and deionized water with 5 µM calcein as feed, cf. the table below:

Draw, FO chamber	J <sub>w</sub> [L/m <sup>2</sup> h]	J <sub>s, total</sub> [g/m <sup>2</sup> h]	R <sub>calcein</sub> [%]	Run time Min
2 M KCl, CF042	10.3	3.08	99.94	900
2 M KCl, CF042	11.47	3.32	99.95	900
2 M KCl, CF042	10.87	3.91	99.95	900
2 M KCl, CF042	12.56	3.69	99.97	900
2 M KCl, CF042	11.87	3.03	99.78	900
2 M KCl, CF042	10.32	3.49	99.96	900
2 M KCl, CF042	10.86	3.36	99.94	900

The table clearly shows that a consistent low reverse salt flux of average 3.41 [g/m<sup>2</sup>h]

5 can be obtained for the potassium salt KCl.

In addition, the present invention provides a low-energy means of reducing freshwater consumption in agriculture by as much as about 40% through the utilization of lower-grade water supplies such as polluted groundwater, brackish water and even seawater.

10 The water extraction system of the invention with its unique aquaporin membrane, such as in the form of a TFC membrane as prepared according to the experimental section herein, is used in combination with a liquid concentrated fertilizer draw solution to selectively extract clean water from the lower-grade water supplies herein utilized as feed source. The end result is a diluted liquid plant nutrient solution, which 15 requires up to a calculated 40 % less freshwater to be ready for use for agricultural irrigation and fertilization. In the example below we describe how membrane tests have shown proof-of-concept in the case where the lower-grade water supply is relatively low-salinity of about 10 to 15 o/oo seawater from Øresund in Denmark.

## 20 **A water extraction system with separation of urea from urine in space**

We have together with scientists from the NASA Ames facilities in Palo Alto (CA, US) performed first real field tests with the system comprising an -aquaporin membrane. Tests concluded that the water extraction system comprising the specific TFC-aquaporin membranes show superior rejection values to urea (>90%) when

compared to existing forward osmosis membranes, cf. Hill & Taylor (2012). The water extraction system of the invention will contribute to the major effort of reducing the mass needed to transport into space on manned space missions, i.a. by re-circulating bodily fluids from the astronauts. It was concluded that a water extraction system

5 according to the invention comes very close to fulfilling the requirements for a simple, lightweight and reliable system to extract potable water from body fluids in space.

In May 2012, scientists from Aquaporin and NASA Ames successfully repeated testing at the NASA Ames facilities with up-scaled TFC-aquaporin membrane samples ( $500 \text{ cm}^2$ ). The up-scaled membrane samples performed identically to the

10 initial samples thus proving the stability of the membrane production protocols. Based on the successful second tests, Aquaporin and NASA Ames are investigating how to produce the first prototype system for yellow water re-use in space.

#### **A water extraction system with separation of urea from RO permeate in dairy**

#### **industries**

Background: Many industrial effluents contain high concentrations of compounds including non-polar solutes such as urea, which are not removed by de-ionized water processes or reverse osmosis membranes. Said non-polar solutes are often chemically stable, and therefore not easily destroyed by UV sterilization processes. The state of

20 the art treatment of urea wastewaters generally involves two steps: first, the

hydrolysis of urea into ammonia and carbon dioxide and, second, the elimination of ammonia. Current methods mostly rely on anaerobic conditions for the biological treatment of high-strength urea wastewaters. However the required nitrifying bacteria have slow growth rates, a small acceptable pH-range, and are often inhibited by other

25 wastewater contaminants (e.g. dicyandiamide). An advantage of the present system is that it is based on the use of a flow cell equipped with a membrane having

immobilized aquaporin water channels, said membranes have shown very high urea removal in lab scale, cf. Example 7 below. This will eliminate the need for bioreactor technology and in principle allow for simple retrofitting of existing unit operations

30 (e.g. polishing steps) currently employed in urea removal.

The high rejection and water flux properties of the aquaporin membrane and the intrinsic low fouling propensity makes it feasible and valuable to employ these biomimetic membranes into large scale industrial systems for urea removal, where

there is a potential for fouling and/or a need to up-concentrate small neutral solutes (e.g. urea) – not readily achievable with current technology – membrane based or other. The high rejection towards urea enables the system of the invention to be used for treatment of wastewater streams containing high amounts of urea, such as is present in process water from dairies. In one embodiment of the water extraction system of the invention, the aquaporin membrane, such as a TFC-membrane comprising immobilized aquaporin water channels, will be used together with a high osmolarity draw solution (e.g. seawater e.g. from Kattegat) to extract close to urea-free water from the wastewater streams. This low-energy water extraction system will effectively reduce disposal costs through wastewater volume reduction.

**Water extraction system for up-concentration of solutes in a wide range of aqueous solutions by forward osmosis, cf. Fig. 6**

In this system a high osmolarity or osmolality draw solution, such as brine, is used in combination with an aquaporin membrane, such as the TFC membrane prepared as described herein, to up-concentrate aqueous solutions in a forward osmosis process. Aqueous solutions of interest include difficult wastewater streams, pharmaceutical and biological product solutions and liquid foodstuffs. An exemplary embodiment is a system for up-concentration of organic molecules of a wide range of molecular sizes, such as amino acids and oligopeptides to proteins including membrane proteins which are normally concentrated to a desirable degree by centrifugal concentrators, e.g. using Pierce Concentrators that are available for 3K, 10K, 30K, and 100K molecular-weight cutoff (MWCO), and which concentrate and desalt biological samples with polyether sulfone (PES)-membrane ultrafiltration centrifugal devices. Advantages of the system according to the invention include a very gentle extraction of water, low peptide or protein loss, ability to concentrate a wide range of molecular sizes from amino acids to small peptides to large membrane proteins, a concentration process that is controllable and can be automated for high throughput in contrast to centrifugal concentrators presently on the market, or alternatively, concentrating the sample solution by vacuum drying, which is, however, often followed by severe loss of sample material and in additional contaminations. The system of the invention may be set up with a concentrator cell with either fixed aquaporin membrane for single use or with a removable aquaporin membrane as shown in Fig. 8. Thus, where the aquaporin membrane can be removed, e.g. for cleaning, and refitted into the cell, it is suggested

that an EDTA or citric acid treatment as described in examples 4 and 5 below could be applied to the membrane while preserving the water extracting properties of the system.

## 5 Definitions

"Feed solution" means a solution of solutes in water.

"Draw solution" means a solution of higher osmotic pressure, relative to that of the feed solution. The draw solution may comprise a draw solute selected from at least one of: water-soluble inorganic chemicals and water-soluble organic chemicals. The water-soluble inorganic chemicals may include at least one of  $\text{Al}_2(\text{SO}_4)_3$ ,  $\text{MgSO}_4$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{K}_2\text{SO}_4$ ,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{Fe}_2(\text{SO}_4)_3$ ,  $\text{AlCl}_3$ ,  $\text{MgCl}_2$ ,  $\text{NaCl}$ ,  $\text{CaCl}_2$ ,  $\text{NH}_4\text{Cl}$ ,  $\text{KC}_1$ ,  $\text{FeCl}_3$ ,  $\text{Al}(\text{NO}_3)_3$ ,  $\text{Mg}(\text{NO}_3)_2$ ,  $\text{Ca}(\text{NO}_3)_2$ ,  $\text{NaNO}_3$ ,  $\text{NO}_3$ ,  $\text{NH}_4\text{HCO}_3$ ,  $\text{KHCO}_3$ ,  $\text{NaHCO}_3$ ,  $\text{KBr}$  and their relative hydrates; and wherein the water-soluble organic chemicals include at least one of methanol, ethanol, acetone, glucose, sucrose, fructose, dextrose, chitosan, dendrimer and 2-methylimidazole-based chemicals.

"Forward osmosis" (FO) is an osmotic process in which an osmotic pressure gradient across a semi-permeable membrane results in extraction of water from dissolved solutes. The driving force for inducing a net flow of water through the membrane is an osmotic pressure gradient from a draw solution of higher osmotic pressure relative to that of the feed solution.

"Reverse osmosis" (RO) is a process of extracting water through a semi-permeable membrane from a feed solution against a gradient of osmotic pressure, by applying a mechanical pressure that is higher than the osmotic pressure of the feed solution.

"Semi-permeable membrane" is a membrane that will allow certain molecules or ions to pass through it.

"Osmotic pressure" is the pressure that must be applied to prevent the net flow of solvent through a semipermeable membrane from a solution of lower solute concentration to a solution of higher solute concentration.

The osmotic pressure of a solution depends on the amount of particles in the solution.

For an ideal solution the osmotic pressure is directly proportional to the molality.

"Osmolality" is a measure of the moles (or osmoles) of osmotic active solutes per kilogram of solvent, expressed as osmole/kg. The osmolality of an ideal solution of a non-dissociated compound equals the molality.

Osmolality is typically measured by freezing point depression. A one osmol/kg

aqueous solution has a freezing point of -1.858°C. As an example: a 1 mol solution of eg sugar in 1 kg of water lowers the freezing point with 1.858°C whereas the freezing point depression will be obtained by 0.5 mol in 1 kg of water.

"Osmolarity" is a measure of the osmoles of solute per liter of solution.

5 The "osmotic pressure" can be calculated from the osmolality by using the formula:

$$\pi(\text{bar}) = \text{osmolality} \left( \frac{\text{osmole}}{\text{L}} \right) \times R \times T(K)$$

wherein R is the gas constant (8.3144621 L bar K<sup>-1</sup> mol<sup>-1</sup>).

"Aquaporin" as used herein refers to selective water channel proteins, including AqpZ and SoPIP2;1 prepared according to the methods described by Maria Karlsson et al.

10 (FEBS Letters 537 (2003) 68-72) or as described in Jensen et al. US 2012/0080377 A1.

"Asolectin" as used herein refers to a soybean lecithin fraction [IV-S] which is a highly purified phospholipid product containing lecithin, cephalin, inositol phosphatides & soybean oil (synonym: azolectin).

15 "Block copolymer" as used herein refers to membrane forming or vesicle forming di- and tri-block copolymers having both hydrophilic (A or C) and hydrophobic (B) blocks; the diblock copolymers being of the A-B or C-B type which are able to form bilayers and the triblock copolymers being of the A-B-A or A-B-C type that form monolayers by self assembly, where all of the membranes have the hydrophobic layer 20 in the middle. Examples of useful diblock copolymers and examples of useful triblock copolymers are the following (all from the supplier Polymer Source):

Species	Formula	n <sub>(hydrophobic)</sub>	n <sub>(hydrophilic)</sub>
P7258	EO <sub>48</sub> DMS <sub>70</sub>	70	48
P5809	EO <sub>15</sub> BO <sub>16</sub>	15	16
P8365	EO <sub>25</sub> DMS <sub>8</sub>	8	25
P7259	EO <sub>48</sub> DMS <sub>14</sub>	14	48
P7261	EO <sub>114</sub> DMS <sub>14</sub>	14	114
P3691B	MOXA <sub>6</sub> DMS <sub>35</sub> MOXA <sub>6</sub>	35	12
P8061	MOXA <sub>15</sub> DMS <sub>67</sub> MOXA <sub>15</sub>	67	30
P9548	MOXA <sub>15</sub> DMS <sub>119</sub> MOXA <sub>15</sub>	119	30

where EO-block-DMS-block represents poly(dimethylsiloxane-block-ethylene oxide-block), EO-block-BO-block represents poly(butylene oxide-block-ethylene oxide-

block), and MOXA-block-DMS-block-MOXA-block represents poly(2-methyl-oxazoline-block-dimethylsiloxane-block-2-methyloxazoline).

“Thin-film-composite” or (TFC) membranes as used herein refers to a thin film membrane active layer having an additional aquaporin component, said layer being

5 prepared using an amine reactant, preferably an aromatic amine, such as a diamine or triamine, e.g. 1,3-diaminobenzene (m-Phenylenediamine > 99%, e.g. as purchased from Sigma-Aldrich) in an aqueous solution, and an acyl halide reactant, such as a di- or triacid chloride, preferably an aromatic acyl halide, e.g. benzene-1,3,5-tricarbonyl chloride (CAS No. 84270-84-8, trimesoyl chloride (TMC), 98%, e.g. as purchased from Sigma-Aldrich) dissolved in an organic solvent where said reactants combine in an interfacial polymerization reaction, cf. US 4,277,344 which describes in detail the formation of a polyamide thin film formed at the surface of a porous membrane support, e.g. a polyethersulfone membrane. More specifically, benzene-1,3,5-tricarbonyl chloride can be dissolved in a solvent, such as a C6 – C12 hydrocarbon including hexane (>99.9%, Fisher Chemicals), heptane, octane, nonane, decane etc. (straight chain or branched hydrocarbons) or other low aromatic hydrocarbon solvent, e.g. Isopar™ G Fluid which is produced from petroleum-based raw materials treated with hydrogen in the presence of a catalyst to produce a low odor fluid the major components of which include isoalkanes. Isopar™ G Fluid: Chemical Name: 10 Hydrocarbons, C10-C12, isoalkanes, < 2% aromatics; CAS No: 64742-48-9, chemical name: Naphtha (petroleum), hydrotreated heavy (from ExxonMobil Chemical). Alternatives to the reactant 1,3-diaminobenzene include diamines such as hexamethylenediamine etc., and alternatives to the reactant benzene-1,3,5-tricarbonyl chloride include a diacid chloride, adipoyl chloride etc. as known in the art. To make 15 the active layer a thin film composite layer, an additional component, herein aquaporin water channels, that facilitates water transport are added to the reactant solutions before interfacial polymerization takes place. Said component may or may not participate in the reaction, but preferably is inert to the reaction and becomes immobilised in the thin film formed. Herein, the aquaporin water channels are 20 preferably contained in vesicles, such as proteoliposomes and proteopolymersomes, formed from amphiphilic compounds.

“Proteoliposomes” as used herein are vesicles that typically have a lipid to protein ratio (LPR calculated on a mole basis) of between 25 to 500, such as about 100 to about 200.

“Proteopolymersomes” as used herein are vesicles that typically have a polymer to protein ratio (POPR calculated on a molar basis) of between 25 to 500, such as about 50 to about 100 when using a triblock copolymer and a polymer to protein ratio of between 25 to 500, such as about 100 to about 200 when using a diblock copolymer.

5 “Aquaporin membrane” as used herein refers to a membrane comprising an active layer comprising immobilised aquaporin water channels and a support layer. In said aquaporin membrane the aquaporin water channels are immobilized or more or less embedded or partly embedded in or even supported in or on said active layer. Said active layer is preferably created in close contact with a support layer, such as a  
10 typical polysulfone or polyether sulfone support membrane.

In one embodiment, the membrane comprises an active layer being a thin film composite (TFC) layer comprising aquaporin water channels. In a further embodiment the aquaporin water channels are incorporated in vesicles before incorporation into the TFC layer. In a further embodiment the vesicles into which the aquaporin water  
15 channels are incorporated are liposomes or polymersomes. In a further embodiment liposomes are prepared from lipids such as DPhPC, DOPC, mixed soy bean lipids, asolectin or E. coli mixed lipids. In a further embodiment the polymersomes comprise triblock copolymers of the hydrophile-hydrophobe-hydrophile (A-B-A or A-B-C) type or diblock copolymers of the hydrophile-hydrophobe type (A-B).

20 Said aquaporin water channels are preferably AqpZ channels, but, in principle, all water selective aquaporins, e.g. such as aquaporin Z (AqpZ), Aqp1, GlpF or SoPIP2;1, are useful in the invention. In a further embodiment the aquaporin water channels are AqpZ channels or SoPIP2;1 water channels.

In a further embodiment TFC layer is formed through interfacial polymerization of an  
25 aqueous solution of a di- or triamine with a solution of di- or triacyl halide in an organic solvent, and wherein the aquaporin water channel vesicles are incorporated in said aqueous solution.

The membrane may be manufactured at described by Zhao, Y. et al (2012).

“Flow cell” as used herein represents a filter (or membrane) module with a feed compartment and a non-feed compartment. The flow cell may be adapted for RO, e.g. having a feed solution inlet and a permeate outlet, or the flow cell may be adapted for FO where an inlet and an outlet for feed solution is fitted on one side of the cell to allow fluid communication with the membrane, and an inlet and an outlet for draw solution is fitted on the opposite side of the cell to allow fluid communication with the

opposite side of the membrane. Examples of useful flow cells include the following from Sterlitech Corp, WA, US. (<http://www.sterlitech.com>):

FO cell: CF042-FO (Delrin Acetal or Acrylic)

RO cell: CF042 Crossflow Cell

5 Membranes of size 5.5 cm x 11cm fit into the CF042 cells.

FO/RO cell: SEPA CF II

This cell can have an RO top or an FO top. Membranes of size 13.5 cm x 19 cm fit into the SEPA CF II cell.

## 10 **Cleaning of the membrane in the systems**

Membrane fouling can cause flux decline and affect the quality of the water extraction process. Thus, the systems for water extraction may also include means for maintenance purposes, such as for introducing air or a cleaning solution. The degree of fouling may be controlled such as by measuring flux decline as determined by flow rates of feed and draw solutions at specific points in the water extraction system.

15 With respect of chemical cleaning, Al-Amoudi et al. (2007) gives an overview of cleaning systems for nanofiltration membranes and Porcelli et al. (2010) gives a review of chemical cleaning of potable water membranes. One example of cleaning reagent is citric acid that can provide buffering and has chelating abilities. Further

20 citric acid can disrupt biofilm formation by removing minerals from foulant layers. A second example of cleaning reagent is EDTA (ethylenediamine tetraacetic acid) which provides chelation capacity for metals such as calcium and dispersed minerals in general.

Physical methods for cleaning membrane of the water extraction system include

25 forward and reverse flushing, backwashing, air flushing (also called air scouring) and sponge ball cleaning (Al-Amoudi 2007). In one embodiment, the water extraction system may be cleaned by introducing bubbles into the cleaning solution for air scouring.

## 30 **Robust operation conditions**

The water extraction system of the invention is useful under varied pH and temperature conditions due to the robustness of the aquaporin membrane, which can tolerate pH values as low as pH=2 and as high as pH=11 and temperatures as high as 65 °C and as low as 10 °C. The water flux becomes reversibly reduced during very

high and very low pH and temperature feed values, so that the membrane regains its high initial performance, cf. the tables below:

Results for FO experiments using TFC-AqpZ membrane in a CF042 cell at high and

5 low feed pH:

Amphiphile	n	$J_w$ (L/m <sup>2</sup> h)	$J_s$ (g/m <sup>2</sup> h)	$R_{Ca}$ (%)
P8061 – pH 6.3	14	12.60 ± 1.21	3.88 ± 0.83	99.80 ± 0.22
P8061 - pH 2.0	3	5.60 ± 0.79	-	-
P8061 re-run pH 6.3	3	12.22 ± 0.95	4.32 ± 0.26	99.71 ± 0.19
P8061 - pH 11.0	3	7.44 ± 0.57	-	-
P8061 re-run pH 6.3	3	11.49 ± 2.42	4.17 ± 0.49	99.55 ± 0.16

The results in the table above clearly shows that the FO system is pH sensitive and pH tolerant and that the membrane performance as measured by water flux ( $J_w$ ), reverse salt flux ( $J_s$ ) and calcein rejection ( $R_{Ca}$ ) is reversible at neutral pH.

10

In addition, the water extraction system of the invention is heat tolerant. However, it was found that operation at both 10°C and 65°C has an impact FO performance. At 65°C extremely high water fluxes are accompanied by higher reverse salt flux and lower forward rejection values. Operation at 10°C results in a lower water flux and a

15

high retention. Operation at 50°C obtains water fluxes and salt rejection values that are comparable to the performance standards of the reference system at 22°C for a TFC-aquaporin membrane using P8061 as amphiphilic vesicle forming material (amphiphile) and in a system where the feed solution contains dissolved calcein as a trace material. Finally, it was found that membrane exposure to 10°C and 65°C for 20 about 1200 minutes does not cause any damage to the membrane and that successive standard FO operation of the system was not negatively influenced. Results are given in the table below:

Results for FO experiments using TFC-AqpZ membrane in a CF042 cell at high and low feed temperatures

Amphiphile	n	$J_w$ (L/m <sup>2</sup> h)	$J_s$ (g/m <sup>2</sup> h)	$R_{Ca}$ (%)
P8061 Reference - 22°C	14	12.60 ± 1.21	3.88 ± 0.83	99.80 ± 0.22
P8061 - 65°C	3	22.09 ± 3.93	7.49 ± 3.4	99.75 ± 0.29
P8061 Re-run - 22°C	1	11.55	4.08	99.81
P8061 - 50°C	3	20.16 ± 6.20	3.67 ± 2.41	99.92 ± 0.06
P8061 Re-run - 22°C	1	12.37	2.43	99.70
P8061 - 10°C	3	7.02 ± 0.16	2.43 ± 0.89	99.95 ± 0.02
P8061 Re-run - 22°C	1	13.16	3.30	99.95

The results in the table above clearly shows that the FO system is heat sensitive and  
5 heat tolerant and that the membrane performance as measured by water flux ( $J_w$ ),  
reverse salt flux ( $J_s$ ) and calcein rejection ( $R_{Ca}$ ) is reversible at room temperature.  
The present invention is further illustrated by the following examples which should  
not be construed as further limiting the scope of the invention.

## 10 EXPERIMENTAL SECTION

Vesicle preparation:

Preparation of 1mg/mL Asolectin proteoliposomes, and lipid to protein ratio (LPR) 200 using AqpZ Mw 27233 according to the following protocol:

1. Fill a 50 mL glass evaporation vial with 5 mL of a 2 mg/mL stock solution of asolectin (mW 786.11 g/mol, Sigma) in CHCl3.
- 15 2. Evaporate the CHCl3 using a rotation evaporator for at least 2h to complete dryness.
3. Add 0.8 mL of buffer solution (1.3% octylglucoside (OG) in PBS pH 7.4) to rehydrate the film obtained in the evaporation vial in step 2.
4. Shake the vial at maximum rpm on a platform shaker (Heidolph orbital platform shaker Unimax 2010 or equivalent) until the lipid is dissolved.
- 20 5. Add 1.73 mg of AqpZ in a protein buffer containing Tris pH8, glucose and OG, 10 mg/mL, and rotate vial for 15 min at 200rpm, the AqpZ being prepared according to description above.
6. Slowly add 9.03 ml PBS (pH 7.4 without OG), and shake vial for 15 min at 200rpm.

7. Freeze/thaw the combined solution/suspension on dry ice/40 °C water bath for three times to eliminate possible multilamellar structures.
8. Add 250 mg of hydrated Biobeads (SM2 from BioRad) and rotate vial for 1h at 200rpm at 4°C to adsorb detergent (OG).
- 5 9. Add further 250 mg of hydrated Biobeads and rotate vial for 2 to 3 days at 200 rpm at 4°C.
10. The Biobeads with adsorbed OG are then removed by pipetting off the suspension.
11. Extrude the obtained suspension for about 11 times through a 200 nm polycarbonate filter using an extruder, such as from at least 1 time and up to about 22 times to obtain a uniform proteoliposome suspension (vesicles) suspension.

Instead of using BioBeads, the detergent can be removed on a typical resin column, such as an Amberlite XAD-2.

- 15 15. Protocol for 1mg/ml proteo-polymersomes, protein to polymer ratio (POPR) 50 Polyoxazoline Based Triblock Copolymers, Poly(2-methyl oxazoline-b-dimethyl siloxane-b-2-methyl oxazoline), Moxa 30: DMS 67, Mw 7319 (P8061 purchased from Polymer Source™, Quebec, Canada), AqpZ Mw 27233
  1. Fill a 50 ml glass evaporation vial with 5 ml of a 2 mg/ml stock solution of P8061 in CHCl3.
  2. Evaporate the CHCl3 using a rotation evaporator for at least 2 h to complete dryness.
  3. Add 3.0 mL of buffer solution (1.3% O.G.; 200mM Sucrose; 10mM Tris pH 8; 50mM NaCl) to rehydrate the film obtained in the evaporation vial in step 2.
- 20 25. 4. Shake the vial at 200 rpm on a platform shaker (Heidolph orbital platform shaker Unimax 2010 or equivalent) for 3 hours to obtain dissolution of the copolymer.
5. Add 75 µL of AqpZ in a protein buffer containing Tris, glucose and OG, and rotate vial over night at 200rpm and 4°C.
6. Add 6.88 ml buffer (10 mM Tris pH 8; 50 mM NaCl) slowly while mixing up and down with pipette.
- 30 7. Add 180 mg hydrated Biobeads and rotate for 1h at 200 rpm.
8. Add 210 mg hydrated Biobeads and rotate for 1h at 200 rpm.
9. Add 240 mg hydrated Biobeads and rotate O.N. at 200 rpm 4°C.
10. Add 240 mg hydrated Biobeads and rotate O.N. at 200 rpm 4°C.

11. The Biobeads with adsorbed OG are then removed by pipetting off the suspension.
12. Extrude the suspension for about 21 times through a 200 nm polycarbonate filter using an extruder, such as from at least 1 time and up to about 22 times to obtain a uniform proteopolymersome suspension (vesicles) suspension.

5

TFC active layer preparation:

Materials:

Apolar solvent: Hexane or an isoparaffin solvent, such as Isopar G, ExxonMobil

Chemical

- 10 TMC: 1,2,5 Benzenetricarbonyltrichloride from Aldrich 147532
- MPD: m-Phenyldiamine from Aldrich P23954
- Vesicles: Proteopolymersomes or proteoliposomes prepared as described above, e.g. using p8061- MOXZDMSMOXZ (Poly(2-methyloxazoline-*b*-dimethylsiloxane-*b*-2-methyloxazoline) from Polymer Source Inc., Quebec, Canada, with AQPZ (POPR 50)
- 15 Support membrane: MICROPES 1FPH or 2FPH manufactured by Membrana GmbH.

Interfacial Polymerization:

Interfacial polymerization is a polymerization reaction that is taking place at the interface between two immiscible liquids with different monomers dissolved. Here,

- 20 MPD is dissolved in water and vesicles are added. The porous PES support membrane, e.g. a MICROPES 1FPH or 2FPH membrane from Membrana GmbH is cut in rectangular shape, e.g. 5.5 cm x 11 cm, 13.5 cm x 19 cm, or 20 cm x 25 cm, and soaked in the aqueous solution and the surface is dried just enough to have a dry surface with aqueous solution filled pores. TMC is dissolved in an apolar solvent (hexane or Isopar<sup>TM</sup>) and applied to the surface of the semidried soaked support membrane. The MPD and TMC react at the interface between the two liquids and form a highly cross-linked network of aromatic polyamide. TMC reacts with water to give a carboxylic acid group and HCl, thus the TMC is broken down in the aqueous phase. MPD reacts readily with TMC, thus it does not diffuse far into the apolar solvent. The resulting layer is a highly cross-linked aromatic polyamide film embedded in the support membrane surface with a thickness of approximately 100-700nm. The vesicles become immobilized by being trapped or embedded in the cross-linked polyamide film.
- 30

**Example 1. System for removal of boron contamination in a fresh water source using RO and/or FO**

Fig. 4 shows a system for water extraction with boron removal using a Washguard SST pump (16) and an osmotic cell (Sterlitech CF042) for RO filtration, where said cell holds a 5.7 cm x 11.3 cm TFC-AqpZ membrane prepared as described herein, and wherein a boron contaminated fresh water feed source created by dissolving boric acid to about 5mg/L B in tap water having a mean content of 187 µg/L B, 0.20 µg/L As, 113 mg/L Ca, pH=7.5 (source: HOFOR, Copenhagen 2011) is filtered through said membrane during RO operation mode at a pressure of 125 psi. The resulting 10 permeate can be sampled for ICP-MS boron elemental analysis, e.g. according to Nagaishi & Ishikawa (2009), giving a calculated rejection range based on the obtained analytical data of from about 45% to about 55% rejection comparable to the results obtained by Kim et al. 2012.

Fig. 2 shows a system for water extraction with boron removal using the same feed 15 source as in the RO experiment above and a draw solution of 35g/L NaCl in tapwater (same tap water source as for the feed) in a closed circuit. The FO system uses a Sterlitech CF042P osmotic cell adapted for FO mode, where said cell holds a TFC-AqpZ membrane prepared as described herein, cf. the figure. The FO system is operated with counter-current flow velocities of 50.03 ml/min corresponding to 0.85 20 cm/s, and both active side of membrane against draw and active side of membrane against feed solutions were tested. After 1300 min operation samples for ICP-MS boron elemental analysis were taken from the draw solutions giving a calculated rejection range based on the obtained analytical data of from about 60% to about 85% representing potential for improved rejection during FO compared to the results 25 published by Kim et al. 2012.

**Example 2. System for removal of arsenic contamination in a fresh water sources using RO and/or FO**

The same RO system as described in Example 1 was used except that an artificially 30 created feed solution of 5mg/L As (arsenic acid dissolved in MilliQ water and adjusted to pH 9.5 using 1N NaOH) is filtered through said membrane during RO operation mode at a pressure of 125 psi. The resulting permeate can be sampled for ICP-MS arsenic elemental analysis, e.g. as described by Grosser (2010), giving a

calculated rejection range based on the obtained analytical data of about 100 % rejection.

The same FO system as described in Example 1 was used except that a feed solution of 5mg/L As in MilliQ water, pH 9.5, and a draw solution of 2M NaCl in MilliQ

5 water was used. After 1300 min operation samples for arsenic elemental analysis were taken from the draw solutions for ICP-MS analysis. The results show that a calculated arsenic rejection based on the obtained analytical data of about 100% can be obtained using FO filtration (both when using the active side of the TFC membrane against the draw solution and using the active side of the TFC membrane against the feed

10 solution).

**Example 3. System comprising an FO concentrator module, e.g. for peptides.**

Method:

FO module is prepared by the following steps:

15 1. water tight fastening, such as gluing with silicone glue or otherwise clamped tight, of a plastic measuring cylinder (such as having a diameter of 1 cm and the like depending on volume to be up-concentrated) to a Plexiglas surface with a corresponding hole of area 0.5 cm<sup>2</sup> or 3.14 cm<sup>2</sup>, where the feed solution will be exposed to the membrane.

20 2. A mesh support is glued immediately underneath.

3. A TFC-AqpZ membrane, such as prepared using 1FPH support membrane and P8061 amphiphilic copolymer for the polymersomes, was prepared as described above, where active side on top is glued under the support or, alternatively, water tight fastened with O-ring.

25 4. Optionally, a rubber gasket may be glued after the membrane.

5. An additional rubber gasket can be added as a cushion when the top part is assembled with the bottom part where the tubing is placed, cf. Fig. 7 or 8 below.

6. The module is now connected to a pump, such as a peristaltic pump where draw solution is recirculated through the system, typically at flow speed of 40 mL/min. An osmotic gradient created by using 2M NaCl in MilliQ water as draw solution drives the movement of water from the feed solution in the measuring cylinder to the draw.

**Detection of feed solute (peptide or protein or other sample):**

In this example a concentrated feed solution of the custom made peptide GGGSGAGKT (available from Caslo Laboratory as a lyophilized trifluoroacetate salt, molecular weight measured by MS of 690.71, purity 98.87%) or of the amino acid L-

5 lysine (from Sigma Aldrich, molecular weight 146.1 g/mol, 97 % purity)) was mixed with equal volumes of LavaPep kit (from gelcompany.com, the kit binds to lysine residues in peptides and is used herein experimentally also to detect the free amino acid) and incubated for 1h in the dark at room temperature. Detection of peptides and L-lysine is done on QuBit with the setting “ssDNA”. Detection range of ssDNA on

10 QuBit: excitation: 400-490 nm, 500-645 nm; emission: 570-645 nm.

Generation of standard curve:

Peptide/lysine in 6 different concentrations ranging from 1000 to 1 $\mu$ g/mL in 9.3 x TES buffer is analysed, the concentrations being suitable due to feed getting concentrated about 2 to 6 times during the up-concentration.

15 Quantification: 10  $\mu$ L of concentrated solution (2 to 5 x conc.) + 90  $\mu$ L 10 x TES buffer to end up at 9.3 x buffer in the dilution + 100  $\mu$ L kit.

Detection range of LavaPep kit: excitation: 405 - 500 nm (green 543, 532 nm, blue 488 nm, violet 405 nm or UVA); emission: max 610 nm (band pass or 560 long pass) Excitation: 540 +-10 nm; emission: 630 +- 10 nm

20 The concentrated feed solution of the peptide/lysine is detected and measured as follows:

1. Start feed: about 50  $\mu$ g/mL peptide or lysine in 1 x TES buffer
2. Run assay
3. Collect concentrated solution

25 4. 10  $\mu$ g/mL conc. peptide sol. + 90  $\mu$ g/mL 10 x TES buffer + 100  $\mu$ g/mL kit

5. Incubation in the dark for 1 h at room temperature

6. Measure fluorescence counts in QuBit

7. Read concentration from standard curve

30 Solutions:

Feed: 200  $\mu$ g/mL L-lysine (amino acid example), or 50  $\mu$ g/mL – 500  $\mu$ g/mL of peptide in 1x TES buffer, or 500  $\mu$ g/mL of bovine serum albumin (BSA) used as a protein example in PBS buffer (0.303 Osm)

Draw solution: 2M NaCl (200 mL) in MilliQ water

Peptide, protein and L-lysine kit: LavaPep kit (fluorescent compound: epicocconone, binds to lysine, and is used for quantification of lysine residue in peptide). Preferably, Lysine (and other amino acids) may be quantified using HPLC.

Results for the up-concentrations are as follows

5

Experimental conditions: A large scale experiment using 1 L of feed and 1 L of draw solutions in the Sterlitech CF042 chambers

Feed: 200 µg/mL L-lysine in 1x TES buffer

Draw: 2M NaCl

10 Operation time: about 1175 min

End concentration L-lysine is concentrated about 7 times

Experimental conditions: A large scale experiment as above

Feed: 200 µg/mL L-lysine in 1x TES buffer

15 Draw: 2M NaCl

Operation time: about 1175 min

End concentration L-lysine is concentrated about 6 times

Experimental conditions: small scale, 1 mL

20 Feed: 50, 200 or 500 µg/mL GGGSGAGKT in 1x TES buffer

Draw: 2M NaCl

Operation time: about 1175 min

The upconcentration of the volumes and peptide concentrations are in the table below:

Concentration of feed, start [µg/mL]	Volumen upconcentration [times]	Peptide upconcentration [times]
50	2.3	1.9
200	5	6
500	4.3	4.8

25

Conclusion: the results clearly show that during less than 20 hours of forward osmosis operation in the system the feed L-lysine solutes can be concentrated up to about 6 to

7 times, and for the feed peptide solutions these can be concentrated up to 6 times with the feed volume being concentrated in the same order of magnitude.

**Example 4. Treatment of the membrane with citric acid**

5 Membranes were prepared as described in the experimental section above and were tested for robustness against treatment with citric acid. The membranes were submerged in a 0.3 % citric acid solution and left soaking for 15 minutes (n=3).

10 Before and after the soaking process the membranes were run in FO mode (with 5 $\mu$ M calcein feed and 2 M NaCl as draw solution) in a CF042 flow cell for 900 min.

The results of the tests are in the table below:

	$J_w$ [L/m <sup>2</sup> h]	$J_{s, total}$ [g/m <sup>2</sup> h]	$R_{calcein}$ [%]
Before treatment (n=3)	10.33	2.26	99.94
After treatment (n=3)	11.43	3.40	99.76

wherein  $J_w$  is the water flux through the membrane,

15  $J_{s, total}$  is the reverse salt flux through the membrane and  
 $R_{calcein}$  is the calcein rejection.

As can be seen from the table, the treatment does not influence the water flux negatively and the calcein rejection is maintained at a very high level.

20 **Example 5. Treatment of the membrane with EDTA**

Membranes were prepared as described in the experimental section above and were tested for robustness against treatment with EDTA. The membranes were submerged in a 0.8 % EDTA solution and left soaking for 15 minutes (n=3).

25 Before and after the soaking process the membranes were run in FO mode (with 5 $\mu$ M calcein feed and 2 M NaCl as draw solution) in a CF042 flow cell for 900 min.

The results of the tests are in the table below:

	$J_w$ [L/m <sup>2</sup> h]	$J_{s, total}$ [g/m <sup>2</sup> h]	$R_{calcein}$ [%]
Before treatment (n=3)	10.06	2.23	99.94
After treatment (n=3)	10.99	3.51	99.00

wherein  $J_w$  is the water flux through the membrane,

$J_{s, total}$  is the reverse salt flux through the membrane and

5  $R_{calcein}$  is the calcein rejection.

As can be seen from the table, the treatment does not influence the water flux negatively and the calcein rejection is maintained at a very high level indicating an intact membrane.

10

#### **Example 6. Water extraction system for FDFO**

In this example the principle of Fertilizer Drawn Forward Osmosis (FDFO) was tested in a forward osmosis water extraction system according to the present invention with the objective of studying rejection rates of typical plant nutrient salts contained in

15 fertilizer and achievable water flux values.

Protocol:

A concentrated nutrient solution of 66.62 g/L was prepared by dissolving in water e.g. tap water or MilliQwater, a dry NPK granulate from Danish Agro having the following composition: total N 14.0%, nitrate-N 5.7%, ammonium-N 8.3%,

20 phosphorus (citrate and water soluble) 3.0%, Potassium (water soluble) 15.0%, Magnesium total 2.5%, sulfur total 10.0% and boron total 0.02%.

The resulting solution can be used as the draw source in a combined FDFO/desalination system, cf. Fig. 3. Alternatively, a commercial concentrated liquid plant nutrient solution, Blomin (The Scotts Company (Nordic), Glostrup DK) can be used. This nutrient solution consists of following nutrient salt composition and concentration: nitrogen (N) - 4.4%; Phosphorus (P) - 0.9%; potassium (K) - 3.3%; Boron (B) - 0.0002%; copper (Cu) - 0.006%; iron (Fe) - 0.02%; Manganese (Mn) -

0.008%, Sulfur (S) - 0.0003%; Molybdenum (Mo) - 0.0002%; and Zinc (Zn) - 0.004%.

With reference to Fig. 3 the system comprises a seawater feed source (10), the water being sampled from Øresund close to the coast at Tuborg Harbour, Copenhagen, said

5 water having an approximate salinity of about 8.7 g/L; (13) is a contained with the concentrated fertilizer solution prepared as described above (optionally fitted with a magnetic stirrer or the like); (1) is a Sterlitech CF042 flow cell with a TFC-AqpZ membrane (active area 0.003315 m<sup>2</sup>) prepared as described in the experimental section above using P8061 copolymer; (12) is a container with the partly diluted 10 fertilizer solution which can be re-circulated to achieve higher degree of dilution; (14) is the additional freshwater tank (normal tap water can be used) for final adjustment of the degree of dilution of the fertilizer solution; (11) is the concentrated feed stream, e.g. up-concentrated seawater; (15) is the diluted fertilizer solution ready for use. The system will initially run for about 900 min and is expected to result in a sufficiently 15 diluted plant nutrient solution ready for use or ready for use after further dilution, cf. Fig. 3 and explanations to Fig. 3 herein.

**Example 7. A water extraction system with separation of urea from RO permeate in dairy industries**

20 With reference to Fig 4 the system comprises a feed tank (18) with dairy process water having between 45 to 75 mg/L total N corresponding to about 110 mg/L urea; (16) is a pump; (17) is a valve; (19) is the permeate and (20) is the permeate tank. The flow from the pump (Washguard SST) through the Sterlitech CF042 flow cell and back to the valve is a pressurized flow of 125 psi and cross flow speed 0.26m/s; the 25 remaining flows are not pressurized flows. The permeate content of urea is expected to be reduced by at least 50 %.

With reference to Fig. 5 the system comprises a feed stream (21) having the same composition as above in (18); (1) is the Sterlitech CF042P flow cell with the aquaporin membrane (2) prepared as described in the experimental section above; 30 (22) is the concentrated feed stream; (23) is the concentrated draw solution; (8) the draw solution, e.g. 35g/L NaCl in tapwater corresponding to typical Kattegat salinity, in fluid communication with the flow cell; (24) the diluted draw solution; (9) the draw solution recovery system; and (25) the desalinated product water free of draw solution solutes. Both feed and draw streams are pumped through the flow cell in counter-

current mode at a flow speed of 50.03ml/min. The resulting urea rejection in this system is expected to be about 75 %.

## References

5 Zhao, Y et al, Synthesis of robust and high-performance aquaporin-based biomimetic membranes by interfacial polymerization-membrane preparation and RO performance characterization, *Journal of Membrane Science*, Volumes 423–424, 15 December 2012, Pages 422-428.

Kim et al. *Journal of Membrane Science* 419–420 (2012) 42–48.

10 Branislav Petrushevski, Saroj Sharma, Jan C. Schippers (UNESCO-IHE), and Kathleen Shordt (IRC), Reviewed by: Christine van Wijk (IRC). Arsenic in Drinking WaterMarch 2007, IRC International Water and Sanitation Centre Nagaishi & Ishikawa (Geochemical Journal, Vol. 43, pp. 133 to 141, 2009)

Grosser, Z., October 13, 2010 (downloaded from internet on 20130219): <url: 15 <http://www.watertechonline.com/articles/the-challenge-measure-arsenic-in-drinking-water>>

Hill & Taylor, 15 July - 19 July 2012, Use of Aquaporins to Achieve Needed Water Purity on the International Space Station for the Extravehicular Mobility Unit Space Suit System. In: (ICES) 42<sup>nd</sup> International Conference on Environmental systems, San 20 Diego, California.

Al-Amoudi et al, *Journal of Membrane Science* 303 (2007) 4-28.

Porcelli et al, *Separation and Purification Technology* 71 (2010) 137-143

Achilli et al. Selection of inorganic-based draw solutions for forward osmosis applications. *Journal of Membrane Science* 364 (2010) 233–241

25 Phuntsho et al. A novel low energy fertilizer driven forward osmosis desalination for direct fertigation: Evaluating the performance of fertilizer draw solutions. *Journal of Membrane Science* 375 (2011) 172–181.

**PATENTKRAV**

1. Et vandekstraktionssystem til anvendelse i et gødningsvandningssystem, hvilket vandekstraktionssystem omfatter:

5 a) en flowcelle (1) indeholdende en membran (2); hvor membranen indeholder et aktivt lag (3), der indeholder immobiliserede aquaporin vandkanaler og et understøttende lag (4), hvilken membran har en fødeside (5) og en ikke-fødeside (6); og hvori membranens ikke-fødeside fungerer som trækside;

10 b) en vandig kildeopløsning (7) i flydende forbindelse med membranens fødeside;

c) en vandig trækopløsning (8) i flydende forbindelse med membranens trækside, og

15 som yderligere indeholder en koncentreret opløsning af plantenæringsstoffer (13).

15

2. Vandekstraktionssystemet ifølge krav 1 hvori det aktive lag er en krydsbundet aromatisk amid-tyndfilm, hvori aquaporinvesikler dannes ved selvsamling af amfifile lipider eller blokcopolymerer i nærvær af en aquaporinproteinsuspension.

20

3. Vandekstraktionssystemet ifølge krav 1 eller 2 hvori det aktive lag er et krydsbundet aromatisk amidlag fortrinsvis dannet ved grænsefladepolymerisation, og vesiklerne er dannet fra en amfifil lipid- eller triblokcopolymeropløsning, såsom asolectin eller en PMOXAA-PDMSB-PMOXAc copolymer.

25

4. Vandekstraktionssystemet ifølge et hvilket som helst af kravene 1 til 3, hvori aquaporinet er udvalgt fra et planteaquaporin, f. eks. SoPIP2;1, et pattedyrsaquaporin, f. eks. Aqp1; og et bakterielt aquaporin, f. eks. aquaporin-Z.

30

5. Vandekstraktionssystemet ifølge et hvilket som helst af kravene 1 til 4 hvori det understøttende lag er en polysulfon- eller polyethersulfonmembran.

6. Vandekstraktionssystemet ifølge et hvilket som helst af kravene 1 til 5, hvor systemet yderligere omfatter:

d) en koncentreringseenhed for trækopløsning (9).

7. Vandekstraktionssystemet i følge et hvilket som helst af kravene 1 til 6 der yderligere indeholder midler til regenerering eller anti-fouling af membranen, hvor midlerne omfatter en rensevæske med et pH på omkring 2 til 11, hvor rensevæsken er udvalgt fra en opløsning af en organisk syre, såsom citronsyre, eller et kelateringsmiddel, såsom EDTA.

8. Vandekstraktionssystemet ifølge krav 1 yderligere indeholdende de følgende træk:

10 i) en fødestrøm (10),  
ii) en pumpe (16),  
iii) en flowcelle (1), fortrinsvis en krydsflowcelle, med en aquaporinmembran, fortrinsvis en TFC-aquaporinmembran (2),  
iv) en fortyndet trækopløsning (12) i flydende forbindelse med membranens  
15 trækside (8),  
v) en valgfri ferskvandskilde (14) til yderligere fortynding af fortyndet trækopløsning, og  
vi) en resulterende fortyndet plantenæringsopløsning der er klar til brug (15);  
og  
20 hvori den fortyndede trækopløsning kan recirculeres gennem flowcellen til opnåelse af en højere grad af fortynding.

1/7

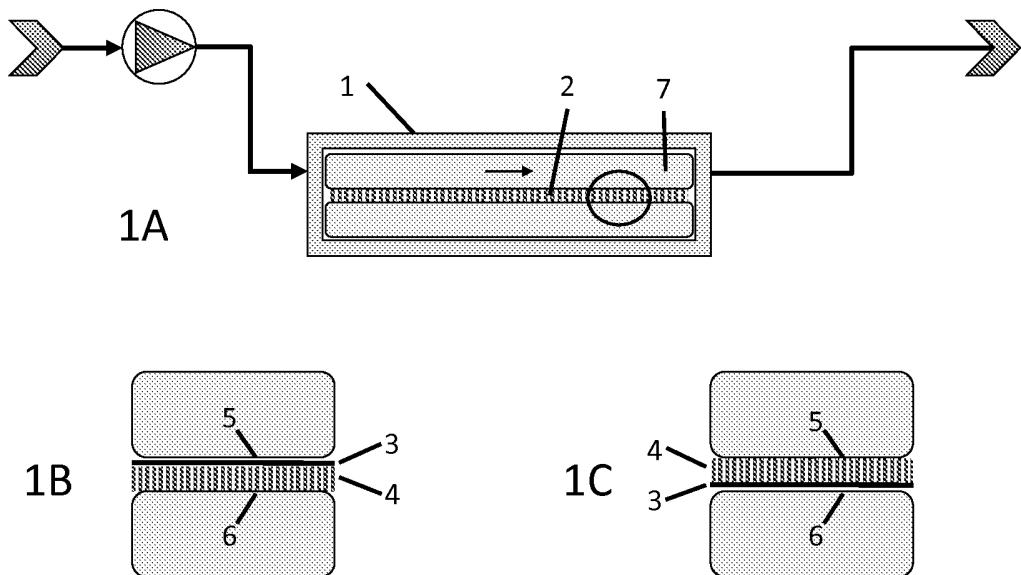


Fig. 1

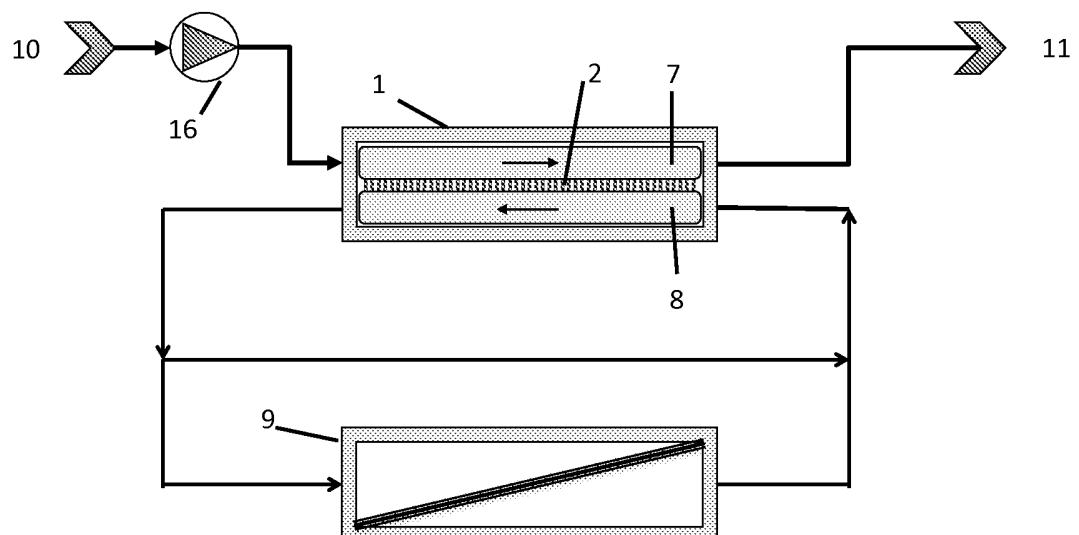


Fig. 2

2/7

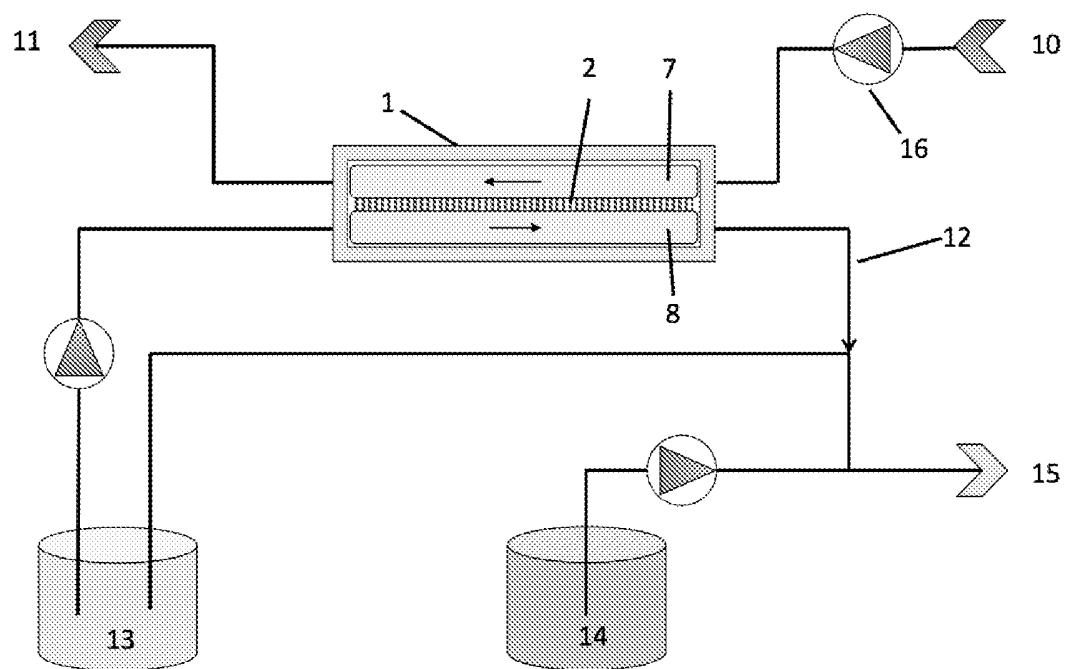


Fig. 3

3/7

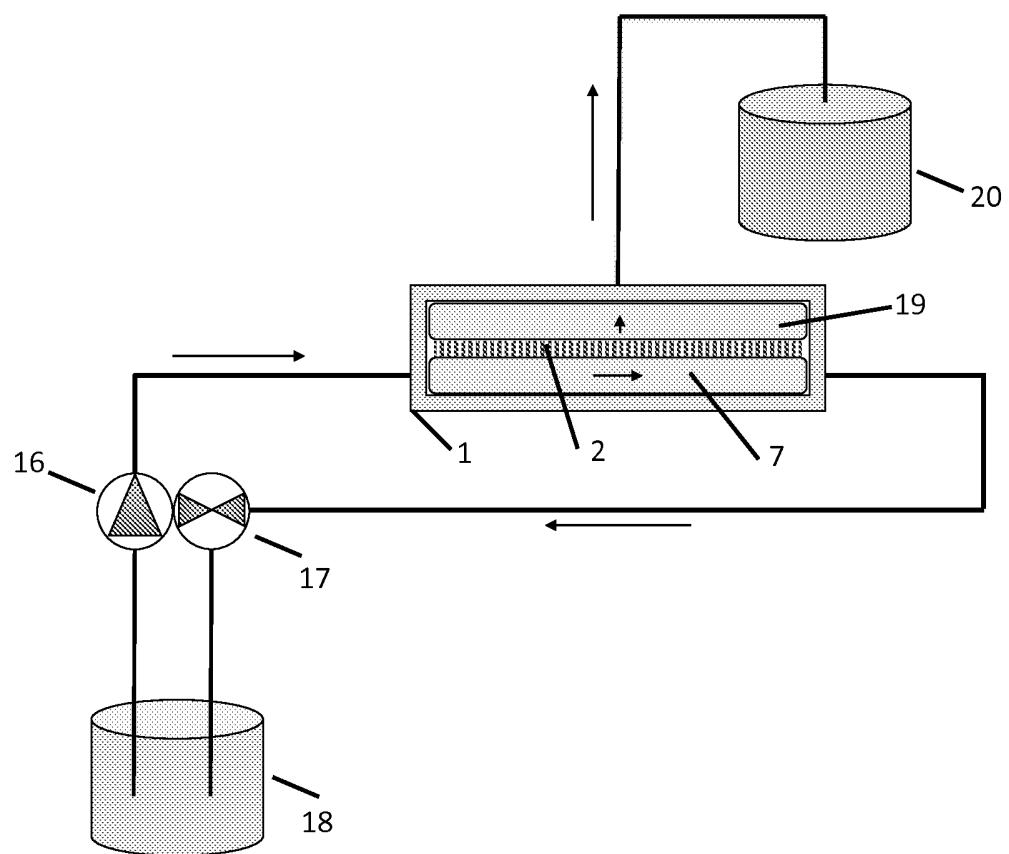


Fig. 4

4/7

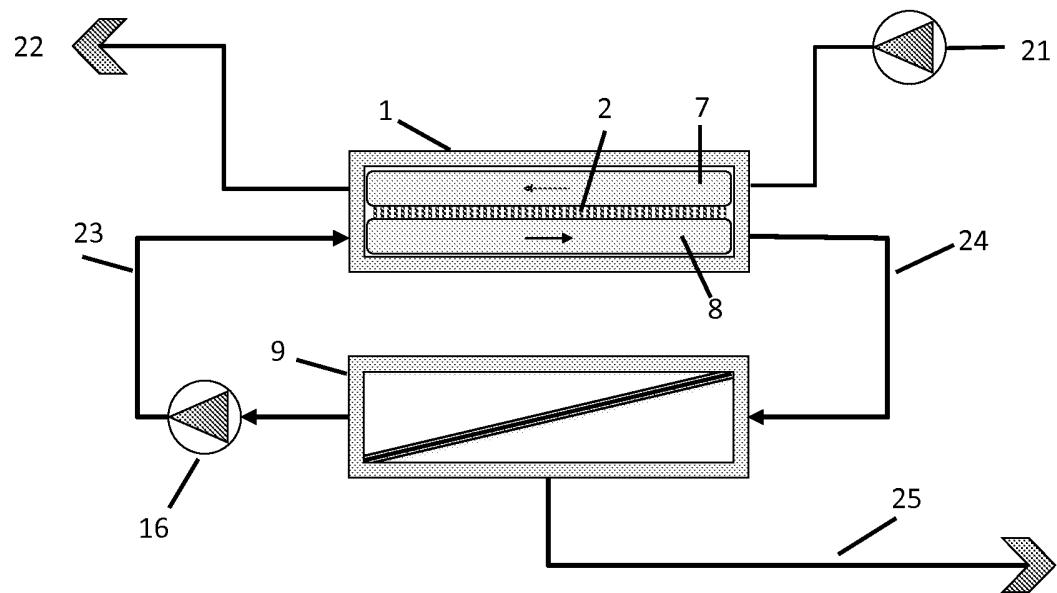


Fig. 5

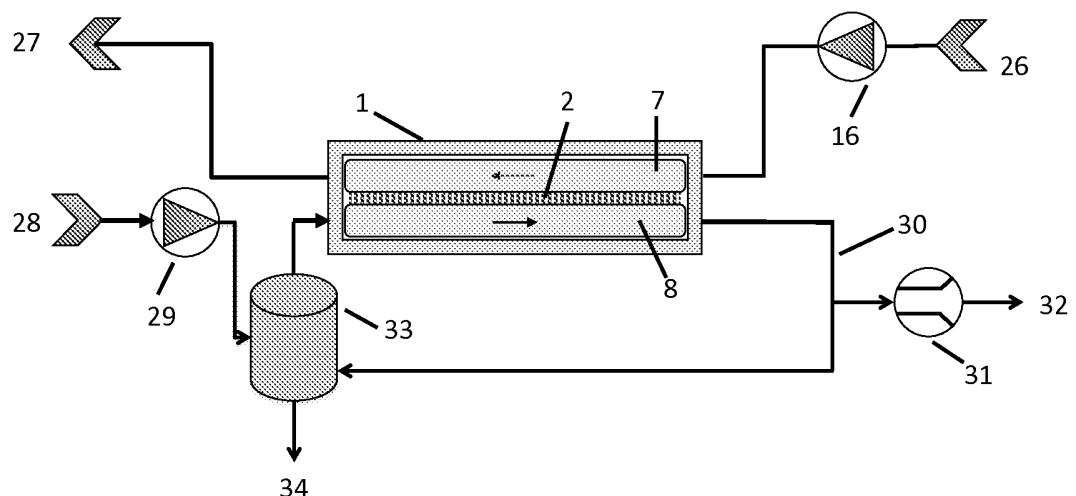


Fig. 6

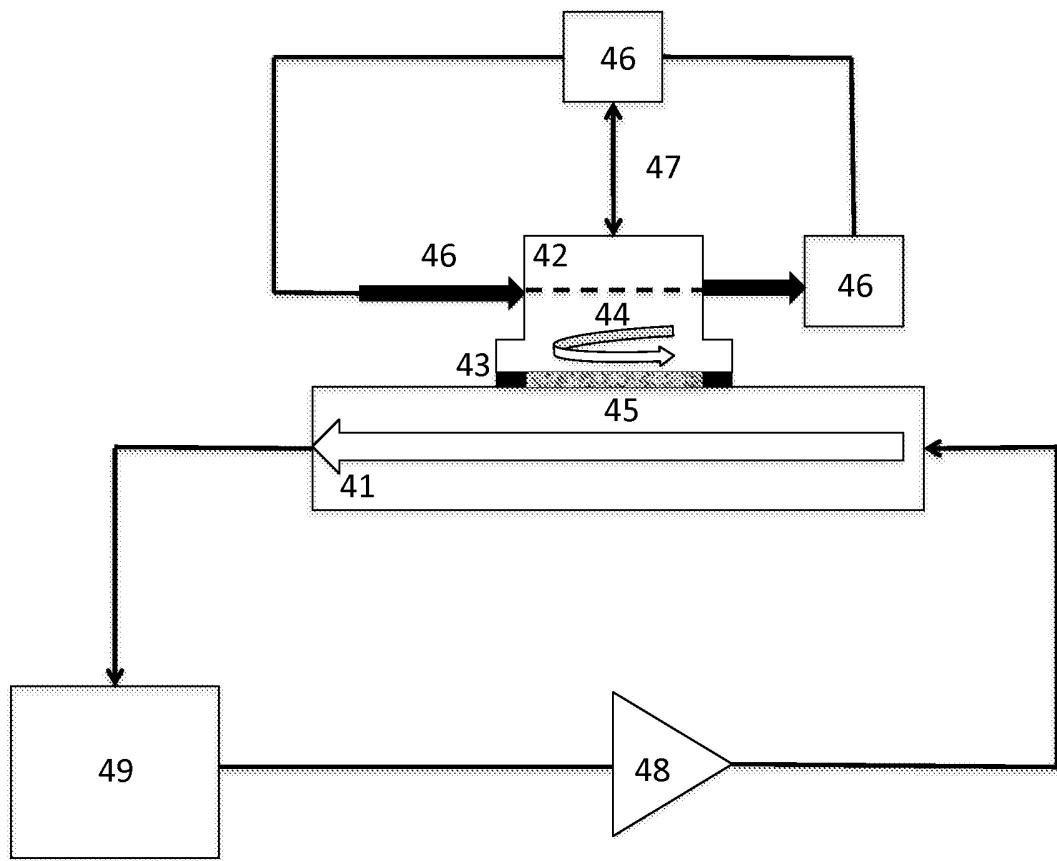


Fig. 7

6/7

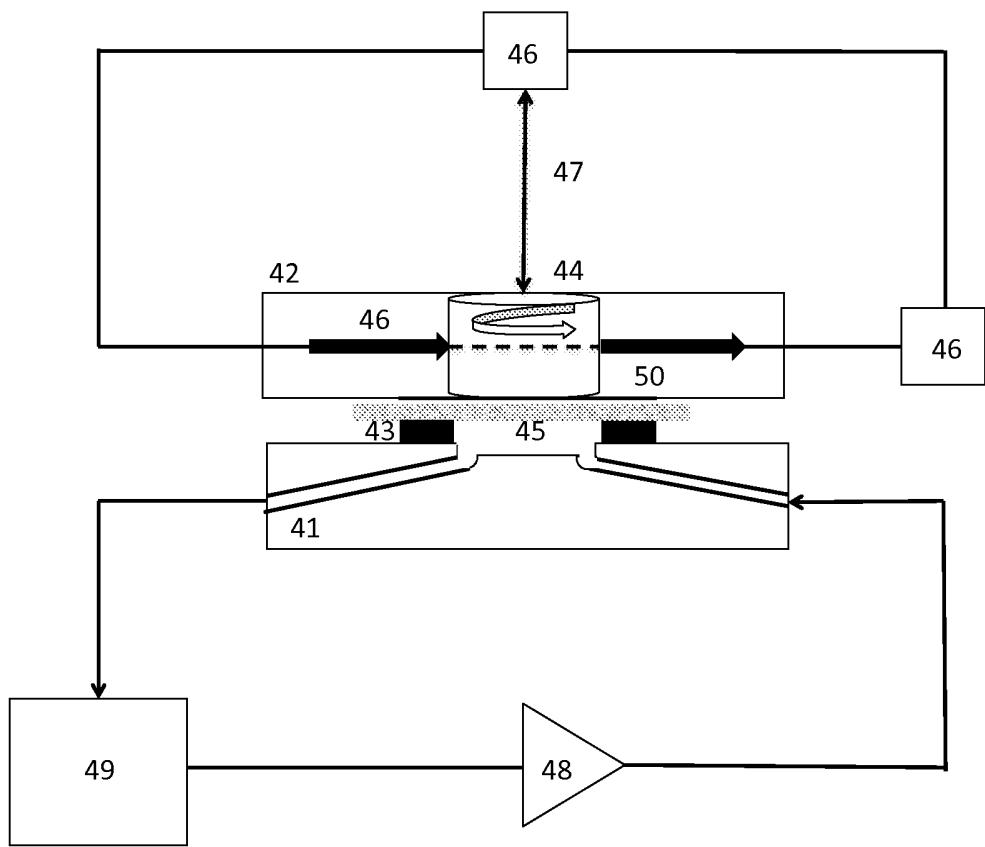
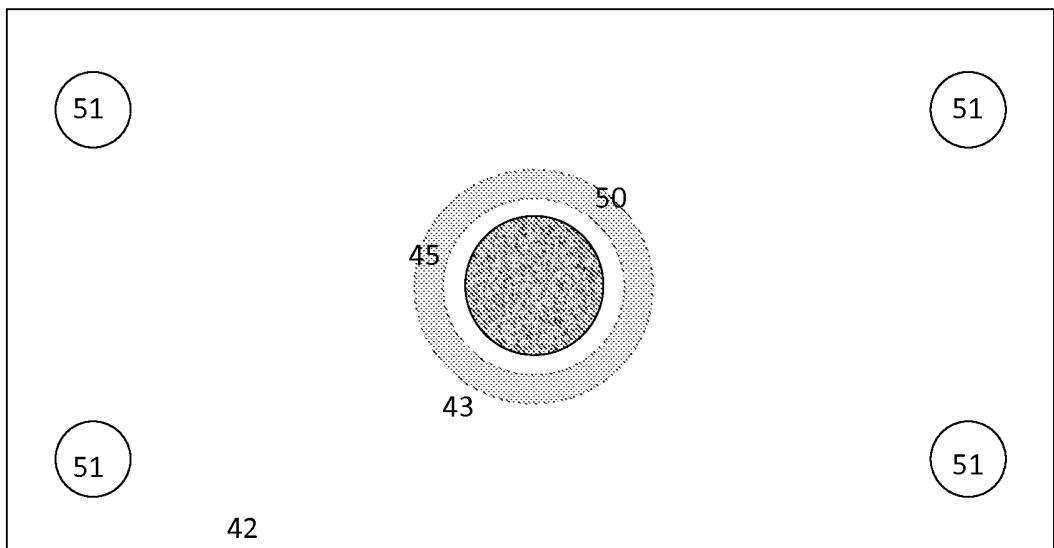
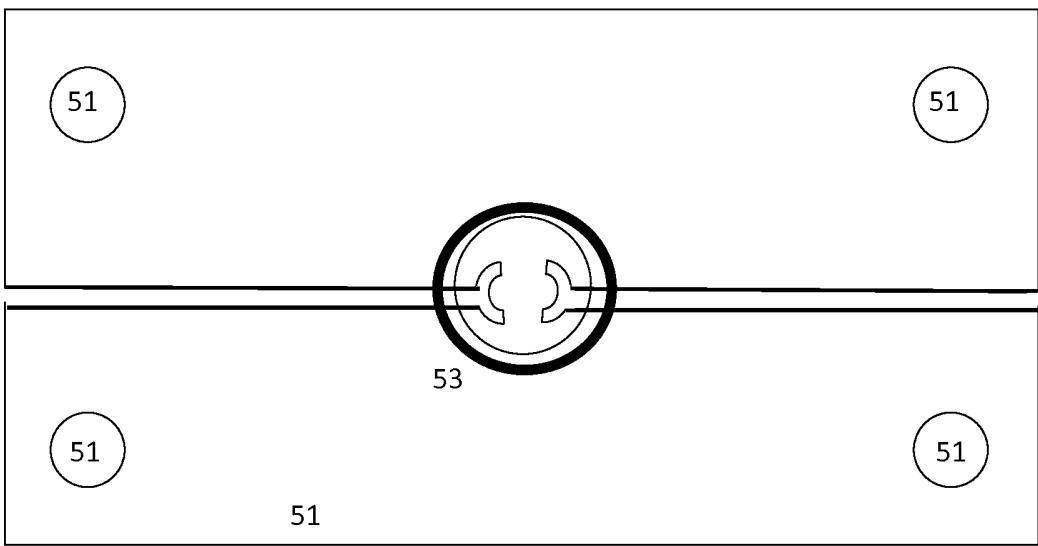


Fig. 8



**Fig. 9**



**Fig. 10**

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<b>SEARCH REPORT - PATENT</b>		Application No. PA 2013 00107
<p><b>A. CLASSIFICATION OF SUBJECT MATTER</b>            B 01 D 71/74 (2006.01); B 01 D 71/56 (2006.01); C 02 F 1/26 (2006.01); C 02 F 1/44 (2006.01)            According to International Patent Classification (IPC)</p>		
<p><b>B. FIELDS SEARCHED</b></p> <p>PCT-minimum documentation searched (classification system followed by classification symbols)            IPC, CPC: C02F, B01D</p>		
<p>Documentation searched other than PCT-minimum documentation            DK, FI, NO, SE: same as above</p>		
<p>Electronic database consulted during the search (name of database and, where practicable, search terms used)            EPODOC, WPI, FULL TEXT: ENGLISH, GERMAN, FRENCH            CAPLUS, SCISEARCH, PASCAL, PQSCITECH, COMPENDEX, DISSABS</p>		
<p><b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b></p>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant for claim No.
Y	WO2007033675 A1 (AQUAPORIN APS [DK]) 29 March 2007, see whole document, especially section [0123] and fig. 5.	1-7, 9-13 and 17-19
Y	Tang et al., "Desalination by biomimetic aquaporin membranes: Review of status and prospects, Desalination (2012), doi: 10.1016/j.desal.2012.07.007; see especially the fourth paragraph of section 3 and fig. 5.	1-7, 9-13 and 17-19
A	WO2007035987 A1 (US FILTER WASTEWATER GROUP INC [US]) 5 April 2007, whole document, especially the claims.	6
A	US2012152841 A1 (VISSING et al.) 21 June 2012, see whole document, especially fig. 7 and sections [0043] and [0134].	-
E,X	WO2013043118 A1 (UNIV NANYANG TECH [SG]; AQUAPORIN AS [DK]) 28 March 2013, see whole document, especially p. 4, l. 21-23; p. 5, l. 34-35; p. 8, l. 6-33 and fig. 5.	1-4
<p>* Special categories of cited documents:            "A" Document defining the general state of the art which is not considered to be of particular relevance.            "D" Document cited in the application.            "E" Earlier application or patent but published on or after the filing date.            "L" Document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified).            "O" Document referring to an oral disclosure, use, exhibition or other means.</p>		
<p>"P" Document published prior to the filing date but later than the priority date claimed.            "T" Document not in conflict with the application but cited to understand the principle or theory underlying the invention.            "X" Document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone.            "Y" Document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.            "&amp;" Document member of the same patent family.</p>		
Danish Patent and Trademark Office Helgeshøj Allé 81 2630 Taastrup Denmark  Tel.: +45 4350 8000 Fax: +45 4350 8001	Date of completion of the search report 20 June 2013  Authorized officer Isabelle Rivas	