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Application of forward osmosis in pretreatment of seawater for small reverse osmosis desalination units

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ABSTRACT

Recently, membrane pretreatment has been accepted as a technically and economically viable alternative to conventional pretreatment in SWRO processes. MF, UF and NF membranes were used in these pressure driven processes, with UF most commonly recommended. Amongst these, forward osmosis (FO) driven process has been used in different water treatment operations including membrane pretreatment of wastewater. In this study, the application of a FO driven membrane process in seawater pretreatment was evaluated. This application is particularly important for small autonomous RO desalination units as it eliminates the need for chemicals which are conventionally used in pretreatment steps, the concomitant need for disposal of the chemical laden waste resulting from the process, and the requirement of qualified expertise for unit operation. The performance of commercially available FO membrane cartridges (Hydration Technologies Inc.) in terms of water flux and salt flux was evaluated using tap water and seawater as feed. Refined sea salt was used for preparing highly concentrated osmotic draw solutions at three salinity levels up to 100,000 ppm. Profiles of water and salt fluxes versus osmotic draw solution concentrations were established and analyzed. A conceptual system design for an integrated desalination unit consisting of a closed FO and RO process was presented in which RO brine is used as the osmotic draw solution and thus the chemical energy stored in the RO brine is recovered and utilized by the FO membrane process. In addition, the energy contained in the excess RO brine pressure is used for brine circulation in the FO process loop. The main operating parameters and relationships of the conceptual FO-RO system are described.

Keywords: Forward osmosis; Seawater pretreatment; Membrane pretreatment; Autonomous RO desalination units

1. Introduction

Membrane pretreatment of seawater/brackish water prior to reverse osmosis (RO) desalination is an innovative pretreatment technology which has gained wide acceptance since its inception more than one decade ago. Unlike conventional pretreatment, which is based on chemo-mechanical processes and characterized by its sensitivity to changes in raw water quality parameters such as turbidity and chemical composition, membrane pretreatment

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does not rely on chemical processes or settling to remove particles because the membranes physically block contaminants and other fouling materials from entering the RO feed stream.

Several studies have been conducted to evaluate the effect of membrane pretreatment on RO performance at laboratory, pilot and full scale using different types of membrane technologies [1–5]. Mostly, pressure driven membrane processes using microfiltration (MF), ultrafiltration (UF) and nanofiltration (NF) membranes were used with UF most commonly recommended [6–8]. Also, a forward osmosis (FO) driven process has been used for membrane pretreatment of wastewater [9–11]. Generally, the studies concluded that membrane pretreatment is a technically and economically viable alternative to conventional pretreatment in wastewater and seawater RO desalination. The major advantage of membrane pretreatment is its ability to guarantee a high quality of RO feed water independently of the raw feed water quality. The high quality of feed water results in lower cleaning requirements, higher flux rates and extended RO membrane life and therefore reducing cost of desalinated water.

For brackish water and specifically wastewater feeds, UF/MF pretreatment resulted in much less fouling of RO elements and reduced chemical usage [12]. For seawater, NF pretreatment prevented RO membrane fouling and scaling and offered an increase in production and recovery, and cost savings in water production [13]. UF provided water to seawater reverse osmosis (SWRO) with a stable quality of Silt Density Index (SDI) values well below 2 [14]. Significant reduction in fouling as expressed in terms of SWRO flux decline was observed when UF was used and this decline decreases with decreasing membrane pore size [15]. The cost of membrane pretreatment, which was a prohibiting factor, became comparable to the cost of conventional pretreatment on average to bad feed water qualities and has lower capital expenditures and other operating costs than conventional pre-treatment [16,17].

The application of FO process in pretreatment of feed water will improve the overall desalination process in two ways. While FO membranes are less susceptible to fouling due to low pressure operation [18], the membrane pretreated feed has less fouling threats on RO system. Membrane pretreatment eliminates the need for chemicals which are conventionally used in pretreatment steps and the concomitant need for disposal of the chemical laden waste resulting from the process. It also simplifies process operation and maintenance. The second improvement is related to the process energy requirement. The potential energy of RO brine could be recovered in the FO process. Recovery of potential energy of RO brine for utilization in the pretreatment process will result in lowering overall energy requirement for desalination. This is particularly important for desalination units installed in remote areas where the energy cost represents even an greater share of total desalination cost. Few projects have been implemented for development of small-scale, stand-alone desalination systems powered by renewable energy with fresh water output in the range of 0.1 m^3/d to 10 m^3/d [19, 20]. Among other aspects, these projects focused on chemical pre-treatment minimization as a means of performance enhancement of desalination units.

In this study, the application of a FO driven membrane process in seawater pretreatment was evaluated. A conceptual system design for an integrated small desalination unit consisting of a closed FO and RO process is also presented and analyzed.

2. Materials and methods

2.1. Membrane module description and specifications

A spiral-wound membrane cartridge manufactured by Hydration Technologies (HTI), Albany, Oregon, was used in the experiments. This cartridge is used in one of the company's products (HydroWell), which is basically marketed as a portable passive water treatment device for pathogen reduction. The membrane is cellulose triacetate embedded on a polyester screen, which acts as a support. The rejection layer of the membrane is facing the feed solution which is typically the dirty- or fouling-water side. This mode of membrane orientation, called the FO mode, is the primary orientation considered for water treatment processes such as FO desalination [21]. The cartridge has a diameter of about 75 mm and length of 305 mm and is made from only one 2.43-m-long membrane leaf. The actual membrane area is 1.5 m^2, but the effective area could be estimated at only 1.0 m^2 after accounting for area taken by glue lines. The pressure drop in the membrane envelop is around 7 kPa/(l/h) while the pressure drop in the open side (feed channel) is around 2 kPa/(l/h) for water at 20°C. The maximum pressure in the membrane envelop should not exceed 70 kPa to avoid glue breaking. Fig.1 shows the cartridge configuration and flow paths of liquids inside and outside the membrane

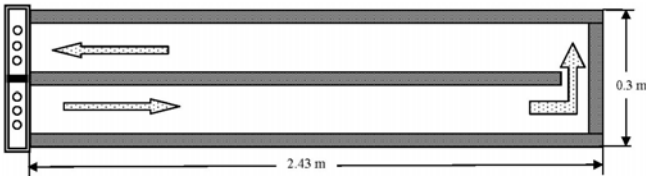

Fig. 1. Flow pattern in an unrolled HydroWell spiral-wound cartridge. Arrows show flow path of osmotic solution in the membrane envelope. Contaminated water flows tangentially across outer side of the membrane envelope [22].

envelop [22]. Similar membrane configuration was used in an osmotic driven process by Mehta [23].

2.2. Experimental set-up

An experimental set-up was constructed for carrying out the FO experiments. The HTI membrane cartridge was placed in specially fabricated housing and was connected to the draw solution (DS) and feed water flow loops. Bourdon pressure indicators, variable area flow meters (McMaster), conductivity and temperature probes (Vernier Software & Technology) were installed at various locations in the flow loops to monitor and collect data during experimentation. Conductivity and temperature data were stored in a personal computer (PC) every 0.2 min for the duration of each test using LabPro computer interface provided by Vernier Software & Technology. A small gear pump (Greylor) and a submersible centrifugal pump were used to recirculate the DS and feed water, respectively. Heat emitted by the submersible pump to the feed water was taken out by coolant (tap water) flowing in a cooling coil. The temperatures of DS and feed water were kept at 20±1°C by controlling the flow rate of coolant. A schematic drawing of the experimental set-up is illustrated in Fig. 2.

Water permeating through the membrane was carried out along with the DS to reservoir B. The overflow from reservoir B was consistently collected in a beaker placed on a digital balance D (accuracy 1 g) and its weight was recorded at 1-min time increments. This flow represents the permeate volume. The change of weight on the balance represents the permeate flux through the membrane.

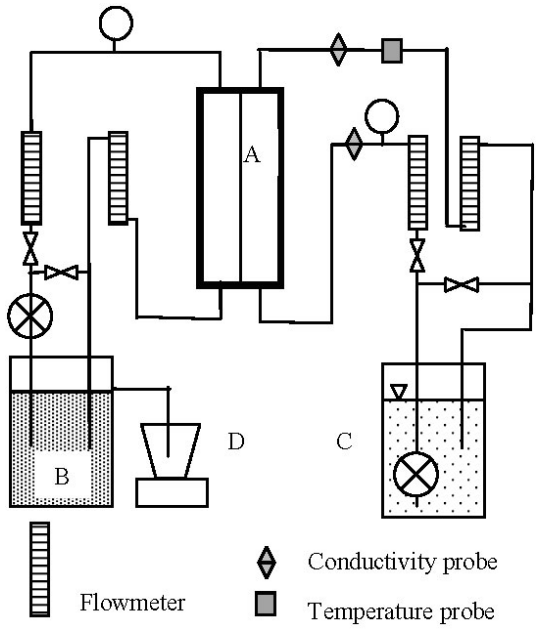

Fig. 2. Experimental set-up used for membrane module test. A, membrane module; B, osmotic solution reservoir; C, feed water reservoir; D, beaker on digital balance.

Bench-scale experiments were conducted to evaluate membrane flux and salt rejection of the HTI membrane cartridge under different concentrations of DS. Tap water and seawater were used as feed waters. A volume of 18 l feed water was stored in the feed water reservoir C. During tests, reservoir C was replenished constantly with tap water to keep the water in the reservoir at a constant level. Pacific Ocean seawater (taken at Newport, Oregon) was used in tests.

The osmotic draw solution was prepared using refined table salt (99.9% NaCl) dissolved in tap water. Four concentrations of DS were tested. These concentrations are 35, 50, 75 and 100 g/l. After adding table salt to tap water, it was observed that the clarity of solution was low, indicating the presence of suspended solids. Therefore, the solution was stored for 48 h to allow settling of the solids, and the clear solution was drained thereafter. No filtration was used. The duration of each experiment was 2 h. At the end of each experiment, the DS and feed water were flushed out of the setup and the membrane was cleaned. Membrane cleaning was accomplished by allowing tap water to flow in the system on both loops until no salt residues were detected (i.e. until the conductivities of the inlet and outlet flows were equal). Under the conditions of this study, it was observed that the membrane flux rate under RO mode (i.e. by allowing tap water to flow under pressure (100 kPa) on the membrane open side only) was recovered up to a similar level as when the membrane was first used.

The flow velocity of osmotic DS was limited by the allowable maximum pressure in the membrane envelope. Tests with the membrane cartridge indicated that the pressure exceeds 35 kPa (5 psi) when the flow rate reached 0.3 l/min. Therefore, a flow rate of 0.25 l/min was selected to assure safe operation of the membrane. The flow rate of feed water was selected at 0.7 l/min which is almost three times higher than the flow rate of DS. The feed water and DS flow velocities were calculated as 1 cm /s and 10 cm/s, respectively.

2.3. Variation of concentration of draw solution

Since the DS volume was kept constant, and as water permeated through the membrane, the DS gradually diluted. The change in DS concentration with time is easily determined using permeate volume measurement. Let C_0 and V_c be the initial levels of DS concentration and DS constant volume in the DS reservoir, respectively. Let also $V_p(\Delta t_i)$ be the volume of water permeated at the ith time increment, Δt_i. Assuming full mixing of the perme-

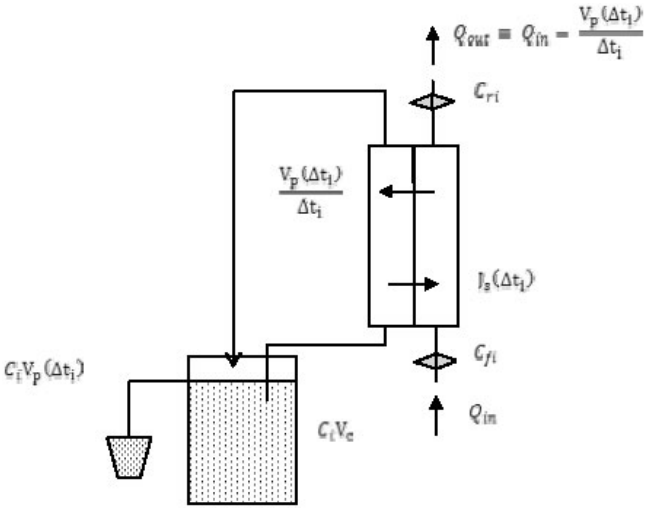

Fig. 3. Schematic representation of mass transfer between draw solution and feed water loops of the system.

ated water with the DS in the reservoir, the principle of conservation of mass gives the following (Fig. 3):

- At the end of the first time increment the amount of salt remaining in the DS reservoir will be:

$$C(\Delta t_1)V_c = C_0 V_c - C(\Delta t_1)V_p(\Delta t_1) - J_s(\Delta t_1)$$

- at the end of second time increment:

$$C(\Delta t_2)V_c = C(\Delta t_1)V_c - C(\Delta t_2) - J_s(\Delta t_2)$$

- and the end of *i*th time increment:

$$C(\Delta t_1)V_c = C(\Delta t_{i-1})V_c - C(\Delta t_i)V_p(\Delta t_i) - J_s(\Delta t_i)$$

where $J_s(\Delta t_i)$ is the salt flux from DS to the feed water during *i*th time increment, and $C(\Delta t_s)$ is the solute concentration during *i*th time increment.

Therefore, the concentration of the OS at *i*th time increment would be calculated as follows:

$$C(\Delta t_i) = \frac{C(\Delta t_{i-1})V_c - J_s(\Delta t_1)}{V_c + V_p(\Delta t_i)},\ i = 1,\ldots n$$

2.4. Salt transport from draw solution to feed water

Salt transport was measured in experiments with tap water as the feed only. In the feed water loop, the salt concentration increases as a result of the diffusion of ions from the DS under high concentration gradient. The reading of inlet conductivity probe represents the concentration of salt in the bulk feed water, while reading of outlet conductivity probe represents the concentration of salt in the retentate before its mixing with the bulk feed water in the reservoir. Salt concentration was calculated based on conductivity data and calibration curves of conductivity probes. The change of solute concentration in any given period of time indicates the amount of salt flux occurring during that time.

Let C_{f1}, C_{r1} be the concentrations of feed water and retentate at the end of first time increment, respectively. Assuming these values represent average concentration for this time increment, then the increase of amount of salt in retentate during the first time increment will be (Fig. 3):

$$J_s(\Delta t_1) = C_{r1}Q_{out}\Delta t_1 - C_{f1}Q_{in}\Delta t_1$$

$$J_s(\Delta t_1) = Q_{in}\Delta t_1(C_{r1} - C_{f1}) - C_{r1}V_p(\Delta t_1)$$

where Q_{in} and Q_{out} are feed water inflow and outflow rates, respectively, and

$$Q_{out} = Q_{in} - \frac{V_p(\Delta t_1)}{\Delta t_1}$$

At the end of the second time increment:

$$J_s(\Delta t_2) = Q_{in}\Delta t_2(C_{r2} - C_{f2}) - C_{r2}V_p(\Delta t_2)$$

At the end of the *i*th time increment:

$$J_s(\Delta t_i) = Q_{in}\Delta t_i(C_{ri} - C_{fi}) = C_{ri}V_p(\Delta t_i)$$

3. Results and discussion

3.1. Effect of draw solution concentration on water flux

Figs. 4 and 5 show the relationships between permeate water flux and draw solution concentration for tap water and seawater, respectively. All the relationships show a linear decrease in water flux from an initial highest value corresponding to the highest concentration difference. It is expected that the water flux decreases with the decrease in concentration difference because the osmotic driving force is lower at lower concentrations. The concentration of draw solution decreases as a result of permeation of water from the feed water, while the concentration of feed water increases as result of diffusion of salt from draw solution. With tap water as feed water and at draw solution concentration of 75 g/l, the initial water flux obtained was 11.1 LMH (liters permeating 1 m^2 of membrane each hour)

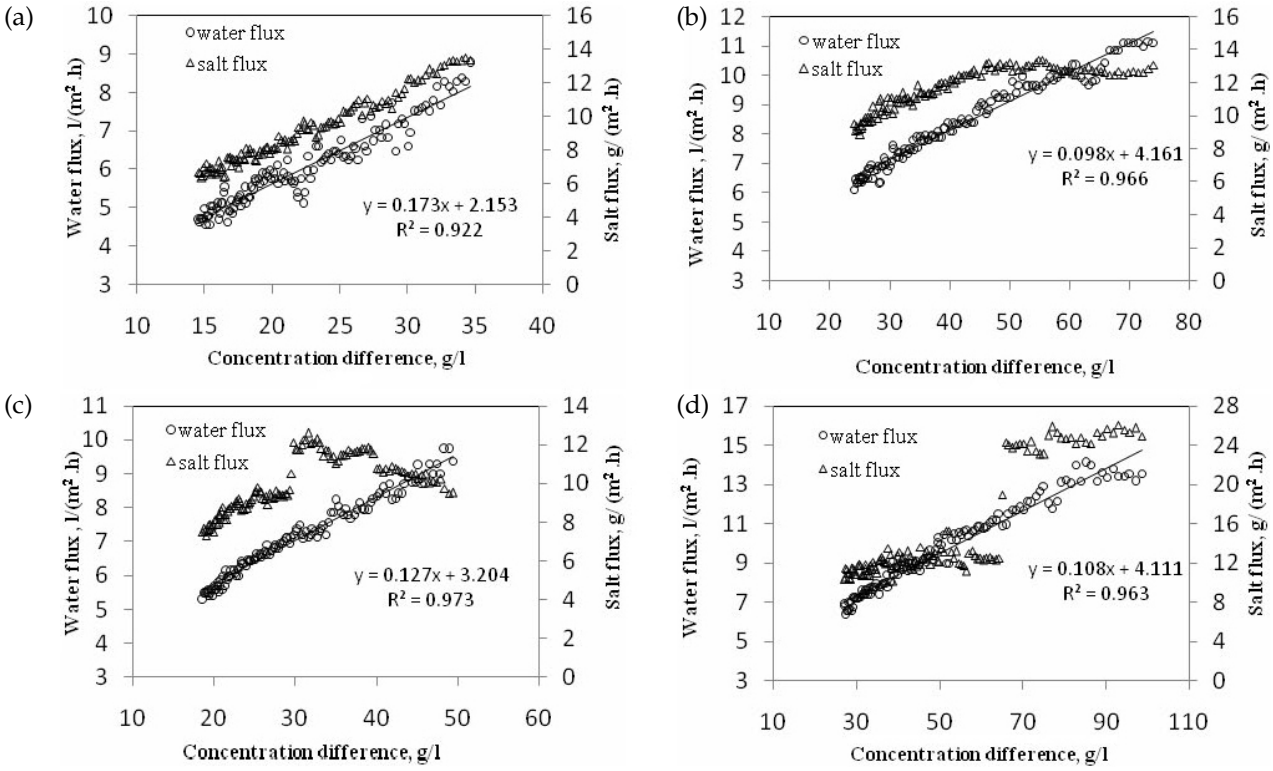

Fig. 4. Profiles of permeate water flux and salt flux at draw solution initial concentrations of (a) 35 g/l, (b) 50 g/l, (c) 75 g/l, and (d) 100 g/l against tap water as feed.

[6.5 gfd (gallons permeating 1 ft^2 of membrane each hour)] at an osmotic pressure difference of 53 atm and temperature of 20°C. This value is a little lower than the value reported by McCutcheon et al. [24] at the same osmotic pressure difference and at temperature of 50°C, which was 11.9 LMH (7 gfd). With seawater as feed water the initial water flux obtained was 6.7 LMH (4 gfd) when the draw solution concentration was 75 g/l which results in osmotic pressure difference 28 atm. Other experimental conditions are the same as with tap water. It could be noted that at similar values of transmembrane pressure differences, the water fluxes through osmotically driven FO membranes are far less than water fluxes in pressure driven RO membranes. Low water fluxes in FO membranes were attributed to the structural characteristics of the support layer of the membrane, such as thickness and porosity, and chemical properties of its material, such as hydrophobicity. The structure of support layer induces internal concentration polarization which reduces the effective osmotic driving force [21]. Membrane support layer hydrophobicity significantly hinders water flux in osmotically driven membrane processes and the membrane support layer must fully wet to ensure effective water transport [25].

Since the standard water flux models assume a linear relationship between concentration (or osmotic pressure) and water flux, it is expected that the linearity coefficients will be similar for all concentration levels. However, data analysis showed that the coefficients decreased significantly with the increase in initial concentration of draw solution. Therefore, the coefficients of regression lines were presented vs. initial draw solution concentrations in Fig. 6. This figure suggests the existence of an optimal range of concentrations, beyond which the water flux only increases marginally with any increase in concentration. The higher salinity levels of draw solution reduce membrane permeability coefficient [26] and therefore, impact negatively on water flux.

Although it was expected that membrane fouling agents present in seawater would cause lower flux values, the data show that the type of feed water does not affect water flux. For the same draw solution concentration difference values, there were no significant differences between water flux values achieved with tap water and seawater. It should be noted that these tests were conducted for short intervals of time and no attempt was made to separate the flux decline component caused by fouling from the flux decline component caused by the osmotic driving force decrease. Also, under test conditions, the influence of osmotic driving force is more dominant on water flux decline because it kept decreasing due to the dilution of draw solution and concentration of

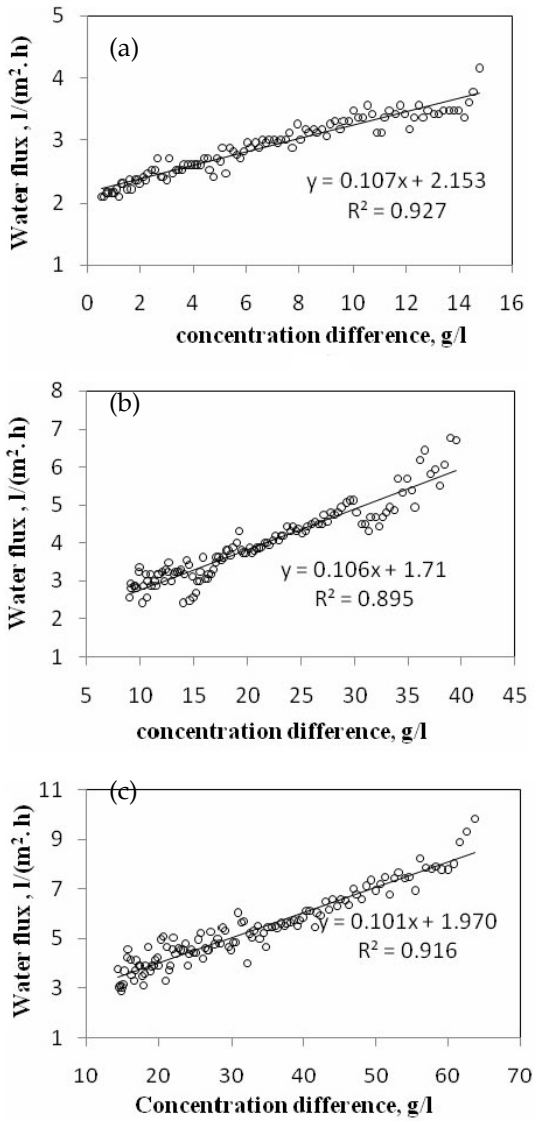

Fig. 5. Profiles of permeate water flux at draw solution initial concentrations of (a) 50 g/l, (b) 75 g/l and (c) 100 g/l against seawater as feed.

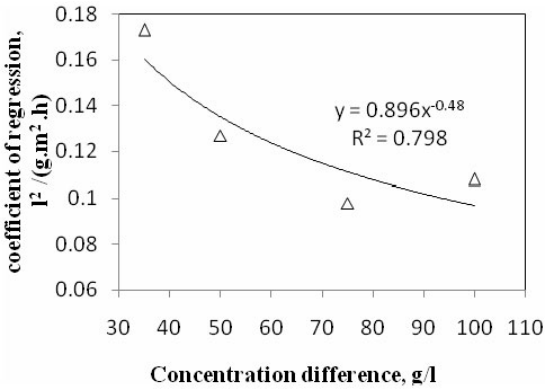

Fig. 6. Effect of initial concentration of draw solution on values of coefficients of regression.

feed solution. Previous studies on FO membrane fouling demonstrated a slower flux decline rate in FO than in RO when treating wastewater concentrate [27], and FO membrane fouling can vary from foulant to foulant depending on intermolecular adhesion forces [28]. A systematic and long-term studies are required to assess the fouling behavior of seawater on FO membranes.

Since the high pressure seawater RO systems can generate RO brine with concentrations between 60–70 g/l, the maximum water flux of FO membrane that can be achieved using this brine as draw solution will not exceed 7 LMH (4 gfd) for seawater pretreatment, which is far less than desired values for practical application . However, in case of pretreatment of brackish water having concentrations less than 5000 ppm, a maximum water flux of about 11 LMH (6.4 gfd) can be achieved with the same RO brine.

3.2. Effect of draw solution concentration on salt flux

The transport models of salts across the membrane assume that the salt flux is independent of water flux and the transport is proportional to the concentration difference. In the FO process, the water flow direction across the membrane is opposite to the solute transport direction, which implies that diffusion will be the dominant transport type [25]. The salt flux profiles show the same trends as water flux profiles (Fig. 4) for tap water as a feed. It could be observed from this figure that at high concentration gradient, the high water flux is coupled with high salt flux and vice versa at low concentration gradient; the low water flux is associated with low salt flux.

The normalized salt flux, calculated as the ratio of salt flux to water flux, was calculated and is presented in Fig. 7 against concentration gradients for all levels of initial concentration of draw solution. There is no consistent trend between normalized salt flux and concentration gradients and also there is no significant difference between average values of normalized salt flux at different levels of initial concentration of draw solution. The average normalized salt flux remained almost constant and ranged between 1.4 to 1.5 g/l.

In a previous FO study on similar CTA membranes for reclamation wastewater, it was observed that salt diffuses from the draw solution the wastewater side at an average rate of 300 mg NaCl for every liter of wastewater recovered [11]. This value is much less than the value observed in this study. The reason for this variation could be linked to the difference in chemistry nature of feeds used in both cases. The feed water in this study was tap water with a very low concentration of TDS (35–50 ppm), while the feed water used by Cath et al. was a synthetic wastewater with concentration varied between 6.4 to

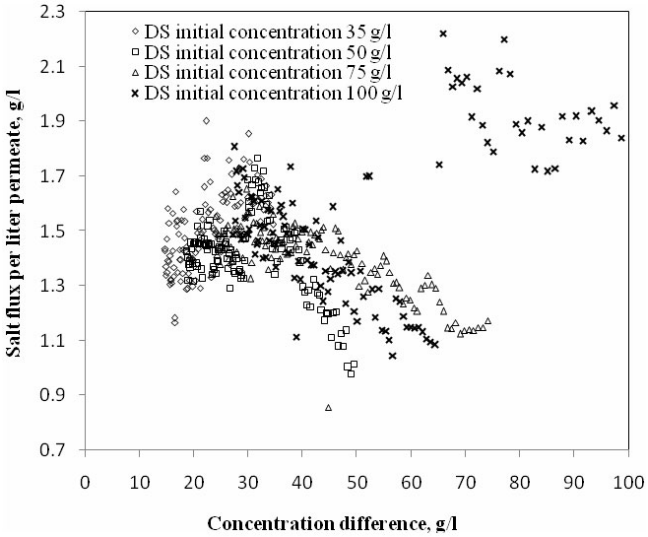

Fig. 7. Salt transported from a draw solution into feed water per liter permeated water.

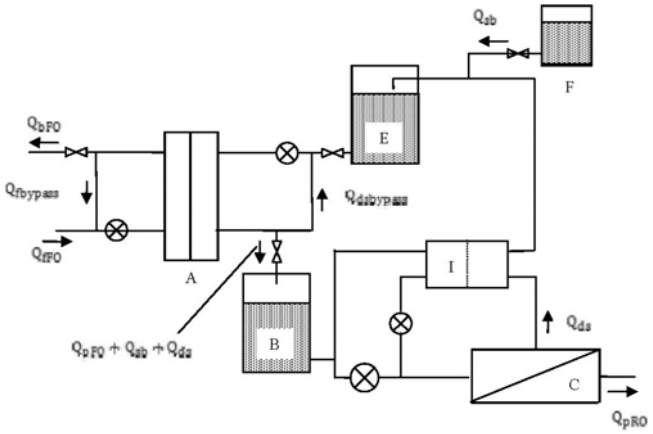

Fig. 8. Main components and design parameters of a conceptual integrated FO–RO system. A, FO module; B, FO permeate reservoir; C, RO module; D, pressure exchanger; E, RO brine reservoir; F, concentrated salt brine reservoir.

19.2 g/l (6,400 to 19,200 ppm). It is expected that concentration polarization effects on the feed side of the membrane will be more predominant under higher feed water concentrations. Due to the concentrative effect on the feed side, the concentration gradient decreases and subsequently salt diffusion from the draw solution side into feed side is retarded.

As a result of continuous loss of salt from the draw solution, the osmotic driving force diminishes gradually. It is necessary to add salt to the draw solution in the form of high concentration brine and at the same rate of salt loss to keep the concentration at constant level. Based on salt transport data the FO system will require resupply of 1.5 kg salt to be added to the draw solution for each 1 m^3 of product water. This amount of salt will be lost with feed brine and cannot be recovered and therefore the cost of salt lost and its environmental impact must be considered.

Although NF and MF membranes were most recommended for RO pretreatment, these types of membranes will not be suitable for use in FO pretreatment since the loss of solute will be considerably high.

3.3. Design parameters of a conceptual integrated FO–RO system

A schematic representation of proposed components of an integrated FO-RO system is shown in Fig. 8. The system consists of one open-end loop for feed water and one closed loop for the draw solution. The draw solution loop connects the FO and RO subsystems. Due to expected variations in water fluxes of the FO and RO subsystems two reservoirs, B and E, are included in the draw solution loop to function as buffer storage for flux variations. Salt should be added regularly to the draw solution in a form of highly concentrated brine to balance the loss in draw solution concentration. The concentrated salt brine is stored in reservoir F which is equipped with a flow regulator to control the flow rate of the salt brine. Since any autonomous desalination unit should be designed with high energy efficiency, a pressure exchanger is incorporated into the system for recovery of hydraulic energy of the RO brine. In addition, the energy contained in the excess RO brine pressure is used for elevating RO brine to the draw solution reservoir, E, which is placed on a higher level to the FO modules to take advantage of gravity assisted flow.

The main design variables for an integrated FO–RO system consisting of a closed loop are feed water and draw solution concentrations, water and salt fluxes and recovery rates for each subsystem. These parameters should be correlated and optimized over a wide operational range to assure high performance operation of the system. Many manufacturers produce small seawater RO systems which were well tested and proved practical in many applications and the parameters of these systems are well established. The RO system parameters can be considered as input parameters for further analysis and optimization of the integrated FO-RO system. A high recovery ratio for the FO system could be achieved either by using several membrane elements arranged in series arrangement or by flow circulation of draw solution and feed water into the FO system. While increased number of FO membrane elements insures higher production capacity, it will result in a long filtration channel that requires higher pressure to maintain flow and therefore the membrane envelope will be subjected to breaking risk. On the other side, excessive circulation of fluids requires more energy inputs for pumping. Therefore, the rate of circulation and the number of FO membrane elements should

be carefully balanced to achieve a system of reasonable size and simplicity.

For steady operation of the system, the RO permeate flux, $Q_{p\,\mathrm{RO}}$ should be equal to the sum of FO permeate flux, $Q_{p\,\mathrm{FO}}$ and the flow rate of concentrated salt brine, Q_{sb}, i.e.,

$$Q_{p\mathrm{RO}} = Q_{p\mathrm{FO}} + Q_{sb}$$

The flow rate of salt brine, Q_{ab}, is calculated as follows:

$$Q_{sb} = \frac{J_{s\mathrm{FO}} Q_{p\mathrm{FO}} + J_{s\mathrm{RO}} Q_{p\mathrm{RO}}}{C_{sb}}$$

where $J_{s\mathrm{FO}}$ is the salt flux in the FO system per liter of permeate water, g/l; $J_{s\mathrm{RO}}$ the salt flux in the RO system per liter of permeate water, g/l and C_{sb} is the concentration of salt brine, g/l.

Also, the following equations were assumed for calculating water recovery ratios of the FO, R_{FO}, and RO, R_{RO} subsystems, respectively.

$$R_{\mathrm{FO}} = \frac{Q_{p\mathrm{FO}}}{Q_{f\mathrm{FO}}}$$

$$R_{\mathrm{RO}} = \frac{Q_{p\mathrm{RO}}}{Q_{p\mathrm{RO}} + Q_{ds}}$$

Considering the equations given above, the FO permeate flux, $Q_{p\mathrm{FO}}$, and the FO recovery ratio, R_{FO}, could be correlated to the RO parameters as follows:

$$Q_{p\mathrm{FO}} = \frac{Q_{p\mathrm{RO}}(C_{sb} - J_{s\mathrm{RO}})}{(C_{sb} + J_{s\mathrm{FO}})}$$

$$R_{\mathrm{FO}} = \frac{R_{\mathrm{RO}}(C_{sb} - J_{s\mathrm{RO}})}{(1 - R_{\mathrm{RO}})(C_{sb} + J_{s\mathrm{FO}})} \frac{Q_{ds}}{Q_{f\mathrm{FO}}}$$

4. Conclusions

The application of the FO driven process in pretreatment of brackish and seawater prior to RO desalination provides many advantages for small autonomous RO desalination units. These advantages include reducing RO fouling, recovery of osmotic energy of RO brine and avoiding the use of chemicals required in conventional pretreatment.

Tests with FO membrane cartridges using osmotic draw solutions of refined sea salt showed a linear decrease in water flux from an initial highest value corresponding to the highest concentration difference when low concentrations of draw solution were used. However, a nonlinear trend was observed between water flux and concentration differences at higher concentrations of draw solution suggesting the existence of an optimal range of concentrations beyond its limit the water flux will only increases marginally with any increase in concentration.

Since the high pressure seawater RO systems can generate RO brine with concentrations between 60–70 g/l, the maximum water flux of FO membrane that can be achieved using this brine as draw solution will not exceed 7 LMH (4 gfd) for seawater pretreatment. In the case of pretreatment of brackish water having concentrations less than 5000 ppm, a maximum water flux of 11 LMH (6.4 gfd) can be achieved with the same RO brine.

The average normalized salt flux, which is the ratio of salt flux to water flux, remained almost constant at different levels of initial concentration of draw solution and ranged between 1.4–1.5 g/l. For a steady and continuous FO–RO process, it is necessary to add salt to the draw solution to keep the concentration at constant level. This amount of salt will be lost with feed brine and therefore the cost of salt and its environmental impact must be considered. In view of low water flux and high slat loss of currently available FO membranes, the FO-RO systems would be suited only for desalinating water with high fouling or scaling risk such as brackish and wastewater.

The main operating parameters and relationships of an integrated FO–RO system in which the FO and RO subsystems are connected in a closed loop are advised. Further analysis and optimization of the operation parameters of the integrated FO–RO system is recommended, and the results should be verified experimentally in a pilot plant. New configurations for FO spiral wound membranes that are intended to avoid glue breaking of the membrane envelope are also recommended.

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