This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This Accepted Manuscript will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Deep eutectic solvents as new class of draw agent to enrich low abundant DNA and proteins using forward osmosis

Dibyendu Mondal, Ashesh Mahto, Polisetti Veera Babu, Jitkumar Bhatt, Kamalesh Prasad and Sanna Kotrapanna Nataraj

Present study explores potential of green and sustainable deep eutectic solvents (DESs) as an alternative energy source for concentration of protein (>6 times) and DNA (>3 times) using forward osmosis (FO). The distinct colligative properties like high osmotic pressure (II >300 atm) and low water activity (aw ≤0.1) of the DESs make them ideal draw solution in FO process. These novel draw solutions in combination with composite semi-permeable membrane were successfully tested for enriching biomacromolecules. Recovery of draw solution was achieved by chilling the diluted solution after FO and reused in the process.

Introduction

The extraction and purification of low-abundance biomacromolecules such as proteins and DNA from a complex mixture involve a series of processes. Many protein enrichment techniques are available for the concentration and easier identification as well extraction of the bio-molecules. The enrichment step significantly improves the characteristic function, structure and interaction of particular or specific bio-macromolecule source media. Since, DNA and proteins are generally sensitive and stable under set of conditions, maintaining their structural integrity at the end of concentration process is vital and helps in accurate analysis. Therefore, green process like FO which uses no pressure or thermal or any other external force for its operation is attractive considering increased demand for pure protein and DNA in biotechnology, molecular biology, food sciences and clinical research.

There have been several attempts made to enrich proteins using membrane technology which offers preferential concentration of protein molecules. Particularly, pressure driven process like microfiltration (MF), ultrafiltration (UF) and nanofiltration (NF) have been popular choice for protein enrichment. But, many of these techniques have inherent drawbacks such as membrane instability (due to fouling) and high capital and operational costs. On the other hand, FO is simple and less capital intensive process operates without any hydraulic pressure, but requires high osmotic energy draw solution as driving force. After the discovery of deep eutectic solvents (DESs) by Abbott (2004) as an alternative to ILs, the application of the DESs is increasing exponentially in various applications. Further the properties of DESs such as non-toxicity, cheaper cost, etc., make them lucrative for number of applications e.g., as reaction media, reactants, and catalysts. In general, DESs can be obtained by complexation of the halide salts of quaternary ammonium or phosphonium cations (as the hydrogen bond acceptor, HBA) with hydrogen bond donor (HBD) moieties such as urea, glycerol, ethylene glycol, mannitol, resorcinol etc. There are reports on the applications of DESs for the dissolution of biopolymers, extraction of phenolic compound from plants, extraction of proteins, and many more applications of the novel liquids are continuously being explored. In the present research work, we attempt to demonstrate the use of DESs, such as choline chloride-ethylene glycol (ChoCl-EG 1:2) and choline chloride-glycerol (ChoCl-Gly 1:2) as efficient and recyclable energy resource in FO process. Although, there are few reports on bovine serum albumin (BSA) enrichment using FO process but to the best of our knowledge, there is no report available on protein and DNA enrichment using DES as draw solution using FO process.

Experimental section

Materials

Deoxyribonucleic Acid extracted from Salmon testes in the sodium salt form (CAS No. 9007-49-2, ca. 20 kbp) was purchased from TCI Chemicals, Tokyo, Japan. The DNA was used as received, since the purity of DNA was sufficiently high as determined from optical measurements. The ratio of the absorbance of the DNA stock solution at 260 nm to that at 280 nm was found to be 1.92, which suggested the absence of proteins. Choline hydrogen carbonate tris (hydroxymethyl) aminomethane (Tris-HCl) and Bovine serum albumin (BSA) was purchased from Sigma-Aldrich. All the other chemicals employed were of analytical grade and were used without further purification.

The deep-eutectic solvents (DESs) were prepared by the method as mentioned in the prior art. In a typical procedure, both the hydrogen bond acceptor i.e., choline chloride (S D Fine chemicals, Mumbai, India) and hydrogen bond donor i.e., ethylene glycol (S D Fine chemicals, Mumbai, India) was mixed in 1:2 molar ratio and heated at 80 °C with constant stirring for 2 h until homogenous and colorless liquid was formed. This liquid was used
as choline chloride-ethylene glycol (ChoCl-EG 1:2) based deep eutectic solvent. For the synthesis of choline chloride –glycerol (ChoCl-Gly) based DES similar procedure was followed except glycerol (S D Fine chemicals, Mumbai, India) was taken in place of ethylene glycol. The physiochemical properties of the DESs used as draw solution in FO are provided in Table 1.

Characterizations

Densities of as prepared deep eutectic solvents (DESs) were measured by taking weight at unit volume using specific gravity bottle of volume 5 mL. The bottle was filled with respective DESs at room temperature (25 °C) and their respective weights were recorded followed by measuring the ratio of the weight of volume to get the density. The viscosity was measured on a Brookfield DV-II+ Pro Viscometer at 25 °C using spindle No 18 at 10 rpm. Conductivity of DES at different dilution was measured on a conductivity meter (EUTECH instrument, USA) at 25 °C. The UV–Vis absorption spectra of standard DNA and protein solutions and their concentrated solutions were recorded on a Varian CARY 500 UV-Vis-NIR spectrophotometer. Osmotic pressures of the DESs were measured at different dilution using Vapour Pressure Osmometer (WESCOR, VAPRO) at room temperature. FT-IR of the samples was carried out on a Perkin-Elmer FT-IR machine (Spectrum GX, USA). Circular dichroism (CD) spectra were recorded on a Jasco model J-815 CD Spectrometer, using measurement range at 230–350 nm at a scanning speed of 10 nm/min and band width of 1 nm. The spectra were acquired in a 1.0 cm path-length quartz cuvette at 25 °C.

SDS-PAGE

BSA solutions prepared in 0.005 M Tris-HCl buffer and the solution concentrated after 20 h of FO were subjected to SDS-PAGE analysis using hand poured 10% polyacrylamide gels. Gels were electrophoresed for 1 h at 200V using Borad mini electrophoresis system and then stained with Coomassie Brilliant Blue to visualize the protein bands.

Agarose gel electrophoresis

Agarose gel electrophoresis was carried out with salmon testes DNA (10 µl, 200 µg/ml) before and after concentration by 20 h FO run. The absorbance of IR light by water enhances linearly with the sample condensed phase to the reference absorbance A₀ (Eq. 3). 18

\[ a_w = \frac{ERH}{100} = \frac{P}{P_o} = \frac{X}{X_o} = \frac{C}{C_o} = \frac{A}{A_o} \]  

The absorbance \( A \) of IR light by water enhances linearly with the water concentration \( C \) in the gas phase as described by the Beer-Lambert law (\( A = \varepsilon dC \)). Thus, the \( ERH \) or \( aw \) can be calculated via the ratio of the absorbance \( A \) in the gas in equilibrium with the sample condensed phase to the reference absorbance \( A₀ \) (Eq. 3). 18

\[ \text{Osmotic pressure (} \pi \text{ in bar)} = \frac{i}{MRT} \]  

where, \( i \) is van't Hoff factor of the solute, \( M \) is molar concentration in mol/L, \( R \) is universal gas constant (0.082 L.atm.K⁻¹.mol⁻¹) and \( T \) is absolute temperature in K.  

Fabrication of thin-film composite polyamide membrane:

Laboratory made thin film composite (TFC) polyamide membrane was used in the present study. 19 Thin-film composite (TFC) polyamide membranes for which the technology was developed at RO-Division, CSIR-CSMCRI, were used as FO membrane to test the performance of draw solutions. TFC membrane is comprised of polysulfone-ultrafiltration (15% polymer concentration) supporting layer with polyamide selective skin made up of trimesoyl chloride (TMC, 0.125% aqueous solution) and m-phenylenediamine (MPD, 5 % in hexane) through interfacial polymerization. Post treatments were carried out by treating with citric acid (2%) and water wash followed by glycerol coating (20%). In all the experiments, before fitting the membrane to testing kit glycerol was washed to regenerate original polyamide layer. Before using TFC-polyamide membrane for FO applications, membranes were characterised for separation performance for reverse osmosis standards. When operated in RO mode at 5 bar pressure, a flux of 26.45 L.m⁻².h⁻¹ (LMH) and rejection of 94.9 % were observed for desalination of 2000 mg L⁻¹ (ppm) brackish water (BW). High rejection efficiency was observed for desalination of seawater (SW) also, albeit at high applied pressure. In view of the satisfactory flux and rejection even at low (5 bar) operating pressure in experiments with BW, the membrane was considered suitable for FO application and results obtained with the membrane are discussed below. The TFC membrane as described above was treated with isopropyl alcohol (IPA) by dipping for 5-7 minutes. The IPA treated TFC membrane was then kept overnight in RO water (Conductance 170 µS). Before using the membrane for FO process it was washed thoroughly with RO water. Nano filtration experiments of different concentration of MgSO₄, NaCl, reactive Black5 (Dye content- 55%; Sigma Aldrich), BSA protein (minimum assay- 97%; SDACL, Mumbai, India) were performed using IPA treated TFC membrane at 6 bar applied pressure. ESI Table S1 provides the data of flux and rejection for nano filtration experiment. This pretreated TFC membrane was utilized for some specific applications employed in the present study.

FO testing kit: A flat sheet membrane testing kit with 0.0057 m² active membrane surface area was fabricated in the Institute’s workshop. Pressure booster pumps capable of maintaining a pressure between 0–10 bars were used for circulation of feed solution and draw solution taken in suitably sized glass containers. After several trials, an optimized inlet operating pressure of 1 bar was adopted. A restricting needle valve was provided on the feed outlet of the membrane kit to pressurize the feed solution. For the circulation of both feed solution and draw solution, RO booster pumps (KEMFLO) were used with nominal flow rate of 1.8 LPM and maximum input pressure capacity of 60 psi. Figures of FO
experiment and membrane morphology are provided in Figure S1 (ESI).

**Flux and Rejection**

The solvent flux \( (J_s) \) across the FO membrane in FO was calculated using water permeability factor for every one hour interval and the osmotic pressure difference as the equation 5:

\[
J_s = \frac{V_o - V_p}{A(t_o - t_a)}
\]

Where, \( J_s \) is fluxes in \((L.m^{-2}.h^{-1})\), \( V_o \) and \( V_p \) are volume of feed solution at zero time and at interval \( n \), respectively and \( A \) is area of the membrane. All optimized experiments were carried out at 1 bar applied pressure on FS.

The rejection of salts in FO is calculated using equation 6

\[
\% R = \left( \frac{C_f - C_p}{C_f} \right) \times 100
\]

Where \( C_f \) and \( C_p \) are the concentration of feed and permeate solutions, respectively.

**FO process for DNA concentration**: In a typical FO procedure, 0.5 L of 500 ppm DNA solution in 0.01 M Tris-HCl buffer (1.5 gm of CC-EG was added to stabilize DNA) was taken as feed solution and 100 mL of CC-EG was taken as draw solution. IPA treated TFC membrane as described above was fitted in single stack FO cell (1 chamber for feed solution and 1 chamber for draw solution) with active membrane surface area of 0.0057 m² was employed for this experiment. At 1 bar applied pressure and at room temperature operation 1781 ppm of concentrated DNA (i.e. ca 3.5 times) was yielded at an average flux of 3.1 LMH/bar. No loss of DNA was confirmed at the draw side by spectrophotometer and no denaturation of DNA was confirmed by CD spectra.

**FO process for protein enrichment**: In a typical FO procedure, 1 L of 200 ppm bovine serum albumin solution in 0.005 M Tris-HCl buffer was taken as feed solution and 150 mL of CC-EG was taken as draw solution. Isopropyl alcohol treated TFC membrane as described above was fitted in double stack FO cell (2 chambers for feed solution and 1 chamber for draw solution) with active membrane surface area of 0.01265 m² was employed for this experiment. At 1 bar applied pressure and at room temperature operation 1460 ppm of concentrated BSA (i.e. ca 7 times) was yielded at an average flux of 4.67 LMH/bar. No loss of BSA was confirmed at the draw side by spectrophotometer.

**Results and discussion**

There are several advantages of using DES over ionic liquids (ILs) or any other reported draw solutions. Few of such advantages are, DESs are easy to handle, non-toxic and more importantly, they can be efficiently recovered. Many of the reported draw solutes are either toxic or expensive and undergo back diffusion contaminating feed solution. Therefore, delioce bio-macromolecules have to be handled very carefully at room temperature since elevation of temperature or physicochemical parameters often destroy their conformations. Moreover, lack of efficient draw solution recovery steps proposed in the literature limits their sustainability prospects. However with some exceptions where product or draw solutions were recovered through NF or RO processes. Further, draw solution recovery by thermal evaporation further add cost to the process.

Furthermore, magnetically active nanoparticles have to be used in higher concentration to induce effective osmotic pressure.

**Table 1 Properties of DESs as draw solution in FO applications.**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Draw Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ChoCl-EG (1:2)</td>
</tr>
<tr>
<td>Density at RT (g.cm⁻³)</td>
<td>1.13</td>
</tr>
<tr>
<td>Viscosity, ( \eta ) at 25 °C (cP)</td>
<td>33</td>
</tr>
<tr>
<td>Conductance at 25 °C (mS)</td>
<td>11.9</td>
</tr>
<tr>
<td>( T_m ) (°C)⁰</td>
<td>-12.9</td>
</tr>
<tr>
<td>( T_f ) (°C)¹</td>
<td>-66</td>
</tr>
<tr>
<td>( E_2 ) (30) (kCal.mol⁻¹)²</td>
<td>57.3</td>
</tr>
<tr>
<td>Water activity (( a_w ))</td>
<td>0.12</td>
</tr>
<tr>
<td>Osmotic pressure, ( II ) (atm)</td>
<td>365</td>
</tr>
</tbody>
</table>

RT=Room Temperature (25 °C)

Herein, we show the osmotic pull efficiency of ChoCl-EG 1:2 and ChoCl-Gly 1:2 for DNA and protein enrichment. Ever since their discovery in 2003 by Abbott et al,² as mentioned above, although DESs have attracted remarkable interest among research community across chemical sciences, but to the best of our knowledge no attempts so far made to utilize DESs as an alternative energy source. In addition, other than high osmotic energy, DESs offer unique dynamic recovery opportunity during post enrichment process, which greatly enhances the feasibility of FO process for such applications. Moreover, DESs constitute used herein are non-toxic well known for their virtue as food ingredients. Ethylene glycol is used as an antifreeze agent and glycerol is a widely used food additive. All DES constituents used here can be derived from bio-based resources and are readily available in the market at very low cost, EG (<0.2 S/Kg), Gly (~3 S/Kg) and ChoCl (~2 S/Kg). The characteristic properties such as viscosity, melting temperature (\( T_m \)), freezing temperature (\( T_f \)), water activity and other physical parameters of DESs are provided in Table 1. With the formation of ChoCl-EG 1:2 and ChoCl-Gly 1:2, their discovery in 2003 by Abbott et al,² their manifestation changes significantly.

![Fig. 1](image-url)

*(a) Vibrational bending peaks of water, and different concentration of DES in water (ChoCl-EG 1:2) at different dilutions, (b) water activity and area of bending vibration as function of % dilution of DES and (c) osmotic pressure (\( \pi \)) depression pattern as a function of dilution of draw solution.*
Firstly, for sustainable FO operations DS need to exercise substantial osmotic pressure across membrane even after several fold dilution besides retaining low water activity and freezing point depression. Therefore, to understand the colligative properties of the DESs, the main contributing parameters such as conductance, water activity ($a_w$) and osmotic pressure ($\pi$) were measured at different dilutions (Fig. 1). For water, intensity of bending vibration peak in FT-IR centred on $\sim$1600 cm$^{-1}$ and absorbance peak intensity increases with the water concentration in DES (Fig. 1a). Therefore, area of vibrational absorption increases with the dilution, which is equivalent the activity of water in mixture (please refer ESI for details). Though, the sharp rise in area of integrated absorbance was noticed after $\sim$60% dilution, $a_w$ remains $< 0.6$ even after 200% dilution as seen in Fig. 1(a). The dilution effect on osmotic pressure of DESs was calculated and corroborated with experimental studies as shown in Fig 1(b). Pure DES carries chemical potential of 5.74 kJ.mol$^{-1}$ and high osmotic pressure of $\pi>300$ atm (Fig 1(c)). Significant osmotic pressure ($\pi>60$) retention in DES even after 80% dilution makes the DESs promising candidates for maintaining high osmotic flux.

The second most important component in FO for achieving sustainable process is integration with an efficient semi-permeable membrane. All experiments were performed using indigenously developed isopropanol treated thin-film composite polyamide (TFC-PA) membrane. TFC-PA has a three component asymmetric structure consisting polyester fabric of thickness $\sim$90 µm, polysulfone porous layer ($\sim$40 µm), and selective thin-layer of polyamide. The structure of the thus obtained membrane was confirmed using FT-IR (ATR) measurements as shown in Fig 2(a). In TFC-PA, the characteristic C=O, amide-I stretching bands are seen at 1660 cm$^{-1}$ and sharp bands around 1550 cm$^{-1}$ corresponds to N–C=O and C–N–H bending (amide-II), which is absent in polysulfone support matrix. The inset SEM image in Fig 2(a) shows the wave-like surface morphology originated from interfacial selective polyamide layer formation in TFC membrane. Initially, TFC-PA membranes were characterized for their separation for different feed and operational conditions as shown in Table S1.

A study was then conducted to probe the most efficient DES as DS using brackish water as feed solution and DES as DS in a FO experiment. An average flux of 7.85 and 3.71 L.m$^{-2}$.h$^{-1}$.bar$^{-1}$ was recorded using ChoCl–EG 1:2 and ChoCl–Gly 1:2 as draw solution respectively. Thus having higher value of flux and very low freezing point besides high osmotic pressure, ChoCl–EG 1:2 was optimized as effective DS and further studies was conducted with this DS as discussed below using bench-scale experimental setup as shown in ESI Figure S1. However, the recovered water was found to have impurities of choline (2000–5000 mg.L$^{-1}$) marginally higher compared to standard upper limit intake for adult men (Choline, 3500 mg/day).

Maintaining chemical and structural integrity of bio-macromolecules during prolonged enrichment process is a challenging task. Use of green solvent and simple osmosis driven FO process provides typical environment for protein and DNA enrichment. Fig 2(b) shows UV-vis spectra of DNA in feed at different time intervals of enrichment process. The absorbance value at 260 nm for DNA increased markedly, whereas no peak was observed in the final DS indicating zero loss of DNA from FS to DS during FO. The DS, ChoCl–EG 1:2 was
resulted in high rate of osmotic flux of ~3.2 L.m⁻².h⁻¹.bar⁻¹ at 22 ± 2°C leaving behind ~3 times enriched DNA as seen in Fig 2(c). Maintaining simple feed and draw solution circulation ensures loss of valuable bioactive constituents. Intermediate sample photograph in Fig 2(d) collected during FO process visibly corroborate the DNA enrichment. Further, circular dichroism (CD) spectra depicted in Fig 2(e) shows no significant difference between the original feed and concentrated DNA even after 14 h of continue FO operation indicating the conformation of DNA remains intact during the concentration process, signifying no reverse flow of draw solution (noting high concentration of free Cl in diluted DES) in enrichment process. Further, agarose gel electrophoresis of the DNA solutions before and after concentration showed similar elution profile indicating structural integrity of the bio-macromolecule after FO run as shown in Fig 2(f).

Food processing industries such as dairy product manufacturers supply various proteins sources viz., mixture of protein concentrates, isolated products or specialty pure proteins at affordable price in large scale. Membrane processes like ultra and microfiltration process are being used for BSA enrichment. But, under rigours filtration conditions like applied pressure and flow rates, severe protein denaturation and destabilization may occur even at room temperature (~25°C). However, FO without any applied pressure shows promising alternative to process protein enrichment. Fig 3(a) shows the UV-Vis spectra of BSA solutions obtained at different interval of FO run. The absorbance value at 278 nm for BSA was increased markedly for final feed solution in comparison to initial, whereas no peak at 278 nm was observed in the final DS indicating safe and fast enrichment. The absence of any peak in final DS indicated 100% rejection of BSA during FO. Fig 3(b) depicts concentration and osmotic flux profile.

On the other hand, CD spectra of BSA after concentration showed peak at 222 nm for the α-helix identical to the protein before concentration (Fig 3(c)). Furthermore, SDS-PAGE of BSA before and after concentration by FO showed similar electrophoresis profile with no loss of BSA monomers in the concentrated solution indicating stability of the chemical structure of the protein after the concentration as shown in Fig 3(d). Since the flux obtained with the DS is very crucial for determining efficiency of a FO process, the flux rate of ChoCl₂EG 1:2 was compared that with 3M NaCl solution, a widely used DS in FO process. The DES showed about 1.5 times higher flux rate indicating superior property of the new DS for such applications (ESI, Figure S2). In both cases (DNA and BSA), relatively good flux (>2 L.m⁻².h⁻¹.bar⁻¹) was recorded throughout in a batch experiment which is relatively higher than the reported specific fluxes in the literature (ESI, Table S3). Thus, using DES as draw solution > 6 fold concentration of protein and > 3 fold DNA concentration can be achieved in a green and sustainable way as shown in Fig 3(e & f). To make it more sustainable DES as draw solution offers unique recovery pathway. Exploiting the low freezing point behaviour of ChoCl₂EG 1.2 (−66 °C) diluted ChoCl₂EG was further chilled (−5 °C) to separate quality product water and reusable draw solution. The recovered ChoCl₂EG (with ~20 % dilution with water) was further reused for three times in the protein enrichment process maintaining the identical flux that recorded with pure ChoCl₂EG 1:2.

Conclusion

In conclusion, present study describes simple, safe and efficient enrichment protocol for temperature and pressure sensitive bio-macromolecules of low-abundance. Enrichment of proteins and DNA was achieved without compromising chemical and structural integrity of feed constitutes. Energy benefits from freezing of the diluted draw solution to recover product reusable draw solution compared to pressure driven processes and other FO systems are very significant. The proposed process offers significant technological benefits and applicable to biomedical and food processing industries.

Notes and references

a Reverse Osmosis Membrane Division, CSIR-Central Salt and Marine Chemicals Research Institute (CSIR-CSMCRI),
b Marine Biotechnology & Ecology Division, CSIR-Central Salt and Marine Chemicals Research Institute (CSIR-CSMCRI), G. B. Marg, Bhavnagar 364 002, India.
c Academy of Scientific and Innovative Research (AcSIR), Central Salt and Marine Chemicals Research Institute, G.B. Marg, Bhavnagar 364 002, India.

Corresponding Authors: S.K. Nataraj (sknataraj@csmcri.org; sknata@gmail.com) Tel: +91-278-2567563 and K. Prasad Tel: +91-278-2567563 (kamlesh@csmcri.org; drkamlesh@gmail.com)

Acknowledgements

SKN gratefully acknowledges the DST, Government of India for DST-INSPIRE Fellowship and Research Grant (IFA12-CH-84). DM and AM acknowledges CSIR and UGC for Research Fellowships as well as AcSIR for PhD registration. KP thanks CSIR for the Young Scientist Awards project and overall financial support. Dr. Ashish Parida is acknowledged for SDS-PAGE measurements. Analytical Science and centralized instrument facility of the institute is acknowledged for analytical support. This is CSIR-CSMCR communication #176/2014.

1 J. E. Bandow, Proteomics, 2010, 10, 1416.
8 T-W. Kim, Y. Kim, C. Yun, H. Jang, W. Kim, S. Park, Desalination, 2012, 284, 253.
21 D. Li and H. Wang, *J. Mater. Chem. A*, 2013, I, 14049
Deep eutectic solvents as new class of draw agent to enrich low abundant DNA and proteins using forward osmosis

Dibyendu Mondal, Ashesh Mahto, Polisetti Veera Babu, Jitkumar Bhatt, Kamalesh Prasad* and Sanna Kotrappanavar Nataraj*

Present study explores the osmotic energy potentials of deep eutectic solvents as an efficient and sustainable alternative energy source for protein and DNA enrichment using forward osmosis (FO).