Human urine as a forward osmosis draw solution for the application of microalgae dewatering

Federico Volpin[1], Hyeonjung Yu[2], Jaeweon Cho[2], Changsoo Lee[2], Sherub Phuntsho[1], Noreddine Ghaffour[3], Johannes S. Vrouwenvelder[3] and Ho Kyong Shon[1*]

[1] School of Civil and Environmental Engineering, University of Technology, Sydney (UTS), City Campus, Broadway, NSW 2007, Australia.

[2] School of Urban and Environmental Engineering, Ulsan National Institute of Science and Technology (UNIST), UNIST-gil 50, Ulsan 44919, Korea.

[3] King Abdullah University of Science and Technology (KAUST), Water Desalination and Reuse Center (WDRC), Biological & Environmental Science & Engineering Division (BESE), Thuwal 23955-6900, Saudi Arabia.

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[*] Corresponding author: Tel.: (+61) 02 9514 2629; email: Hokyong.Shon-1@uts.edu.au

Abstract

Human urine is a unique solution that has the right composition to constitute both a severe environmental threat and a rich source of nitrogen and phosphorous. In fact, between 4-9% of urine mass consists of ions, such as K^+, Cl^-, Na^+ or NH_4^+. Because of its high ionic strength, urine osmotic pressure can reach values of up to 2000 kPa. With this in mind, this work aimed to study the effectiveness of real urine as a novel draw solution for forward osmosis. Water flux, reverse nitrogen flux and membrane fouling were investigated using fresh or hydrolysed urine. Water flux as high as 16.7 ± 1.1 $L.m^{-2}.h^{-1}$ was recorded using real hydrolysed urine.

Additionally, no support layer membrane fouling was noticed in over 20 hours of experimentation. Urine was also employed to dewater a *Chlorella vulgaris* culture. A fourfold increase in algal concentration was achieved while having an average flux of 14.1 $L.m^{-2}.h^{-1}$. During the algae dewatering, a flux decrease of about 19% was noticed; this was mainly due to a thin layer of algal deposition on the active side of the membrane. Overall, human urine was found to be an effective draw solution for forward osmosis.

Keywords: Human Urine, Forward Osmosis, Draw Solution, Microalgae Dewatering, Nutrient Recovery

1. Introduction

Sustainable urban water management is shaping the design of future water and wastewater infrastructure [1-3]. Specifically, the source-separation of urine, brown water, greywater and stormwater could open up numerous opportunities for the recovery of nutrients, energy and water from wastes [1, 4]. Despite its low volume relative to the overall wastewater flow, human urine on average contributes more than two-thirds of the nitrogen (N) and half of the phosphorous (P) and potassium (K) present in sewage [5-8]. This high concentration of nutrients results in substantial costs during downstream wastewater treatment [9]. However, despite its high nutrient concentration, the direct application of urine as a raw fertiliser is hindered by its non-ideal N/P/K ratio (the concentration of N is much greater relative to that of P and K), its high concentration of NH_3 and organic acids (resulting in the characteristic unpleasant smell), and the possible presence of hormones and pharmaceuticals [5]. Nonetheless, if we could adequately mine and transform these nutrients into marketable products, the potential economic and environmental benefits would be enormous [8, 10, 11].

One approach to simultaneously treating urine and transforming it into a valuable product is microalgae cultivation [12]. In this concept, microalgae are grown in the nutrient-rich urine, which are later harvested and converted into feedstock for the production of valuable chemicals (e.g., Poly-3-hydroxybutyrate, pigments) or biofuel [13-18]. Previous work from de Wilt et al. (2016) has shown that in an algae photobioreactor, 60–100% of pharmaceuticals such as diclofenac, ibuprofen and paracetamol could be removed via biodegradation and photolysis [19]. Consequently, sustainable urban water management and nutrient recycling could also be achieved.

However, the high pH and free ammonia concentration (i.e. 2–8 g N/L) of urine were found to inhibit microalgal growth [13, 14, 16]. A minimum dilution of 100% was found to be necessary for stable algal growth [13]. In addition, a further barrier to the feasibility of algal cultivation is lack of access to a low-cost and efficient algal dewatering process. In fact, 20-40% of the production cost for microalgae is generally associated with the harvesting process [20-22]. Harvesting algae is costly because of low algal density; this makes many commercial dewatering technologies, e.g. centrifugation, belt filtration and flocculation, either very energy- and chemical-intensive or capable of affecting the final biomass quality [22, 23].

Meanwhile, membrane-based filtration processes have gained attention as an alternative dewatering approach due to their lower energy consumption and footprint [23]. However, one of the downsides of pressure-driven algae filtration is the high chance of irreversible membrane fouling [22, 24, 25].

One alternative membrane-based process, which relies on the osmotic pressure gradient rather than the hydraulic pressure difference, is forward osmosis (FO). In FO, the transport of water across the membrane occurs due to the osmotic pressure difference between a solution with low osmotic pressure (i.e. the feed) and one with high osmotic pressure (i.e. the draw). Specifically, water moves from the feed to the draw until the osmotic equilibrium is reached [3, 26-28]. The absence of hydraulic pressure has a number of positive effects on the filtration, including lowering the operational costs and reducing the chance of irreversible fouling [29, 30].

The high salinity of human urine causes its osmotic pressure to range between 1100-1800 kPa when fresh and up to 2200-3000 kPa when hydrolysed, making it a completely unexplored FO draw solution [3, 5, 31]. Moreover, as urine is generally considered a waste product, the cost of using it as a draw solution (DS) is expected to be minimal.

In light of the above, the present work aimed to investigate the feasibility of utilising real urine as an FO draw solution to dewater a *Chlorella Vulgaris* culture.

2. Materials and methods

2.1 Experimental plan

To understand the effectiveness of real urine as an FO draw solution, experiments using fresh and hydrolysed urine (real and synthetic) were initially conducted.

First, the effects of urea hydrolysation on the electric conductivity (EC) and ion concentration of urine were measured. Based on this analysis, the van 't Hoff equation was used to calculate the osmotic pressure of fresh and hydrolysed urine. Next, the effectiveness of different fresh and hydrolysed urine samples, which have distinct EC values, was investigated. FO experiments were performed using 500 mL of real and synthetic urine as DS and 500 mL of de-ionised water as FS. The goal was to identify a correlation between the initial urine EC and the FO water flux.

It should be noted that despite the correlation between the EC and osmotic pressure, a high EC does not necessarily mean a high FO flux, as the diffusivity of the species in the DS has a significant impact on the concentration polarisation effect and, therefore, the flux itself. During these tests, the nitrogen reverse salt flux of fresh and hydrolysed urine was also measured. Given the heterogeneous composition of urine, long-term experiments (i.e., > 20 hours in length) were then conducted to study the support layer fouling (if any) of the membrane [32]. In these experiments, the pristine membrane was first tested with de-ionised water (DI) and 0.5 M NaCl; the experiment was then operated again with real urine instead of NaCl, flushed with water, and finally re-tested with NaCl. The difference between the flux of the first and last NaCl test was used as an indication of membrane fouling.

After measuring the maximum flux achievable with urine as DS, urine was used to dewater a microalgae culture (Figure 1). 500 mL of 0.2 g/L *C. Vulgaris* was employed as FS and 2 L of urine as DS. This concentration was chosen to benchmark the present results against the outcomes from other published studies [21, 22]. Synthetic seawater was also used as a benchmark for the results. During these tests, fouling of the active layer was also investigated, and the Extracellular Polymeric Substance (EPS) was measured before and after the filtration. Analysis of the EPS concentration was performed to elucidate the response of microalgae to the reverse salt flux (RSF) of the compounds present in the urine, in particular to urea and ammonia, as well as to the algal stress occurring due to pumping and FO filtration [33]. To summarise, the following investigations were performed:

- Impact of urine hydrolysis in the EC and osmotic pressure;
- Maximum achievable FO flux using real fresh and hydrolysed urine;
- Membrane support layer fouling when urine DS is used;
- The effectiveness of urine DS in concentrating a microalgae solution.

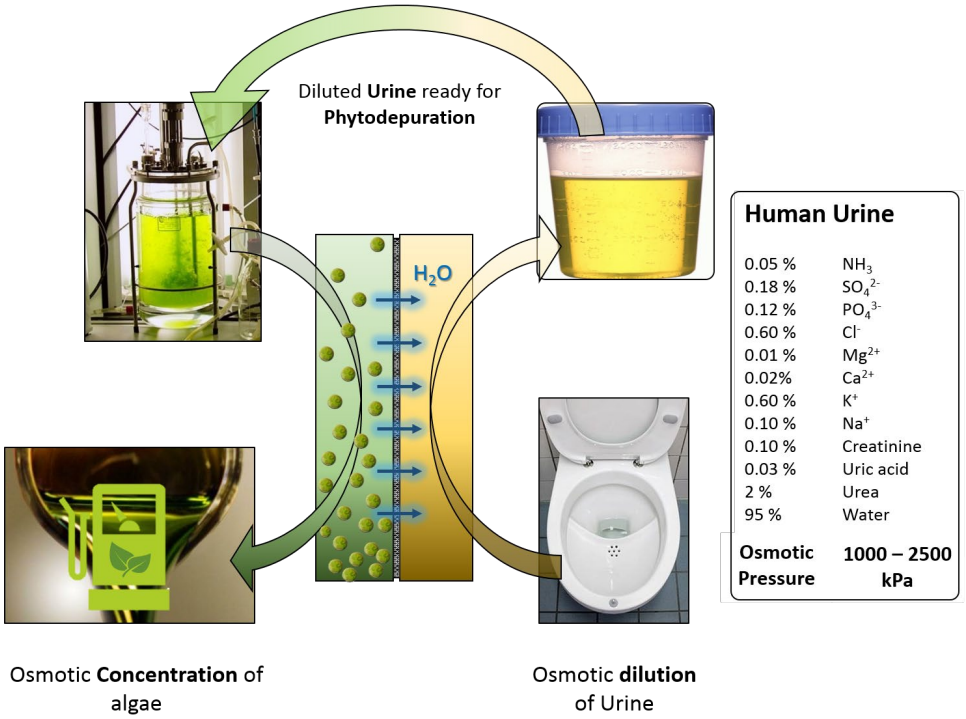

Figure 1. Conceptual design of the process where urine is used as DS to concentrate a microalgae solution.

2.2 Forward osmosis set-up and feed and draw solution preparation

Toray Chemical Korea Inc. supplied the thin-film-composite (TFC) polyamide (PA) FO membranes that were used for the experiments. Measured pure water permeability (A) was found to be 6.64 $L.m^{-2}.h^{-1}.bar^{-1}$, NaCl selectivity (B) was 1.17 $L.m^{-2}.h^{-1}$, and the structural parameter (S) was 409 µm. To measure the transport and structural (i.e., A, B and S) values, the four-step method proposed by Tiraferri et al. was employed, as it does not use hydraulic pressure (which could cause membrane deformation) [34]. Equation 1 is used in the four-step method to predict the structural parameters.

$$J_w = \sigma K_m \ln\left(\frac{A\pi_D + B_s}{A\pi_F + J_w + B_s}\right) \quad \text{(AL-facing FS)} \qquad (1)$$

Here, σ is the reflection coefficient, assumed as unity (complete rejection of the solute), while K_m is the mass transfer coefficient of the selected DS and is given by the ratio between the diffusivity of the salt and the structural parameter (S) of the membrane (i.e. $K_m = D/S$). The transport coefficients for water and solutes are expressed as A and B_s. Finally, π_D and π_F are the DS and FS bulk osmotic pressure, respectively. The measured A, B, and S values were similar to those reported by the supplier. The membrane was tested in an acrylic testing cell that exposed an area of 20 cm^2 to the FS and DS solutions. The system was operated in counter-current mode, with the urine facing the porous support layer and the microalgae or DI-water solutions facing the PA membrane active layer. The DS weight

increase (i.e. water flux) was continuously measured via a digital scale that was connected to a computer for continuous data recording. Each set of experiments was carried out in triplicate. Real urine was compared to synthetic urine to study the effect of the trace organics present in real urine (e.g. carbohydrates and proteins) on the FO flux. The synthetic urine preparation, both fresh and hydrolysed, was carried out according to the values in the literature [35, 36]. The overall composition is presented in Table 1.

Real urine was collected from healthy lab members over two weeks at different times of the day. Directly after sampling, urine was immediately frozen at -80°C to prevent bacterial growth [37]. Following collection, a portion of the sampled urine was kept frozen for use in evaluating the impact of EC in the FO water flux (see Figure 3), while the rest was thawed at 4°C, mixed to homogenise the solution, and separated into two batches. The first batch was for the "hydrolysed urine" experiments. In this case, 10 µL of urease enzyme solution was added to the urine to speed up the hydrolysis process; the urine was hydrolysed at room temperature for 30 days before use, with the result that complete urea hydrolysis was reached. Urea concentration in the hydrolysed urine was measured before its use to confirm that less than 10 mg/L was still present. The second batch was used for the "fresh urine" experiments. In this case, the urine was divided into 1 L plastic bottles and re-frozen at -80°C. No pre-filtration of the urine was performed. Finally, the urine hydrolysation process was continuously monitored to facilitate understanding of the transformation of urea into NH_3. Throughout the monitoring, the urine was continuously analysed for urea, NH_3, EC and pH until it was fully hydrolysed (Figure 2).

Table 1. Composition and characteristics of the synthetic fresh and hydrolysed urine feed solution [36]. *Calculated with OLI System Analyser. ** Based on Udert et al. [36].

Components	Concentration [g/L]		Characteristics		
	Fresh Urine	Hydrolysed Urine			
Urea	16	-	pH* [-]	4.51	8.87
NH_4-Acetate	-	9.6	Osmotic Pressure* [kPa]	1340	3010
NH_4Cl	1.8	-	Alkalinity** [M]	0.02	0.59
Na_2SO_4	2.3	2.3	Ionic Strength** [M]	0.18	0.64
NaH_2PO_4	2.9	2.1			
KCl	4.2	4.2			
$MgCl_2$	0.37	-			
$CaCl_2$	0.51	-			
NaCl	0.183	3.6			
NH_4OHsol (25% NH_3)	-	13.0 mL			
NaOH	0.23	-			
NH_4HCO_3	-	21.4			

2.3 Microalgae cultivation and characterisation

Chlorella Vulgaris KCTC AG 10002 was purchased from the Korean Collection for Type Cultures (KCTC) and cultivated in a modified Bristol medium (per litre distilled water: 157 mg NH_4Cl, 25 mg $CaCl_2{\cdot}2H_2O$, 75 mg $MgSO_4{\cdot}7\ H_2O$, 75 mg K_2HPO_4, 175 mg KH_2PO_4, 25 mg NaCl, and 1 mL trace element solution) [38]. The trace element solution contained (per litre DI-water) 0.49 mg $Co(NO_3)_2{\cdot}6\ H_2O$, 8.82 mg $ZnSO_4{\cdot}7\ H_2O$, 1.44 mg $MnCl_2{\cdot}4H_2O$, 0.71 mg MoO_3, 1.57 mg $CuSO_4{\cdot}5\ H_2O$, 11.42 mg H_3BO_3, 50 mg EDTA, 31 mg KOH, 4.98 mg $FeSO_4{\cdot}7\ H_2O$, and 0.001 mL H_2SO_4 (98%, v/v). The medium was sterilised by autoclaving at 121°C for 15 min. The microalgal cultivation was conducted in 500 mL Erlenmeyer flasks at room temperature (20 – 25°C) under white light-emitting diode illumination (1000 lux) with a 16 h light and 8 h dark cycle. The flasks were continuously aerated with ambient air at a flow rate of 170 mL/min via magnetic stirring at 200 rpm. The *C. Vulgaris* culture was harvested at a volatile suspended solids concentration of 0.2 g/L and adjusted to pH 7.0 ± 0.3 with 3 M NaOH solution before use in the dewatering experiments.

2.4 Extracellular carbohydrates analysis

The EPS in the algae solution was measured before and after the FO filtration, using the phenol-sulphuric acid method with glucose as standard. EPS analysis was conducted to determine how EPS released by the algae varied during filtration with different types of DS. The EPS measurement

approach adopted was the same as that described by Larronde-Larretche and Jin 2017: firstly, the algae suspension was pelletised during centrifugation at 6 × g for 20 min; next, the supernatant was collected and filtered through a 0.2 μm hydrophilic nylon filter (Millipore, Australia) [22, 33]; finally, the EPS concentration was measured and converted from mg/L to mg by multiplying the concentration for the feed volume.

2.5 Analytical methods

Thermo Fisher Scientific ionic chromatography (IC, Australia) was used to measure the anions in the urine (i.e. SO_4^{2-}, Cl^-, PO_4^{3-}), while Agilent Technologies microwave plasma atomic emission spectrometry (MP-AES, Australia) was used for the analysis of cations (i.e. Na^+, Ca^{2+}, Mg^{2+}, K^+). Urea and ammonia were measured spectrophotometrically, at 340 nm wavelength, using a urea/NH_3 Megazime kit (Megazyme, Australia) [3]. Finally, Zeiss Supra 55VP scanning electron microscopy (Carl Zeiss AG, Germany) was used to analyse the membrane coupon before and after filtration. The membrane coupons were dried under compressed nitrogen and then coated with Au. A voltage of 10 kV was used for the image acquisition.

3. Results and discussion

3.1 Urine composition and hydrolysation process

Firstly, the effect of the storage on urine EC and osmotic pressure was investigated. This was crucial in determining the stage of hydrolysation at which the urine performs best as an FO DS. During the enzymatic hydrolysis of one mole of urea, two moles of NH_3 plus one mole of CO_2 are produced (reaction 1). As the osmotic pressure is a colligative propriety of the solution, the increase in the net amount of dissolved species leads to an increase in the osmotic pressure of the urine. This process is inevitable if the urine is stored in non-sterile conditions.

$$NH_2CONH_2 + H_2O + heat \longrightarrow 2\,NH_3 + CO_2 \quad (1)$$

To calculate the osmotic pressure, the urine composition was measured before and after storage. The results are shown in Table 2.

Table 2. Ionic composition, pH and conductivity of fresh and hydrolysed urine.

Urine Composition											
	Urea [mg/L]	NH_3	PO_4^{3-}-P	Cl^-	SO_4^{2-}	K^+	Mg^{2+}	Ca^{2+}	Na^+	Cond. [mS/cm]	pH [-]
Fresh	9049 ± 1489	96 ± 33	420 ± 190	3210 ± 410	723 ± 170	1177 ± 200	70 ± 12	90 ± 13	1943 ± 500	15.6 ± 3.1	6.3 ± 0.5
Hydrolysed	< 10	6272 ± 712	370 ± 190	3210 ± 410	723 ± 170	1177 ± 200	19 ± 7	46 ± 16	1943 ± 500	27.84 ± 4.4	9.1 ± 0.2

Subsequently, the pH, EC, urea, and NH_3 of the fresh urine were continuously measured during the hydrolysis process (Figure 2). Cations and anions were also measured to enable calculation of the osmotic pressure of the solution (Figure 2). The OLI Stream Analyser 3.1 software (OLI Systems Inc., Morris Plains, NJ, USA) was used for computing the osmotic pressure calculations. In theory, one mmole of urea generates about 2.4 kPa, while two mmols of NH_3 plus 1 mol of H_2CO_3 generates 7.2 kPa. This explains why the osmotic pressure of hydrolysed urine was found to be almost double that of fresh urine (Fig. 2 A). Figures 2 A and B also show that the measured parameters follow a semi-logistic function trend; in particular, there is a clear lag-phase and exponential growth phase, which is due to microbial ureolysis [39].

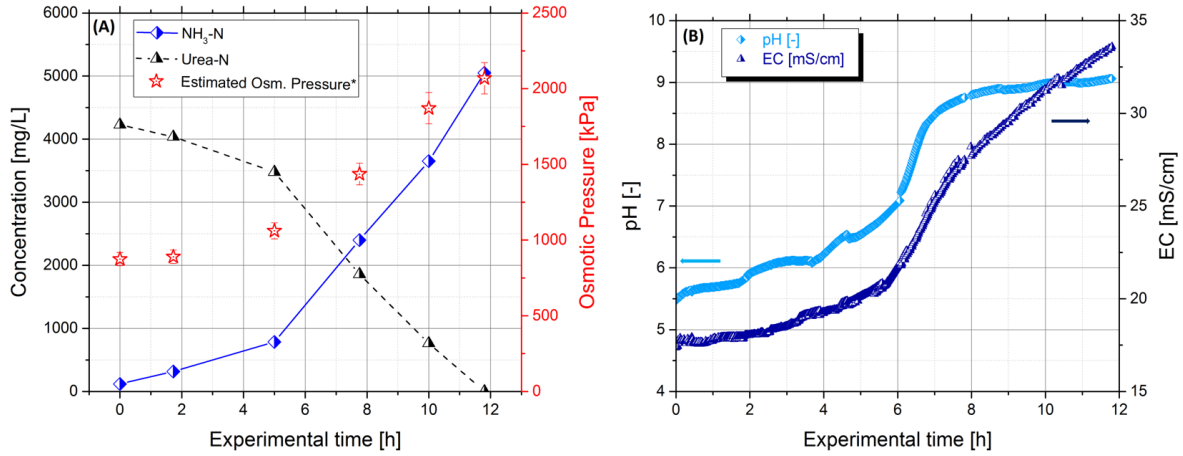

Figure 2. Measured urea and ammonia (A), pH and conductivity (B) during urine storage. * The osmotic pressure of the urine was calculated using OLI Stream Analyser 3.1 software based on the chemical analysis of the urine during storage (OLI Systems Inc., Morris Plains, NJ, USA).

3.2 Real and synthetic urine FO performance

However, the osmotic pressure of a solution is not sufficient on its own to predict the FO water flux. The internal concentration polarisation effect (ICP) and reverse salt flux are major factors hindering the water transport from FS to DS. The ICP, in particular, is a function of the diffusivities of the ionic species in the DS. The higher the diffusivity (D) of the ions, the lower the ICP and, therefore, the higher the water flux (J_w) will be (see equation 1) [40]. In this case:

$$D_{urea} = 1.32 \cdot 10^{-10} \frac{m^2}{s} < D_{NH_3} = 2.21 \cdot 10^{-9} \frac{m^2}{s} < D_{NaCl} = 1.48 \cdot 10^{-9} \frac{m^2}{s} \quad [2]$$

It can readily be observed that hydrolysed urine, compared to fresh urine, has both higher osmotic pressure and a higher concentration of ions with a greater self-diffusivity coefficient. Thus, hydrolysed urine should generate higher fluxes then fresh urine.

By looking at Figure 3, it can be seen that, as predicted, the water flux generated by hydrolysed urine is almost double that generated by fresh urine. More specifically, an average of 9.5 ± 0.7 $L.m^{-2}.h^{-1}$ was achieved with real fresh urine compared to 16.7 ± 1.1 $L.m^{-2}.h^{-1}$ with real hydrolysed urine as DS. Statistically, the water flux generated by fresh urine was the same when using real or synthetic solutions. On the other hand, synthetic hydrolysed urine solution achieved higher fluxes compared to the actual urine (Figure 4 A). One of the reasons might be that, given that $pKa_{NH_4^+} = 9.24$ and that NH_3 is a volatile compound, ammonia volatilisation might have occurred during the urine storage and testing process due to the high pH. This explains why the theoretical concentration of NH_3 in the hydrolysed synthetic urine is often higher than that in actual hydrolysed urine solutions.

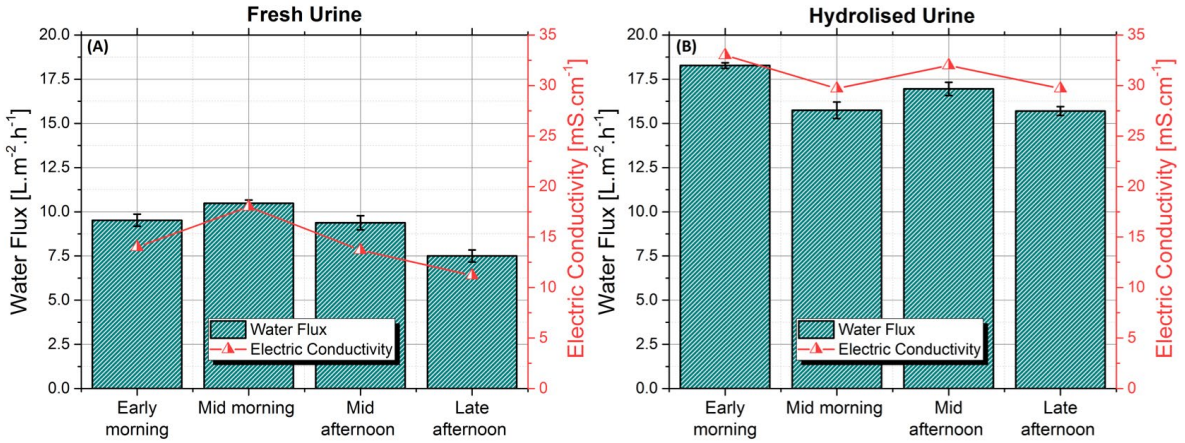

Figure 3. Histograms representing the water flux and EC of several samples of fresh (A) and hydrolysed (B) urine. The red triangles indicate the EC of the samples tested. These experiments were run for one hour using 500 mL of DS (urine) and 500 mL of FS (DI-water).

Figure 4 (B) also depicts the total nitrogen (TN), total organic carbon (TOC) and specific reverse salt flux (SRSF) when different urine solutions are used as DS. Given its low molecular weight and absence of charge, any polyamide membrane often performs poorly at removing urea [41]. This explains why the fresh urine had a higher TN/TOC SRSF. The higher SRSF of fresh urine also contributes to its lower water flux.

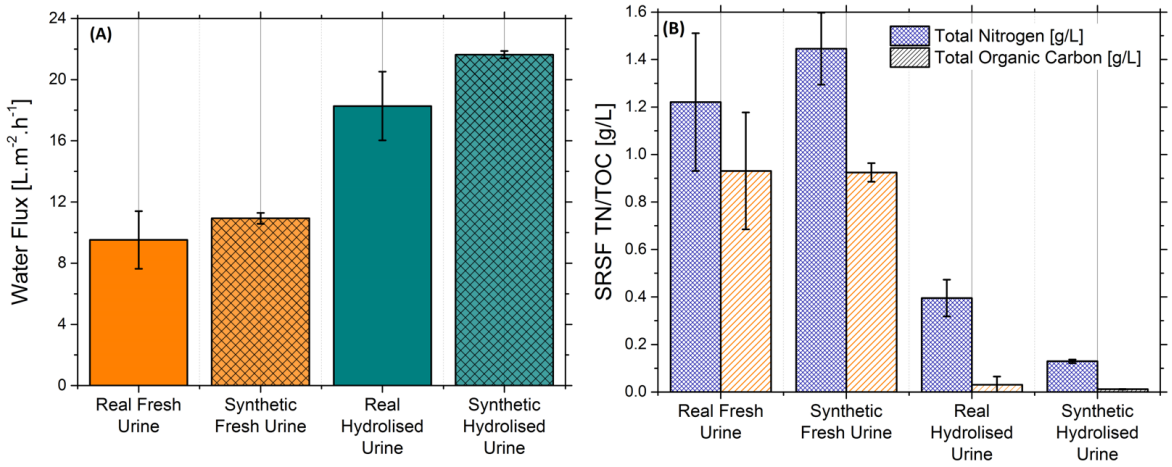

Figure 4. Summary of the experiments conducted with real, synthetic, fresh and hydrolysed urine using DI-water as FS. The water flux is presented in (A), while the reverse salt flux (SRSF) of TN and TOC are displayed in (B).

In summary, hydrolysed urine outperformed fresh urine, with a high water flux (i.e. up to 20 $L.m^{-2}.h^{-1}$) and a lower SRSF (i.e., 0.4 gN/L).

3.3 Support layer fouling while using real urine draw solution

Urine, due to its high concentration of creatinine, carbohydrates, organic acids, and suspended solids (e.g., $CaPO_4$, struvite), is a solution with a high fouling potential [42, 43]. As urine is facing the porous support layer of the membrane in these experiments, it is critical to assess whether this may cause

severe flux decline and irreversible fouling. Recent papers have shown that when urine is facing the active layer of the membrane, the fouling is reversible [35, 44]; however, no investigations into support layer fouling when urine is used as a draw solution have yet been published.

The experiments were run for 20 hours, reaching a final draw dilution over 70%, and benchmarked with the performance of a pure 0.6M NaCl solution. Figure 5 A shows that no rapid flux decline occurred when real hydrolysed urine was used as a draw solution; in fact, the observed flux decline occurred only due to the dilution of the draw solution. Figure 5 B shows that 98%-99% of the initial flux was recovered after urine filtration, meaning that the irreversible fouling on the membrane support is negligible. One explanation for this could be that the transmembrane water flux prevents the foulants from being entrapped in the porous support layer. Therefore, allowing for the shear force exerted by the cross-flow to preventing any fouling compaction [45, 46]. This phenomenon is similar to the effect of osmotic backwashing described by Kim C. et al. when describing the cleaning of a fouled FO membrane operated in AL-DS mode (active layer facing the draw side) [45].

Finally, figure 6 shows the image of pristine membrane support layer (B) and the support layer after filtration. Both the picture of the membrane and the SEM image show that there is no clear deposition of any crystal, gels or colloids on the membrane surface, reinforcing the idea that the support layer fouling is negligible.

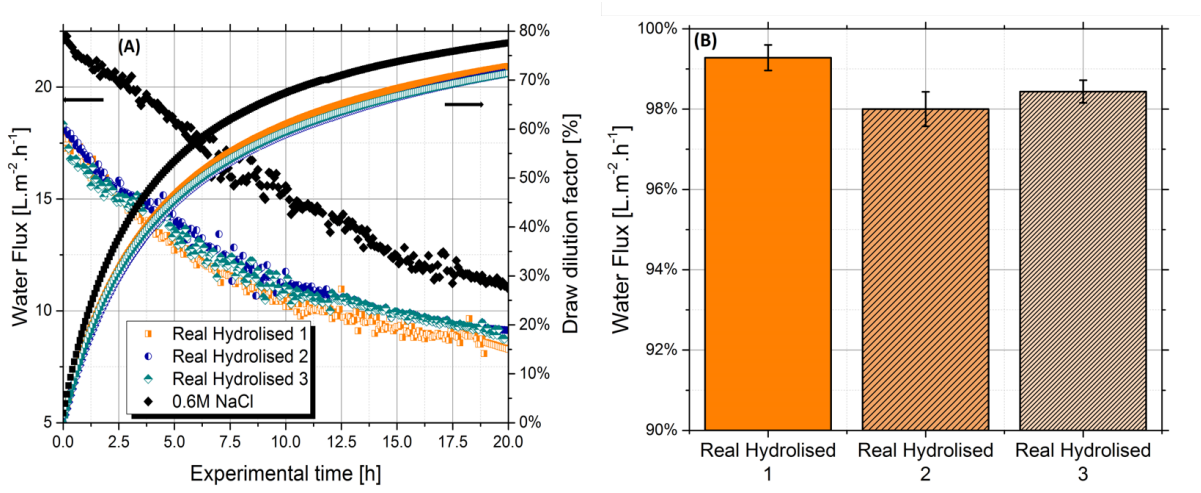

Figure 5. Experiments with real hydrolysed urine as draw solution and deionised water as feed. The tests were performed until > 70 % DS dilution (A). The membrane was tested with standard 0.5 M NaCl as DS and DI – water as FS before and after the experiments. The differences in flux before and after the urine tests are displayed in Figure 5 (B).

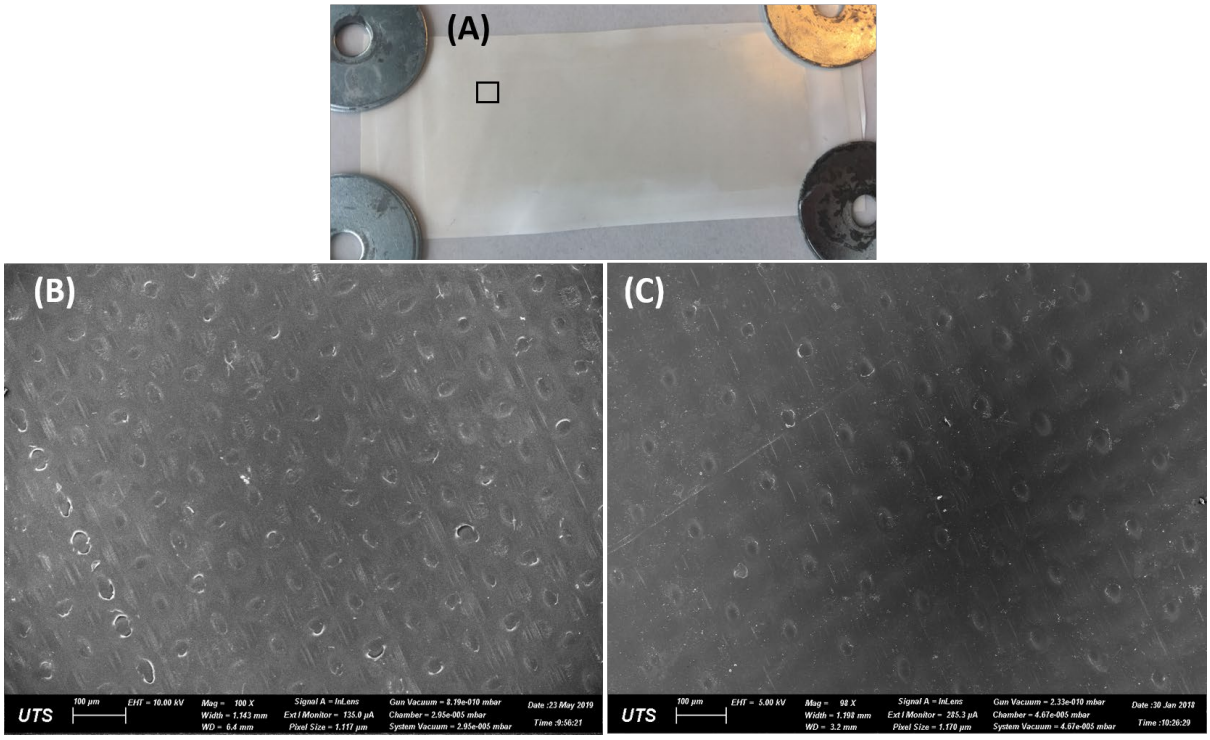

Figure 6. (A) Picture of the support layer after using real hydrolysed urine as DS and DI-water as FS. SEM image of the pristine membrane support layer (B) and SEM image of the support layer after FO experiments with hydrolysed urine as DS and DI-water as FS (C).

3.4 Microalgae dewatering using real human urine as the draw solution

The third leading research question was to understand the effectiveness of urine when used in concentrating a *C. Vulgaris* solution. Both real and synthetic urine were tested. Additionally, synthetic seawater, i.e. 0.6M NaCl, was used as a benchmark. Seawater was chosen because it has been extensively investigated as a suitable draw solution for microalgae dewatering [21, 22, 33, 35, 47].

Synthetic seawater yielded the highest flux, followed by synthetic hydrolysed urine, real hydrolysed urine and fresh urine (Figure 7). Although seawater and synthetic hydrolysed urine have similar osmotic pressures (2700 and 3100 kPa respectively), NaCl, which has very high self-diffusivity (see equation 2), is the predominant constituent of seawater. This is why seawater outperformed all the other urine solutions. However, the difference between real hydrolysed urine and seawater is just 5 $L.m^{-2}.h^{-1}$.

In respect of the membrane fouling, none of the DSs showed a faster flux decline. Nonetheless, Figure 8 shows that a mild layer of algae was deposited on the membrane surface after the filtration. The circular shape of the algae matched with the shape of *C. Vulgaris* [33], which indicates that membrane fouling occurred during algae dewatering, especially when a higher feed concentration was targeted (i.e. > 75%). It should be noted that Honda et al. showed that alkaline cleaning with NaOH at a pH of 11 was enough to fully restore the FO water flux after dewatering *C. Vulgaris* [47]. Overall, the algal concentration was increased fourfold, from 0.2 to 0.8 g/L, with an average flux of 7.6 $L.m^{-2}.h^{-1}$ for fresh urine and 14.1 $L.m^{-2}.h^{-1}$ for hydrolysed urine.

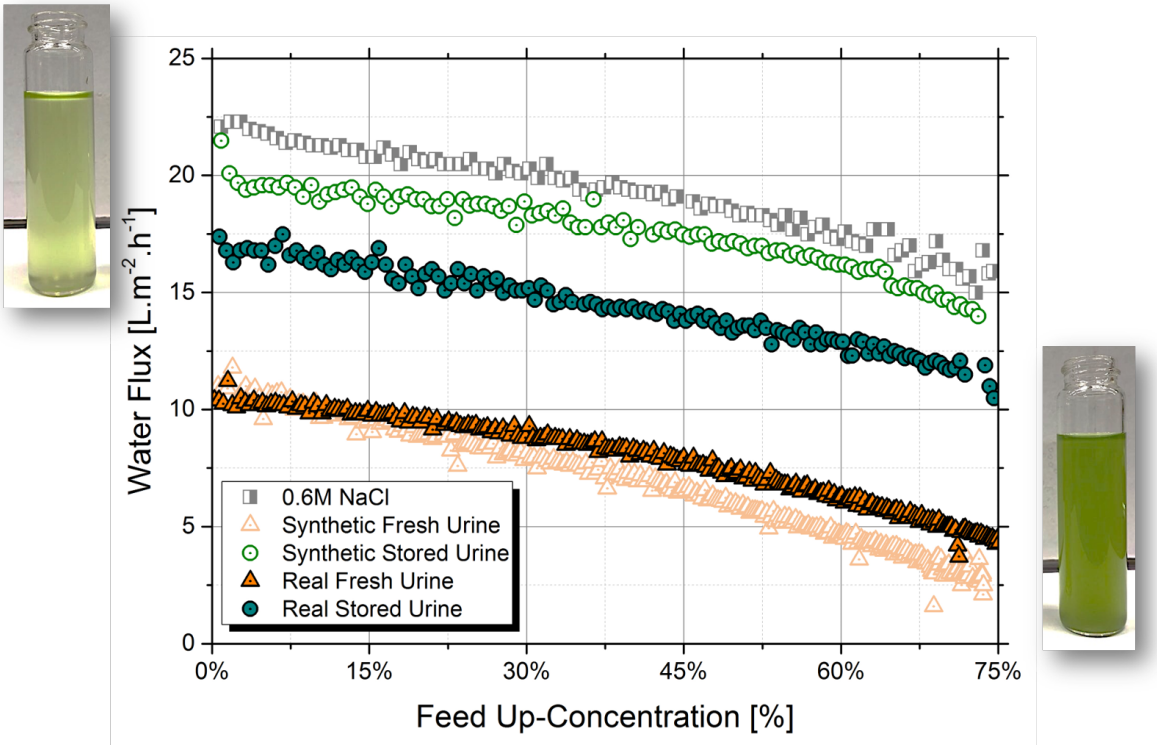

Figure 7. Long-term testing (>20 hours) of real and synthetic urine in dewatering a 0.2 g/L solution of *Chlorella Vulgaris*. The experiments were carried out using 2 L of draw solution and 500 mL of feed until the FS was concentrated four times. Synthetic seawater (0.6 M NaCl) was also tested as a benchmark for the results.

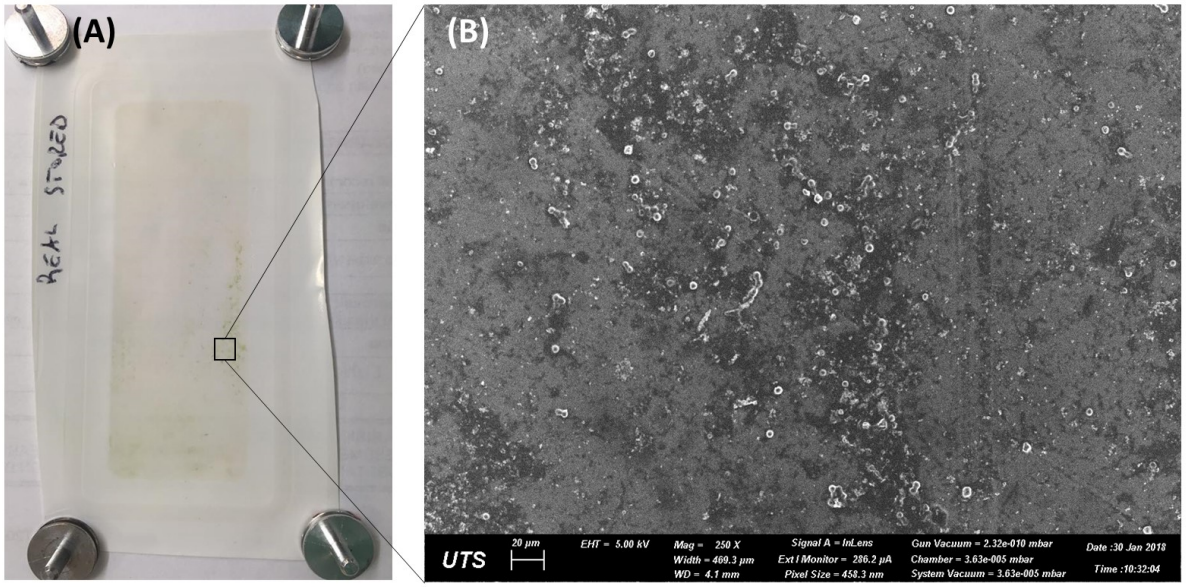

Figure 8. Photo (A) and SEM picture (B) of the active layer of the FO membrane after microalgae filtration.

3.5 Extracellular carbohydrates production during algal filtration

Algae can release storage compounds, such as carbohydrates, to adjust their growth during changing environmental conditions [48]. One explanation for this EPS increase is the accumulation of intracellular carbohydrates with low molecular weight, such as trehalose, sucrose, etc., which puts the internal algal osmotic pressure in equilibrium with the outside [48]. This increase in carbohydrate content protects the algae from salt harms [48]. This means that, in this work, the change in the algae growing solution due to e.g. NH_3-RSF and feed up-concentration could result in EPS release. Particularly in the presence of divalent cations such as Ca^{2+}, EPSs are reported to bind with the carboxylate functional group of the algal cell-EPS interface, creating a gel network [33, 49]. This gel network can provide a surface for the further adherence of organic or inorganic foulants, thereby promoting membrane fouling, which could jeopardise the filtration process. Therefore, it is essential to assess the extent of EPS released by the algal solution when urine is used as DS during the FO process.

Figure 9 shows that there is no noticeable difference between fresh and hydrolysed urine in terms of EPS production. In both cases, the final EPS mass in the FS was about 8-10 times higher than at initialisation. The RSF of NH_3 is suggested as the primary cause for EPS release. Figure 9 shows the difference between the initial and final amount of nitrogen in the culture. High free ammonia concentration can interfere with the photosynthesis process in isolated chloroplasts [50, 51]. M. Larronde-Larretche also speculated that a higher localised concentration of Ca^{2+} could interfere with the "Ca^{2+} signal", which is also involved in the photosynthesis process [33],[52]. Nonetheless, the NH_3

RSF from hydrolysed urine, which was higher compared to fresh urine, appeared not to increase the algal EPS release.

When synthetic urine was tested, 35% fewer EPS were found in the feed. One possible explanation might be the RSF of carbohydrates from the urine to the feed. A low concentration of carbohydrates may be found even in the urine of a healthy individual [53]. However, this argument requires further investigation, as carbohydrates are generally well rejected by the FO membrane due to their large size. Finally, synthetic seawater induced EPS uptake rather than release. This last result was also observed in the literature [22, 33].

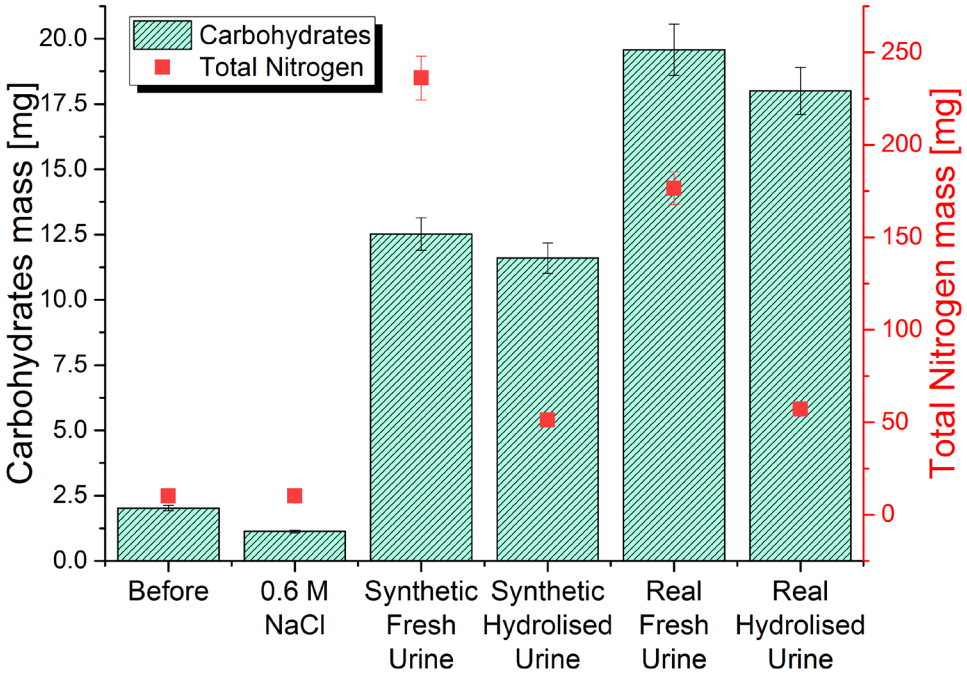

Figure 9. Extracellular carbohydrate content (histograms) and total nitrogen (red squares) in the solution before dewatering and after the FO concentration, using different draw solutions.

3.6 Conclusions

Real urine was investigated as a novel DS for forward osmosis. The experimental results showed that, when hydrolysed, real urine had an osmotic pressure of up to 2000 kPa which led to a water flux of over 16 $L.m^{-2}.h^{-1}$. Support layer fouling was also mild and easy to remove by simple flushing. Additionally, this work proposed the application of a urine draw solution in the field of microalgae biotechnology, specifically in the dewatering of microalgae solutions. That is because, contrary to conventional draw solution such as seawater, the availability of human urine is not constrained by the geographical location of the plant and the exhausted DS, i.e. diluted urine, could itself be used as

microalgae growing media. A solution of 0.2 g/L *C. Vulgaris* was concentrated fourfold (up to 0.8 g/L) using real fresh and hydrolysed urine with an average flux of 7.6 $L.m^{-2}.h^{-1}$ for fresh urine and 14.1 $L.m^{-2}.h^{-1}$ for hydrolysed urine. A mild active layer fouling was confirmed by SEM analysis and attributed to the release of EPS by the algae during the filtration.

Finally, if urine is intended to be used for microalgae dewatering, several research questions are yet to be addressed. Among them, we have identified the following: (I) the effect of pharmaceuticals and hormones on the growth and dewatering of algae solutions; (II) the effect of high urea RSF on the growth rate of the algae culture; finally, (III) membrane fouling and cleaning strategies when longer experiments are performed.

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