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Influence of fertilizer draw solution properties on the process performance and microbial community structure in a side-stream anaerobic fertilizer-drawn forward osmosis – ultrafiltration bioreactor

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Abstract

In this study, a side-stream anaerobic fertilizer-drawn forward osmosis (FDFO) and ultrafiltration (UF) membrane bioreactor (MBR) hybrid system was proposed and operated for 55 days. The FDFO performance was first investigated in terms of flux decline with various fertilizers draw solution. Flux decline was very severe with all fertilizers due to the absence of aeration and the sticky property of sludge. Flux recovery by physical cleaning varied significantly amongst tested fertilizers which seriously affected biofouling in FDFO via reverse salt flux (RSF). Besides, RSF had a significant impact on nutrient accumulation in the bioreactor. These results indicated that nutrient accumulation negatively influenced the anaerobic activity. To elucidate these phenomena, bacterial and archaeal community structures were analyzed by pyrosequencing. Results showed that bacterial community structure was affected by fertilizer properties with less impact on archaeal community structure, which resulted in a reduction in biogas production and an increase in nitrogen content.

Keywords: Anaerobic osmotic bioreactor, Fertilizer-drawn forward osmosis, Biogas production, Salt accumulation, Pyrosequencing

1. Introduction

Freshwater resources are getting scarcer due to the impacts of global warming, and rapid and extensive industrialization and urbanization (Rijsberman, 2006). Agricultural sectors consume about 70% of the accessible freshwater with about 15 -35% of water being used unsustainably (Clay, 2004). Therefore, Mediterranean countries, which are stressed by water shortage, have considered wastewater reuse as a viable alternative water resource for agricultural purposes (Angelakis et al., 1999). However, since wastewater reuse is often limited due to the presence of harmful heavy metals, industrial waste, pharmaceutical and personal care products (PPCPs), and excess salts (Snyder et al., 2003), adequate treatment of wastewater before reuse for irrigation is essential not only to protect the human and plant health but also to enhance the value of the crops grown through wastewater reuse (Ferro et al., 2015). Therefore, advanced treatment processes (e.g., reverse osmosis (RO), nanofiltration (NF) or advanced oxidation) are generally required as a post-treatment process since wastewater could contain pollutants which are not removed by conventional treatment processes (Ahluwalia & Goyal, 2007).

Anaerobic membrane bioreactor (AnMBR) has been studied to treat wastewater and has several advantages including complete rejection of suspended solids, low sludge production, high organic rejection and biogas production (Stuckey, 2012). However, post-treatment processes such as RO and NF exhibit high fouling issues which ultimately increase energy requirements since these processes are driven by hydraulic pressure (Kim et al., 2014). To overcome these issues, osmotic membrane bioreactor (OMBR) has been proposed by integrating AnMBR with forward osmosis (FO) process instead of

conventional pressurized membrane processes (Achilli et al., 2009). OMBR can provide high rejection of contaminants, low fouling propensity and high fouling reversibility but has limitations, such as that pure water should be extracted from draw solution (DS) and reversely transported draw solutes can be toxic or inhibit the biological processes (Achilli et al., 2009; Kim et al., 2016). Moreover, the reverse diffusion of draw solutes has been shown to exacerbate the salt accumulation in the bioreactor, which can, as a side effect, accelerate the inhibition impact on the biological performance (Luo et al., 2017; Qiu & Ting, 2013) by altering the microbial community structure and reduce the water flux in FO (Kim, 2014).

Fertilizer-drawn forward osmosis (FDFO) has received increased attention since the diluted fertilizer solution can be utilized directly for irrigation purpose and thus the diluted DS separation and recovery process is not required (Phuntsho et al., 2011). However the diluted fertilizer solution still requires substantial dilution since the final nutrient concentration can exceed the standard nutrient requirements for irrigation especially using feed water sources with high salinity (Phuntsho et al., 2011). Thus, NF can be employed as a post-treatment process for further dilution to meet the water quality requirements for fertigation (Phuntsho et al., 2013). However, FDFO was shown to be more suitable for the treatment of low salinity impaired water sources such as municipal wastewater (Chekli et al., 2017) so that desired fertilizer dilution can be achieved without the need of a NF post-treatment process. Thus, recently, an anaerobic FDFO membrane bioreactor hybrid system (AnFDFOMBR) was proposed by combining FDFO and AnMBR for simultaneous wastewater treatment for greenhouse hydroponic application (Kim et al., 2016).

Despite of many advantages (i.e., complete rejection of pollutants, low energy requirement and high fouling reversibility) of the AnFDFOMBR hybrid system, it also has critical issues including salt accumulation in the bioreactor similar to OMBR (Kim, 2014; Xiao et al., 2011). When considering the typical submerged AnFDFOMBR hybrid system, salt accumulation takes place in the bioreactor from both the influent and DS. This is due to wastewater being continuously fed into the bioreactor and the FO membrane rejecting almost 100% of ionic compounds and the back diffusion of the DS. Salt concentration will continuously increase and therefore may affect the microbial activity of the anaerobic bacteria as well as FO performances (Kim et al., 2016). Therefore, many researchers have tried to mitigate the salt accumulation by combining OMBR with porous membrane technologies (e.g., microfiltration (MF) and ultrafiltration (UF)) (Wang et al., 2014) and desalting technologies (e.g., capacitive deionization (CDI) and electrodialysis (ED)) (Lu & He, 2015).

In this study, a side-stream anaerobic FDFO-UF-MBR hybrid system (An-FDFO-UF-MBR) is proposed for simultaneous wastewater treatment for greenhouse hydroponic application based on the concept described in **Fig. S1** of the supporting information. Side-stream FO and UF membrane modules are used since membrane fouling in the side-stream system is readily controlled by simple physical cleaning compared to the submerged system. UF plays an important role to reduce and mitigate salt accumulation in the bioreactor. In this system, raw municipal wastewater is utilized as the influent and a highly concentrated fertilizer solution is used as DS. Thus, the diluted fertilizer solution can then be obtained

and supplied to greenhouse hydroponic irrigation. The UF permeate can be also utilized to recover fertilizers due to its high nutrient content (Luo et al., 2016b).

Therefore, this study aims to investigate the impact of various fertilizer DS on the anaerobic FDFO-UF-MBR hybrid process for treating municipal wastewater in order to understand how fertilizers influence the hybrid process, especially the anaerobic process via a microbial community analysis and find out the optimum design for successful and sustainable operation. FO performance in terms of flux decline was firstly evaluated with various fertilizer DS. During the operation, salt accumulation and biogas production were monitored. Besides, bacterial and archaeal community structures of the sludge were characterized through pyrosequencing analysis to investigate the effect of fertilizer DS on variations of bacterial and archaeal structures and their relationship to biogas production and composition. Even though there are some studies available on anaerobic OMBR (AnOMBR), the present study will provide valuable information on the effect of various draw solutes on the performance and microbial community variation in AnOMBR; which has not been investigated yet.

2. Materials and methods

2.1 Feed and draw solutions

Synthetic municipal wastewater with chemical oxygen demand (COD) of 400 ± 10 mg/L consisting of food ingredients, chemical compounds and trace metals was used as FS in this study (**Table S1**). Three different chemical fertilizers (i.e., mono-ammonium phosphate (MAP), mono-potassium phosphate (MKP) and potassium chloride (KCl)) as DS

were prepared by dissolving fertilizers in the deionized (DI) water. Detailed information of fertilizer chemicals is provided in **Table S2**. Osmotic pressure, effective diffusion coefficient and viscosity of three fertilizers were obtained by OLI Stream Analyzer 3.2 (OLI System Inc., Morris Plains, NJ, USA). All chemicals of reagent grade were received in powder form from Sigma Aldrich (Saudi Arabia).

2.2 A lab-scale side-stream anaerobic fertilizer-drawn forward osmosis – ultrafiltration membrane bioreactor

A lab-scale side-stream An-FDFO-UF-MBR unit (**Fig. 1**) consisted of a completely mixing bioreactor (Applikon Biotechnology, the Netherlands) with effective volume of 2 L controlled by a level sensor (temperature 35 ± 1 °C, pH 7 ± 0.1 and stirring speed 200 ± 2 rpm), a side-stream crossflow hollow fibre UF membrane module and a side-stream crossflow flat-sheet FO membrane module. The FO membrane was provided by Hydration Technology Innovations (Albany, OR, USA) and made of cellulose-based polymers with an embedded polyester mesh for mechanical strength. The UF membrane is composed of polyvinylidene fluoride with nominal pore size of 30 nm. The effective membrane surfaces of FO and UF modules were 100 cm^2 and 310 cm^2 , respectively. Operation temperature was 35 ± 1 °C and crossflow velocity of FO and UF modules were 2.3 cm/s and 20 cm/s, respectively. The hybrid system was operated for 55 days with a HRT 20 - 24 hours and a SRT of 160 days. Initially, AnMBR was operated without FO operation for 6 days to stabilize the system. FO was then operated with KCl DS for 6 days and stopped for 9 days to stabilize anaerobic microorganisms. Then, FO was run again with MAP DS for 15 days.

After that, FO operation was stopped and the bioreactor was operated for 7 days to restabilize the system. Finally, FO was operated with MKP DS for 12 days. In this study, the operational period of FDFO was determined according to the anaerobic performance (represented as biogas production). Even though the operation time with each fertilizer was a little short, it was sufficient to achieve our goal (i.e., identify the potential problems of this hybrid study via microbial community analysis). In order to recover water flux, physical cleaning was applied everyday since water flux severely declined within 1 day of continuous operation. Physical cleaning consisted of flushing deionized (DI) water inside the DS and the sludge solution (FS) channels at 3 times higher crossflow velocity (6.9 cm/s) for 30 mins.

2.3 Analytical methods

Chemical oxygen demand (COD) was determined using a COD cell test kit (Hach, Germany) following the standard method (DIN ISO 15705). Total nitrogen (TN) was determined using a TN test kit according to the persulfate digestion method. Total phosphorous (TP) was analyzed by ion chromatography (Dionex ICS-5000). Mixed liquor suspended solids (MLSS) was measured using standard methods (American Public Health et al., 2005). Biogas composition and volume (reported under a temperature of 25 °C and pressure of 1 atm) were measured according to the gas bag method (Ambler & Logan, 2011) based on gas chromatography.

2.4 Pyrosequencing analysis

2.4.1 DNA extraction

DNA was extracted through the FastDNA Spin kit for soil (MP Biomedicals, USA), using 4x the normal bead beating to enable recovery of bacteria that are difficult to lyse (Albertsen et al., 2015).

2.4.2 16S rRNA amplicon library preparation

The procedure for bacterial 16S rRNA amplicon sequencing targeting the V1-3 variable regions is based on Caporaso et al. (2012), using primers adapted from the Human Gut Consortium (Jumpstart Consortium Human Microbiome Project Data Generation Working, 2012). Archaeal V3-5 16S sequencing libraries were prepared by a custom protocol based on the 16S metagenomic sequencing library preparation protocol (Part # 15044223 Rev. B). 10 ng of extracted DNA was used as template and the PCR reaction (25 μ L) contained dNTPs (400nM of each), $MgSO_4$ (1.5 mM), Platinum® Taq DNA polymerase HF (2mU), 1X Platinum® High Fidelity buffer (Thermo Fisher Scientific, USA), and barcoded library adaptors (400 nM) containing Bacteria V1-3 specific primers: 27F AGAGTTTGATCCTGGCTCAG and 534R ATTACCGCGGCTGCTGG and Archaea V3-5 (Pinto & Raskin, 2012): 5'-CCCTAHGGGGYGCASCA (Arch-340F) and 5'-GWGCYCCCCCGYCAATTC (Arch-915R). PCR was set as below: Initial denaturation at 95°C for 2 min, 30 cycles of 95°C for 20 s, 56°C for 30 s, 72°C for 60 s and final elongation at 72°C for 5 min. All PCR reactions were run in duplicate and pooled afterwards. The amplicon libraries were purified using the Agencourt® AMPure XP bead protocol (Beckmann Coulter, USA) with the following exceptions: the sample/bead

solution ratio was 5/4, and the purified DNA was eluted in 33 μ L nuclease-free water.

Library concentration was measured with Quant-iT™ HS DNA Assay (Thermo Fisher Scientific, USA) and quality validated with a Tapestation 2200, using D1K ScreenTapes (Agilent, USA). Based on library concentrations and calculated amplicon sizes, the samples were pooled in equimolar concentrations and diluted to 4 nM.

2.4.3 DNA sequencing

The purified sequencing libraries were pooled in equimolar concentrations and diluted to 4 nM. The samples were paired end sequenced (2x301bp) on a MiSeq (Illumina) using a MiSeq Reagent kit v3, 600 cycles (Illumina) following the standard guidelines for preparing and loading samples on the MiSeq. 10% and 20% Phix control library for respective bacterial and archaeal analyses were spiked in to overcome low complexity issue often observed with amplicon samples.

2.4.4 16S rRNA amplicon bioinformatic processing

Forward and reverse reads were trimmed for quality using Trimmomatic v. 0.32 with the settings SLIDINGWINDOW:5:3 and MINLEN:275. The trimmed forward and reverse reads were merged using FLASH v. 1.2.7 with the settings -m 25 -M 200. The merged reads were dereplicated and formatted for use in the UPARSE workflow. The dereplicated reads were clustered, using the usearch v. 7.0.1090 -cluster_otus command with default settings. OTU abundances were estimated using the usearch v. 7.0.1090 -usearch_global command with -id 0.97. Taxonomy was assigned using the RDP classifier (Wang et al., 2007) as implemented in the parallel_assign_taxonomy_rdp.py script in QIIME (Caporaso et al., 2010), using the MiDAS database v.1.20 (McIlroy et al., 2015).

The results were analysed in R (Team, 2015) through the Rstudio IDE using the ampvis package v.1.24.0 (Albertsen et al., 2015).

3. Results and discussion

3.1 Influence of fertilizer properties on FO performance

The lab-scale side-stream An-FDFO-UF-MBR hybrid system was operated with three different fertilizers (i.e., KCl, MAP and MKP) as DS for a period of 55 days. The water flux data are presented as normalized flux as shown in **Fig. 2**. The effect of each fertilizer DS on the performance of An-FDFO-UF-MBR was first investigated since this three fertilizers have different nutrient (i.e., N/P/K) combinations. All fertilizers showed severe flux decline (i.e., up to almost 80%), even within 1 day operation, with fairly similar initial fluxes (i.e., $9.06 \text{ L/m}^2/\text{h}$ – $9.82 \text{ L/m}^2/\text{h}$). This is because the sludge with high MLSS (i.e., $3.06 \pm 0.06 \text{ g/L}$ at the initial stage) was treated by the FO membrane under quite low cross-flow velocity (i.e., 2.3 cm/s) with no aeration (Nguyen et al., 2013) resulting in severe fouling. In addition, anaerobic sludge is more sticky than aerobic sludge, and thus it can accelerate membrane fouling.

To remove the membrane fouling layer and recover the initial water flux, physical cleaning was applied by flushing the membrane surface for 30 mins (i.e. hydraulic cleaning at crossflow velocity of 6.9 cm/s) as well as replacing DS with DI water to provide the osmotic backwashing effect. Compared to similar flux decline in FDFO regardless of fertilizer types, water flux recovery by physical cleaning was significantly affected by fertilizer DS properties. When considering KCl DS as shown in **Fig. 2a**, water flux was

fully recovered after physical cleaning until the 5th cycle. Besides, the cleaned surface was also clearly visible during the operation, indicating that the membrane surface could be perfectly cleaned by physical cleaning when using KCl DS. With MAP DS, water flux was recovered by about 70% as presented in **Fig. 2b**. It was observed that FO membrane was partially cleaned by physical cleaning. With MKP DS, water flux was fully recovered until the 3rd cycle. However, after 3rd cycle, water flux started to be only recovered by about 80% (**Fig. 2c**).

MLSS of the sludge was measured to elucidate the different behaviour of water flux recovery and evaluate the effect of different fertilizer DS on microorganisms. When FO was operated with KCl, MLSS was 1.64 – 1.71 g/L. With MAP DS, MLSS was initially 1.42 ± 0.03 g/L and slightly reduced to 1.25 ± 0.13 g/L. Moreover, MLSS with MKP DS was lower (i.e., from 1.01 ± 0.01 g/L to 0.92 ± 0.23 g/L) compared to other fertilizers. These results indicate that microorganisms in the bioreactor were damaged by FO operation, consistent with our previous study (Kim et al., 2016).

It is very interesting to compare water flux recovery with MLSS of the sludge. KCl DS exhibited 100% recovery rate even though MLSS was very high. On the other hand, MAP DS exhibited lower recovery rate despite of lower MLSS than KCl DS. To identify the reason behind this different trend, the DNA concentration of the fouling layer was measured. Experiments carried out with MAP DS (i.e., 467.8 ± 28.1 ng/nL) exhibited much higher DNA concentration in the fouling layer than those performed with KCl DS (i.e., 160.2 ± 1.5 ng/nL), indicating that severe biofouling was formed on the FO membrane surface when MAP was used as DS. This is because KCl DS has higher reverse salt flux

(RSF) (i.e., 22.32 ± 1.61 g/m²/h) than MAP DS (i.e., 1.15 ± 0.1 g/m²/h). Therefore, microorganisms attached on the surface of FO membrane could be inactivated due to high salt concentration (Johir et al., 2013). In case of MKP, water flux recovery was fully recovered at the initial stage, but afterward, it was continuously reduced. This could be explained by the fact that the sludge with MKP DS had a low fouling potential (i.e., low MLSS). Thus, the membrane surface was readily cleaned by physical cleaning. Besides, MKP DS has a low RSF (i.e., 2.15 ± 0.25 g/m²/h) due to its low effective diffusion coefficient (i.e., 0.2×10^{-9} m²/s), as shown in **Table S2**. Therefore, biofouling was significant; similar to the results obtained with MAP and consistent with the DNA results (i.e., 467.8 ± 28.1 ng/nL and 504.6 ± 72.2 ng/nL for MAP and MKP, respectively).

3.2 Influence of fertilizer properties on salt/nutrient accumulation in the bioreactor

Salt/nutrient accumulation in the bioreactor is presented in **Fig. 2a**. When the hybrid system was operated without FO process, TN, TP and conductivity were initially stable: 63.06 ± 4.91 mg/L, 12.11 ± 0.71 mg/L and 0.81 ± 0.07 mS/cm, respectively. However, when operating FO with KCl DS, conductivity started to significantly increase up to 5.39 mS/cm. This is because KCl shows a high RSF (i.e., 22.32 ± 1.61 g/m²/h) probably due to its highest solute effective diffusion coefficient (i.e., 0.31×10^{-9} m²/s) as shown in **Table S2** and also lower hydrated diameters of both K⁺ and Cl⁻ species (Kim et al., 2016; Phuntsho et al., 2011). In OMBR, salt accumulation is dominantly governed by the reverse diffusion of draw solutes rather than the rejection of salts from the FS (Kim et al., 2016;

Luo et al., 2016a). Thus, the use of draw solutions with high RSF can accelerate and exacerbate salt accumulation in the bioreactor. To stabilize the anaerobic bioreactor and reduce the conductivity caused by RSF from DS, FO operation was stopped for a few days while UF was continuously operated to extract the salts from the bioreactor. As a result, salt/nutrient concentrations were recovered to the original values (after approxi. 6 days). Then, FO operation was restarted with MAP DS. Results showed that conductivity gradually increased to 2.44 mS/cm with a significant increase in concentrations of both TP and TN from 69.81 mg/L and 83.5 mg/L to 712.87 mg/L and 246.8 mg/L, respectively. Compared to the experiments carried out with KCl DS, those conducted with MAP DS exhibited lower conductivity increase rate (i.e., 0.12 mS/cm/day against 0.91 mS/cm/day for MAP and KCl, respectively). This is because the effective diffusion coefficient of MAP DS (i.e., $0.21 \times 10^{-9} \text{ m}^2/\text{s}$) is lower than that of KCl DS (i.e., $0.31 \times 10^{-9} \text{ m}^2/\text{s}$) due to high molecular weight of phosphate (Valencia & González, 2011), thereby reducing RSF (i.e., $22.32 \pm 1.61 \text{ g/m}^2/\text{h}$ and $1.15 \pm 0.1 \text{ g/m}^2/\text{h}$ for KCl and MAP, respectively) and the salt accumulation (**Fig. 3a**). When stopping FO operation, it was observed that TN, TP and conductivity were rapidly reduced. FO was finally operated again with MKP DS. **Fig. 3a** shows that both conductivity and TP in the bioreactor increased with no change in TN since MKP DS consists of potassium and phosphate. When comparing experiments carried out with MKP DS and MAP DS, interestingly, those with MKP DS had lower conductivity increase rate (i.e., 0.07 mS/cm/day against 0.12 mS/cm/day for MKP and MAP, respectively) as well as lower TP increase rate (i.e., 13.66 mg/L/day and 46.63 mg/L/day for MKP and MAP, respectively). These results are not consistent with their effective diffusion coefficients as shown in **Table S2** suggesting that salt accumulation in the real

operation may be influenced by some other factors, such as membrane fouling since RSF is generally measured under the condition without foulants. Consequently, from these results, it can be concluded that UF is not sufficient to mitigate salt accumulation in the bioreactor when operated simultaneously with FO.

3.3 Influence of fertilizer properties on biogas production

Biogas production from the anaerobic bioreactor was monitored and the results are presented in **Fig. 3b**. When operating AnMBR without FO process, methane content was continuously increased with decreasing nitrogen and carbon dioxide contents. This indicates that the anaerobic activity was gradually taking place in the bioreactor. When FO was operated with KCl DS, the biogas production rate was slightly increased from 0.178 L/g COD to 0.301 L/g COD in 2 days while methane content slightly decreased, which is consistent with another study (Song et al., 2016) where it was shown that the bulk organic removal and biogas/methane production decreased as the bioreactor salinity increased. Interestingly, after only 2 days of FO operation, biogas production completely stopped, which indicates that the increased salt concentration in the bioreactor (**Fig. 3a**) had a negative impact on anaerobic microorganisms (Kim et al., 2016). To recover anaerobic activity, FO operation was stopped while UF operation was continued. Afterward, salt concentration in the bioreactor was reduced to its original value and biogas started to be produced again with an increase in methane content. Thus, FO was operated again with MAP DS and biogas production rate was slightly reduced to 0.084 L/g COD with a small

decrease in methane content (from 36.81% to 30.03%). For the sludge stabilization, FO operation was stopped for 7 days. However, methane content continuously decreased while nitrogen content increased, which implies that anaerobic microorganisms were critically damaged. Lastly, when starting again FO operation with MKP DS, biogas production rate was still low at 0.05 L/g COD and methane concentration kept decreasing to 18.25% which was accompanied by a continuous increase in nitrogen concentration. As a consequence, it was implied that FO operation with inorganic fertilizer DS affects negatively the anaerobic activity.

3.4 Influence of fertilizer properties on bacterial community structure from pyrosequencing analysis

To elucidate the variations in the performance of the bioreactor, the bacterial community structure was analyzed by pyrosequencing and presented in **Fig. 4**. Before operating FO, *Gelria* (18.8%) was the most dominant species followed by *Longilinea*, *VadinBC27*, *Pertimonas*, *Brooklawnia*, and *Comamonas* (i.e., 12.5%, 9.0%, 8.6%, 8.4% and 5.9%, respectively). Based on the literature, *Gelria* is an anaerobic thermophilic glutamate-degrading bacterium which oxidizes glutamate to propionate, H_2 , NH_4^+ and CO_2 (Plugge et al., 2002). *Longilinea* is associated with efficiency in hydrolytic and acidogenetic fermentation (Ambuchi et al., 2016). *VadinBC27* degrades recalcitrant organic contaminants (Riviere et al., 2009) and *Pertimonas* ferments glucose to produce acetate, H_2 and CO_2 as well as reduces elemental sulfur to sulfide and reduces nitrate to ammonium (Grabowski et al., 2005). *Brooklawnia* plays a significant role for hydrolysis and

acidogenesis (Bae et al., 2006). *Comamonas* is a denitrifying bacterium which reduces nitrate to nitrogen gas (Gumaelius et al., 2001). Results from previous studies focusing on bacteria species indicated that all the aforementioned bacteria are essential to ensure the procedures from hydrolysis to acetogenesis and thus to successfully achieve methane production in the bioreactor as shown in **Fig. 3b**.

When FO operation started with KCl DS, the bacterial community structure was significantly changed (**Fig. 4**) and the biogas production stopped (**Fig. 3b**). Results show that *Comamonas* (54.3%) became the most dominant species followed by *Longilinea*, *VadinBC27*, *Curvibacter*, and *Clostridium sensu stricto 1* (i.e., 7.4%, 4.9%, 4.2% and 3.6%, respectively), which indicates that beneficial bacteria for bio-methane production were no longer present or deactivated in the bioreactor. This is because TDS in the bioreactor was significantly increased since KCl has very high RSF (i.e., $22.32 \pm 1.61 \text{ g/m}^2/\text{h}$). Besides, the presence of potassium ions could inhibit the anaerobic process since high potassium concentration can lead to a passive influx of potassium ions which neutralize the membrane cell potential (Kim et al., 2016). Results of the bacterial community structure analysis were quite consistent with another study on OMBR (Qiu & Ting, 2013) even though their operation conditions were different (i.e., anaerobic condition and aerobic condition). They suggested that the deterioration of biological activity resulted from the salt accumulation and almost all the dominant species in the activated sludge were taken over by new high salt-tolerant species. Similarly, in this study, the beneficial bacteria were mainly replaced with *Comamonas* (i.e., the denitrifying bacterium).

To recover the anaerobic activity, the An-FDFO-UF-MBR hybrid system was operated without FO process. Results in **Fig. 4** show that beneficial bacteria (i.e., *Longilinea*, *VadinBC27*, *Brooklawnia*, *Gelria* and *Comamonas*) for methane production became dominant, and biogas production was re-started together with an increase in methane content. When operating FO with MAP DS, *Comamonas* (35.5%) was dominant followed by *VadinBC27*, *Gelria* and *Longilinea* (i.e., 5.1%, 4.8% and 4.1%, respectively), which is consistent with the results of biogas composition. With MAP DS, biogas was continuously produced as shown in **Fig. 3b** but nitrogen content was increased with a decrease in methane content. This is because the population of anaerobic bacteria (i.e., *VadinBC27*, *Gelria* and *Longilinea*) which are beneficial for hydrolysis, fermentation and acetogenesis (Ambuchi et al., 2016; Plugge et al., 2002; Riviere et al., 2009), was reduced while the population of anoxic bacterium (*Comamonas*) which is a denitrifying bacterium (Gumaelius et al., 2001), was increased.

Therefore, FO operation was stopped again to re-stabilize anaerobic bacteria in the bioreactor. Results show that *Comamonas* (40.0%) was the most dominant species followed by *Longilinea*, *VadinBC27*, *f_KD1-131_OTU_3* and *Brooklawnia* (i.e., 5.6%, 5.5% 3.7% and 3.0%, respectively). It is interesting to note that the bacterial community structure was slightly different than the original one, which is different from the results obtained after FO operation with KCl. This implies that the anaerobic bacteria population was seriously damaged and thus nitrogen content was increased with a decrease in methane content. This is because inorganic chemical fertilizers could inhibit anaerobic microbial activity even at low concentration (Kim et al., 2016).

The An-FDFO-UF-MBR hybrid system was operated with MKP DS even though the anaerobic activity in the bioreactor was not restored to its original value. Interestingly, *Gelria* (15.6%) became the most dominant species followed by *f_KD1-131_OTU_3*, *VadinBC27*, *c_SJA-15_OTU_10* and *Longilinea* (i.e., 14.7%, 7.2%, 4.6% and 4.5%, respectively), while *Comamonas* was significantly reduced from 40.0% to 2.8%. This implies that biogas production and methane content should be enhanced. However, biogas production was still low with a continuous reduction in methane content. Therefore, it can be hypothesized that if methanogens were activated with MKP DS, biogas production and methane content would have increased with a longer operation.

To further investigate the effect of fertilizer properties on biogas production, the archaeal community structure was analyzed by pyrosequencing and presented in **Fig. 5**. Initially, *Methanosaeta* (45.7%) was dominant followed by *Methanomethylovorans* and *Methanobacterium* (i.e., 28.6% and 14.4%, respectively) with a total of 97.92% of methanogens. With KCl DS, the archaeal community structure was not significantly changed but its relative abundance was slightly affected. During operation, *Methanosaeta* was getting more abundant in the sludge while the population of *Methanomethylovorans* was reduced. This indicates that the archaeal community structure is affected by inorganic fertilizers in the bioreactor. However, from these results, it was observed that methanogens were still the dominant species (i.e., 95% ~ 98%) regardless of the types of fertilizer DS. Nevertheless, methane content was continuously decreased with low biogas production. This is because the anaerobic bacterial community was seriously influenced by fertilizer DS and thus biodegradation and biogas production mechanisms did not work effectively.

Findings from this study have significant implications for optimizing the proposed An-FDFO-UF-MBR hybrid system. The present hybrid system has two major problems: the first is the severe membrane fouling in FDFO resulting in flux decline. The second issue is the negative impact on anaerobic activity caused by reversely diffused fertilizer draw solutes in the bioreactor. The RSF issue is more problematic since membrane fouling in a side-stream FO membrane module could be easily controlled by optimizing the system design (e.g., module design and system configuration) and the operation conditions (e.g., critical water flux, cross-flow rate and cleaning methods). Thus, three solutions can be implemented to overcome the RSF issue. The first would be to reduce RSF by optimizing the DS composition. For example, inorganic DS can be mixed with surfactant such as Triton X-114 (Nguyen et al., 2015) and thus RSF can be significantly reduced. The second would be to enhance the anaerobic activity by taking advantage of the RSF. For instance, inorganic fertilizer DS can be mixed with organics which can enhance anaerobic activity or organic fertilizer with low RSF can be developed. The last solution would be to reduce salt accumulation in the bioreactor. In this study, UF was applied for removing salts in the bioreactor, but this was not effective. Thus, alternative macro porous membrane technology (e.g., MF or membrane cartridge filtration) which can retain sludge or electric desalination technology (e.g., CDI, ED (Lu & He, 2015) or ion exchange) can be employed. The novel hybrid process proposed in this study can therefore be further optimized by implementing the solutions mentioned in this paragraph and be evaluated for long-term operation.

4. Conclusions

Primary findings drawn from this study are summarized as follows:

- Flux decline was very severe regardless of fertilizer DS due to the absence of aeration and the sticky sludge, while flux recoveries were different amongst the tested fertilizer DS since their effect on biofouling was different.
- Nutrient accumulation in the bioreactor was influenced by fertilizer properties and exhibited a significant impact on anaerobic activity as well as the sludge composition.
- Bacterial community structure was affected by nutrient accumulation while archaeal community structure remained fairly stable, implying that anaerobic activity was mainly depending on variations in bacterial community structure.

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Figure Captions

Fig. 1. Schematic diagram of the lab-scale side-stream anaerobic FDFO-UF hybrid system.

Fig. 2. Flux-decline curves obtained during the anaerobic FDFO-UF-MBR hybrid system under AL-FS mode with (a) 1 M KCl DS, (b) 1 M MAP DS and (c) 1 M MKP DS. Initial fluxes were 9.06 L/m²/h, 9.56 L/m²/h and 9.82 L/m²/h for KCl, MAP and MKP, respectively. Experimental conditions of all experiments: synthetic municipal wastewater as FS, crossflow velocity of 2.3 cm/s and 20 cm/s for FO and UF, respectively, pH 7 ± 0.2 , temperature of 35 ± 1 °C, HRT of 24 hrs, SRT of 160 days. Membrane cleaning was conducted every 24 ~ 30 hour by applying crossflow velocity of 6.9 cm/s with DI water as DS.

Fig. 3. Influence of fertilizer DS on (a) salt/nutrient accumulation caused by RSF and (b) biogas composition. Salt and nutrient accumulations were monitored by measuring conductivity (representing potassium ions with KCl DS), total nitrogen and total phosphorous. Biogas composition was monitored by GC-FID.

Fig. 4. Variations of bacterial community structures of the sludge collected from the anaerobic bioreactor.

Fig. 5. Variations of archaeal community structures of the sludge collected from the anaerobic bioreactor.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Graphical abstract

A side-stream anaerobic fertilizer-drawn forward osmosis ultrafiltration bioreactor

Highlights

- Flux decline in FDFO was very severe regardless of fertilizer DS.
- Properties of fertilizer DS significantly affected water flux recovery.
- Fertilizer properties considerably affected nutrient accumulation in a bioreactor.
- Nutrient accumulation had a significant impact on biogas production.
- Bacterial and archaeal community structure was varied by nutrient accumulation.