Advances in Environmental Research, *Vol. 7, No. 1 (2018) 39-52*
DOI: https://doi.org/10.12989/aer.2018.7.1.039

Direct membrane filtration of wastewater under very short hydraulic retention time

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(Received April 2, 2018, Revised April 3, 2018, Accepted April 9, 2018)

Abstract. Direct membrane filtration (DMF) of wastewater has many advantages over conventional biological wastewater treatment processes. DMF is not only compact, but potentially energy efficient due to the lack of biological aeration. It also produces more biosolids that can be used to produce methane gas through anaerobic digestion. Most of ammoniacal nitrogen in wastewater is preserved in effluent and is used as fertilizer when effluent is recycled for irrigation. In this study, a technical feasibility of DMF was explored. Organic and nitrogen removal efficiencies were compared between DMF and membrane bioreactor (MBR). Despite the extremely high F/V ratio, e.g., 14.4 kg COD/m^3/d, DMF provided very high COD removal efficiencies at ~93%. Soluble microbial products (SMP) and extracellular polymeric substances (EPS) were less in DMF sludge, but membrane fouling rate was far greater than in MBR. The diversity of microbial community in DMF appeared very narrow based on the morphological observation using optical microscope. On the contrary, highly diverse microbial community was observed in the MBR. Microorganisms tended to form jelly globs and attach on reactor wall in DMF. FT-IR study revealed that the biological globs were structurally supported by feather-like materials made of secondary amines. Confocal laser scanning microscopy (CLSM) study showed microorganisms mainly resided on the external surface of microbial globs rather than the internal spaces.

Keywords: wastewater treatment; biodegradation; nitrogen; nitrate

1. Introduction

Membrane bioreactor (MBR) has been widely used in municipal and industrial wastewater treatment. The main drivers of this relatively new process include superior effluent quality and smaller footprint comparing to conventional activated sludge (CAS). MBR is often combined with enhanced biological nutrient removal processes (EBPR) to lower the nitrogen and phosphorus concentrations in the effluent to meet stringent discharge limit. In the meantime, demand of recycled wastewater for irrigation and landscaping has been increasing in many geographical regions. Deliberate nutrient removal from wastewater may not be required for these purposes. In fact, the ratio of nitrogen and phosphorous in typical municipal wastewater roughly falls into the ideal N:P ratio for terrestrial plants, i.e., 100:7.4-14.8 in weight basis (Geider and La Roche 2002).

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http://www.techno-press.org/?journal=aer&subpage=7 ISSN: 2234-1722 (Print), 2234-1730 (Online)

Direct membrane filtration (DMF) is one of the simplest forms of wastewater treatment that can produce readily recyclable water for irrigation and landscaping. It has several potential advantages over CAS and MBR due to the lack of biological aeration tanks: 1) lower net energy consumption and smaller footprint, 2) less operational challenges, 3) lower capital costs, 4) greater amount of nitrogen in effluent, etc. (Sutton 2011)

While little has been known about DMF, most known studies have been performed using tubular membranes (Till *et al*. 1998, Bendick *et al*. 2004, 2005). It has been found that the membrane flux was lower than that of regular MBR with aeration tanks despite of the much lower suspended solids in DMF. In addition, the inherently high energy consumption of tubular membranes, e.g., 4-12 kWh/m^3 (Côté 2000), made the technology prohibitively expensive.

Submerged membrane can be a promising substitute of tubular membrane for direct wastewater filtration. The membrane tank holding submerged membrane cassettes can be designed compact enough with a hydraulic retention time (HRT) of 0.5-1.0 hour, which provides comparable compactness with tubular membrane systems in terms of footprint requirement. Submerged membrane adds a few more advantages in addition to the general advantages of direct filtration mentioned above: 1) inadvertently occurring biological activity ensures low soluble COD in the effluent, 2) much less energy consumption than tubular membranes by a factor of 10-40, 3) much lower capital costs due to the inexpensive membrane modules, etc.

Diamantis *et al*. (2009) have tested submerged flat sheet membranes to filter municipal wastewater without a separate aeration tank. It was found that membrane permeability was only 50 L/m^2/hr/bar, which was much lower than that of typical MBR, e.g., 100-300 L/m^2/hr/bar (Yoon, 2016). However, detailed experimental conditions were not revealed that the cause of low membrane permeability was not clear. Mezohegyi *et al.* (2012) also tested submerged flat sheet membranes to concentrate municipal wastewater and observed more significant membrane fouling than that in typical MBR perhaps due to the excess extracellular polymeric substances (EPS). Again, no detailed experimental conditions were revealed in the study.

In this study, DMF was investigated using submerged membranes under a well-controlled lab environment. The main goal of this study was to investigate the treatability of wastewater by DMF with respect to COD and ammoniacal nitrogen. Alongside microbial behavior and membrane performance were investigated to elucidate the effect of short HRT on process operation.

2. Materials and methods

2.1 Experimental setup and operation

Two DMS systems were built by submersing compact hollow fiber membrane modules in clear acrylic tubes with 2.5 cm inner diameter. Membrane fibers were taken from a commercial hollow fiber module (Z10, Suez). The effective membrane length and surface area were 0.55 m and 0.031 m^2, respectively. Effective reactor volume was 250 ml excluding the membrane, diffuser, and tubing volumes. Permeate was drawn intermittently (4 minutes ON and 1 minute OFF) using a peristaltic pump. Trans-membrane pressure (TMP) was tracked by recording vacuum pressure in the permeate line. Water level was tightly controlled at ±1 cm using a pressure switch (ZSE40 model, SMC, Japan) that measured static pressure in the middle of the air tube connected to the reactor bottom as shown in Fig. 1, where lower static pressure than set point triggered wastewater feeding to the reactor. HRT was targeted at 30 minutes. SRT of the two DMF systems were

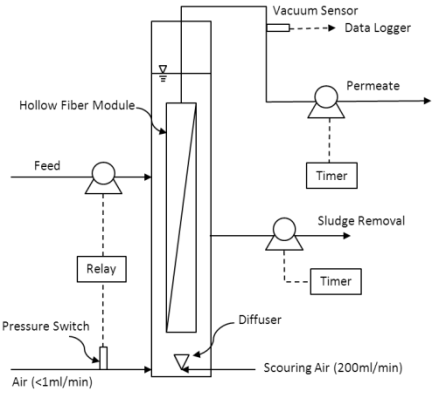


Fig. 1 Schematic of experimental setup

Table 1 Specifications of DMF and MBR used in the experiment

	DMF1	DMF4	MBR
HRT (min)	30	30	300
SRT (d)	1	4	20
Working volume (ml)	250	250	250
Membrane area (m^2)	0.031	0.031	0.003
Membrane length (m)	0.45	0.45	0.27
Net average flux ($L/m^2/hr$)	16	16	16
# of fibers (ea)	12	12	2
F/V ratio (kg $COD/m^3/d$)	14.4	14.4	1.44
Suction cycle (on/off)	4 min/1 min	4 min/1 min	4 min/1 min

maintained at 1 day and 4 days, respectively, by draining the mixed liquor intermittently at two different rates using a timer. The two DMFs with short HRT were called DMF1 and DMF4, respectively.

Simultaneously with the DMF experiments, one lab scale MBR system was built to obtain control data. HRT and SRT were maintained at 5 hours and 20 days, respectively, which were close to those of regular MBR treating municipal wastewater. This MBR was started 2 months earlier than other two MBRs to stabilize the condition under the much longer SRT. Details of operating conditions are summarized in Table 1.

All systems were seeded with return activated sludge taken from a local municipal wastewater treatment plant. Scouring air was supplied through a fine bubble diffuser and the flow rate was fixed at 200 ml/min. All the experiments were performed at 21 ± 1°C. Yeast extract (140 mg/L), sucrose (140 mg/L), urea (43 mg/L), K_2HPO_4 (11 mg/L), $CaCl_2$ (37 mg/L), $MgSO_4$ (50 mg/L), and $FeCl_3$ (5 mg/L) were dissolved in city water to make synthetic feed. Total COD, nitrogen, and phosphorous of the synthetic feed were measured at around 300 mg/L, 32 mg/L, and 3.3 mg/L, respectively, using colorimetric methods (Hach Co., USA). The synthetic feed was prepared daily in order to minimize microbial activities in the feed tank and was continuously stirred at low speed. Membrane cleaning was performed daily for DMF1 and DMF4. Membrane modules were cleaned with water jet and were immersed in warm NaOCl solution (1,000 mg/L) for 30 min.

Before placing the membrane modules back in the reactor, they were immersed in deionized water and 10 ml of permeate was slowly drawn through the permeate outlet using a syringe in order to remove the residual free chlorine in the lumen. The membrane module in MBR was cleaned typically every other day whenever TMP reached 30-40 kPa.

2.2 SMP and EPS analyses

Soluble microbial products (SMP) were quantified by measuring the dissolved proteins and polysaccharides in membrane permeate and mixed liquor supernatant. The supernatant sample was taken by centrifuging mixed liquor for 30 min at 1,050g. After draining supernatant, centrifuge tube was filled up with 0.1 N NaCl solution to the original water level. The solids in the bottom was re-suspended before the sample was placed in water bath at 100 ^{o}C for 30 min. The suspension was centrifuged for 30 min at 1,050 g and the supernatant was taken to measure proteins and polysaccharides for EPS quantification.

Polysaccharides concentrations were measured colorimetrically at 490 nm using phenol-sulfuric acid method (Dubois *et al*. 1956). Protein concentrations were also measured colorimetrically at 562 nm using the bicinchoninic acid (BCA) method with bovine serum albumin (BSA) as a standard (G-Biosciences, USA). For EPS quantification, the measured proteins and polysaccharides concentrations were normalized to the total solids (TS). Using TS instead of mixed liquor suspended solids (MLSS) was particularly compelling for this small scale experiment because measuring TS is much more accurate than measuring MLSS when sample volumes are small. Moreover, the low conductivity of mixed liquor (610 μS/cm) translated to total dissolved solids (TDS) at around 0.3-0.4 g/L according to the correlation found in literature (Atekwanan *et al.* 2004). Therefore, if TS was measured at 3-4 g/L, the deviation from the MLSS was ~10%.

2.3 Excitation-emission (EEM) spectroscopy

Three-dimensional EEM spectroscopy was performed for DMF and MBR permeates and mixed liquor supernatants using a Horiba FluoroMax-3 luminescence spectrometer. All mixed liquor supernatant samples were obtained by centrifuging mixed liquor at 1,050 g for 30 minutes. Excitation wavelength was increased from 200 nm to 500 nm with a resolution of 5 nm. No pH adjustment was performed since sample pH was consistent, i.e., 7.7±0.1 for DMF1 and DMF4, 7.1±0.1 for MBR.

In order to quantify the rejection efficiency of membrane for each category of molecules, EEM spectra were divided by four regions and the emission strengths were integrated according to the method shown in Chen *et al*. (2003). In the original method, emission strengths were integrated over each region in EEM chart. But, if scanning was performed with uniform step sizes for all samples, simply adding up emission strengths in each region is sufficient to compare the relative differences among regions. The exact borders among regions were decided somewhat arbitrarily considering the location of peaks obtained experimentally. Following are the details of the regional breakdown: I) protein-like (200 nm<Ex≤310 nm, 250 nm<Em≤420 nm), II) fulvic-like (200 nm<Ex≤310 nm, 420 nm<Em≤550 nm), III) humic-like1 (310 nm<Ex, Ex≤800 nm-Em), and IV) humic-like2 (310 nm<Ex, Ex>800nm-Em). The Region I includes the strong tryptophan peak (Baker 2001) while the Region II includes the weak fulvic acid peak (Sierra 2005). The rest of the areas were split to Region III and Region IV based on the two strong peaks observed in supernatant samples.

Table 2 The dyes used for staining microbial floc samples and the dyeing conditions

Dye	Target	Excitation (nm)	Emission (nm)	Dilution factor/Concentration	Staining duration
SYTO9	Cell	488	515/30	1,000x	20 min
Con A	Polysaccharide	568	600/50	0.5 mg/ml	

2.4 Floc observation and analysis

The microbial community of mixed liquor was observed using a phase contrast optical microscope (BX51, Olympus Co., Japan) at 100x-1,000x. In addition, confocal laser scanning microscopy (CLSM) was performed to observe the internal structure of microbial floc (LSM5 Pascal model, Zeiss GmbH, Germany) according to the methods described in Hwang *et al*. (2012). For CLSM, large microbial flocs (or globs) were collected and stained with the 1:1 mixture of two diluted dyes, i.e., SYTO9 and Concanavalin A conjugated to tetramethylrhodamine isothiocyanate (Molecular Probes® , Life Technologies Corp., USA). The details of the dyes and the dyeing condition are summarized in Table 2.

Since the core materials structurally supporting microbial flocs did not appear microorganisms in the microscopic and CLSM studies, FT-IR was performed to identify the chemistry of supporting materials. The large microbial flocs captured by hollow fiber membranes were collected from the membrane module and washed using deionized water. After drying the sample at room temperature overnight, FT-IR was performed (Nicolet 6700, Thermo Scientific, USA). The FT-IR instrument was equipped with an internal reflection accessory based on attenuated total reflectance (ATR) technique (SplitPea™, Harrick Scientific Products Inc., USA). The sample was analyzed at four wavenumber resolution and the final spectrum was the result of 64 coadded scans.

3. Results and discussions

3.1 Process performance

Biological conditions were believed mostly stabilized within the first 12 days of operation for DMF1 (SRT=1d) and DMF4 (SRT=4d), which corresponds to 12x and 3x of SRT, respectively. However, it was observed that TS of the mixed liquor started to decrease noticeably at 10^{th}-12^{th} day as a result of microbial attachment on reactor walls. Microorganisms also could grow on membrane surface, but it could not be visually observed due to the daily membrane cleaning. A part of biofilm on reactor wall was irregularly detached mainly due to the physical scratches when membrane module was taken out of reactor during the daily chemical cleaning. As a result, taking representative sample from the reactor became practically impossible and the measured TS fluctuated significantly day by day. In order to minimize the impact of the TS fluctuation on EPS quantification, all the samples were shaken vigorously for 30 seconds to break the large microbial flocs before splitting them to measure TS and EPS contents.

Very large flocs (or globs) with jelly appearance were found on hollow fiber membrane bundle especially in DMF4, which were grown on reactor wall and detached. If there were no daily

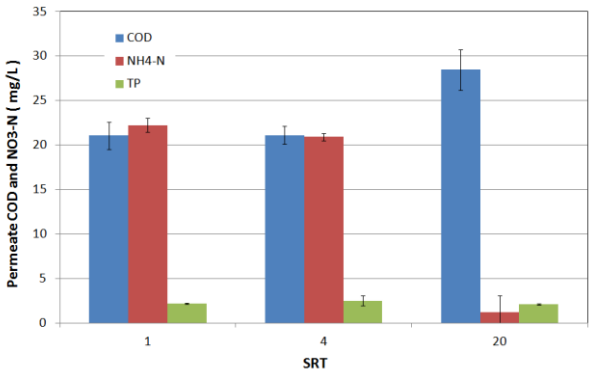


Fig. 2 Permeate quality of MBRs with three different SRT

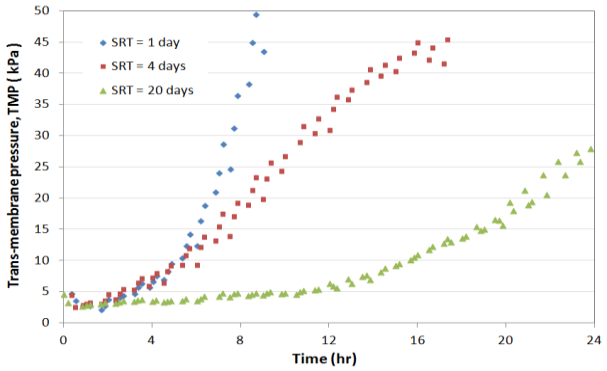


Fig. 3 Trans-membrane pressure (TMP) increase in the three different reactors with different solids retention time (SRT)

membrane cleaning, hollow fiber membranes could be ragged sufficiently by the microbial globs and lose the mobility partially. Diminished fiber movement will eventually reduce membrane performance by promoting the deposition of other materials near them and limiting the freedom of fiber movement. On the contrary, no apparent floc attachment was observed in MBR that had much longer SRT (20d).

Mixed liquor TS were observed at 2.1 ± 0.8 g/L, 3.2 ± 1.0 g/L, and 7.0 ± 0.3 g/L (average ± standard deviation), for DMF1, DMF4, and MBR, respectively. As a result of very low TS and low HRT, average F/M ratio were calculated extremely high, e.g., 7.32 g COD/g TS/d, 4.34 g COD/g TS/d, and 0.2 g COD/g TS/d for the three MBRs, respectively. The effective F/M ratio for DMF1 and DMF4 could be much lower than the apparent F/M ratio, if the attached solids were considered.

Despite of the very high organic loading for DMF1 and DMF4, dissolved oxygen (DO) was maintained greater than 1.0 ppm without exception at an average of 1.6 mg/L in both reactors. It is noteworthy that dissolving sufficient oxygen under such high F/V ratio, e.g., 14.4 kg COD/m^3/d, is rarely possible in large scale MBR. Perhaps three factors contributed to the fast oxygen dissolution: 1) high air flow rate (200 ml/min) for the small reactor (250 ml), 2) the strong wall effect that delays bubble rise and increases contact time (Mukundakrishnan, 2007), 3) low MLSS at 2.1 ± 0.8 g/L and 3.2 ± 1.0 g/L for DMF1 and DMF4, respectively. Meanwhile average DO was

3.2 mg/L in MBR.

The soluble COD in feed wastewater, i.e., 300 mg/L, was lowered to around 21 mg/L in both DMF1 and DMF4 despite the very short HRT (30 min) and low TS (2-3 g/L). Interestingly, COD of MBR effluent was significantly higher at 29 mg/L as shown in Fig. 2 although MBR was operated at much longer HRT (5 hours) at much higher TS (7.0 g/L). As will be shown later, polysaccharides and protein contents were also higher in MBR than in DMF. Nitrification was negligible in DMF1 and DMF4 while it was almost complete in MBR. Therefore, permeate NH_4-N concentrations were much higher in DMF1 and DMF4 than in MBR. Total phosphorus (TP) in DMF and MBR effluent was 2.1-2.5 mg/L, which was 1 mg/L lower than that in feed solution. Overall DMF produced suitable effluent for irrigation with high NH_4-N and phosphorus at a ratio of around 10:1. This is within the ideal range for terrestrial plants, e.g., 100:7.4-14.8 (Geider and La Roche 2002).

TMP rise was the slowest for MBR consistently despite of the highest TS. TMP rise of DMF1 was generally the fastest. Two membrane modules in DMF1 and DMF4 had switched each other after every filtration cycle to eliminate the effect of potentially different membrane performance, but no apparent module effects were observed. Fig. 3 shows typical TMP curves, but the trends of the two curves were reversed at times.

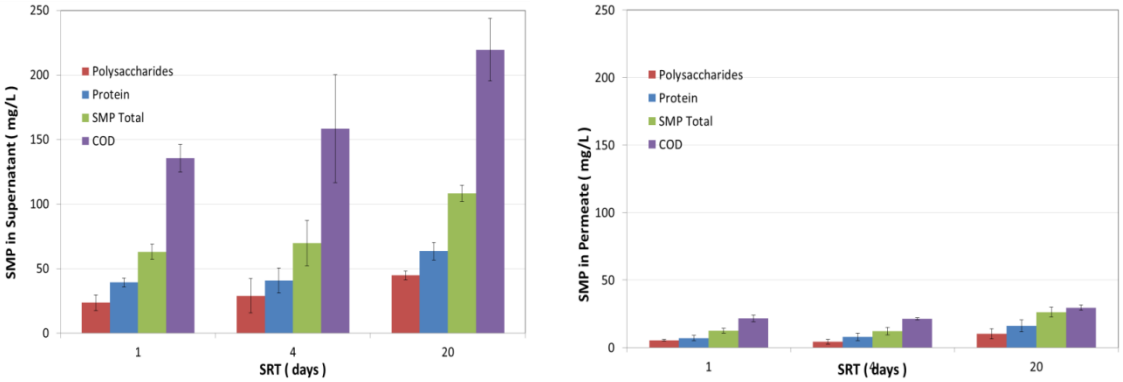
3.2 Effect of SMP/EPS on membrane performance

In all MBRs, protein concentrations were greater than polysaccharide concentrations in mixed liquor supernatant as shown in Fig. 4(a). Both protein and polysaccharide concentrations were clearly the highest in MBR while there were little differences between DMF1 and DMF4. The total SMP concentrations were measured at 63 mg/L, 70 mg/L, and 108 mg/L on average for DMF1, DMF4, and MBR, respectively. The hollow fiber membranes with a nominal pore size of 0.04 μm used in the experiment removed the vast majority of SMP in mixed liquor. As a consequence, average total SMP in permeate were only 12 mg/L, 12 mg/L, and 24 mg/L in two DMFs and one MBR, respectively, as shown in Fig. 4(b).

The rejection efficiencies of proteins, polysaccharides, and COD are summarized in Fig. 5. The rejection efficiencies of total SMP were estimated at 80%, 83%, and 76%, respectively, for DMF1, DMF4 and MBR. In the meantime, COD of supernatant were removed somewhat better at 84%-86% for the three systems. The better removal of SMP than COD suggests that some large molecules/particles are present in the mixed liquor supernatant, which are not detected as SMP.

The normalized EPS in DMF1 and DMF4 were not significantly different as shown in Fig. 6. While polysaccharides levels were nearly identical, proteins levels were somewhat greater in DMF4. Meanwhile the trend of EPS composition in MBR was clearly distinguished from those in DMFs. It was apparent that polysaccharides EPS was substantially greater, yet protein EPS was much lower. Overall, total EPS concentrations were 262 mg/L, 443 mg/L, and 642 mg/L for DMF1, DMF4, and MBR, respectively. If the total EPS concentration is normalized against TS, EPS of MBR became the lowest of all due to ~3 times greater TS in MBR.

Generally high SMP and EPS have been considered an indicator of high membrane fouling potential not only in MBR, but also in RO (Chang and Lee 1998, Jarusutthirak and Amy 2006). However, combining all the data shown in Fig. 2, Fig. 4(a), and Fig. 6, it was apparent that TMP rose slowest in MBR, where SMP and absolute EPS concentrations were the highest. Perhaps the quality of the SMP and EPS played a role, but the biopolymer qualification was out of the scope of this study.



(a) SMP in supernatant (b) SMP in permeate

Fig. 4 SMP concentrations in mixed liquor supernatant and permeate

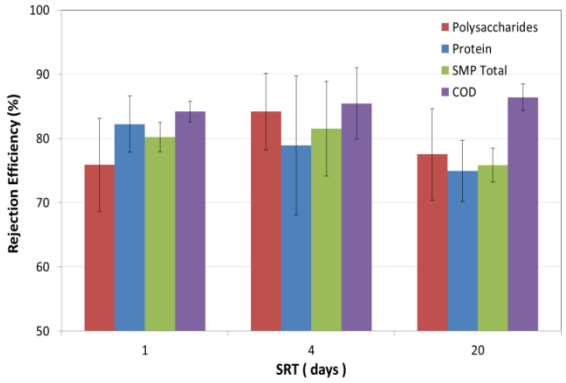


Fig. 5 Rejection efficiencies of SMP and COD by the membrane with a nominal pore size of 0.04 μm

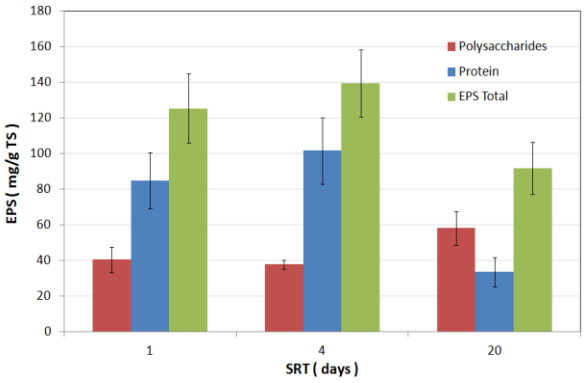


Fig. 6 EPS contents normalized to total solids

3.3 Excitation-emission (EEM) spectroscopy

Three-dimensional EEM spectroscopy was performed for permeate and mixed liquor supernatant in order to investigate which portion of the organics are removed by membrane. As

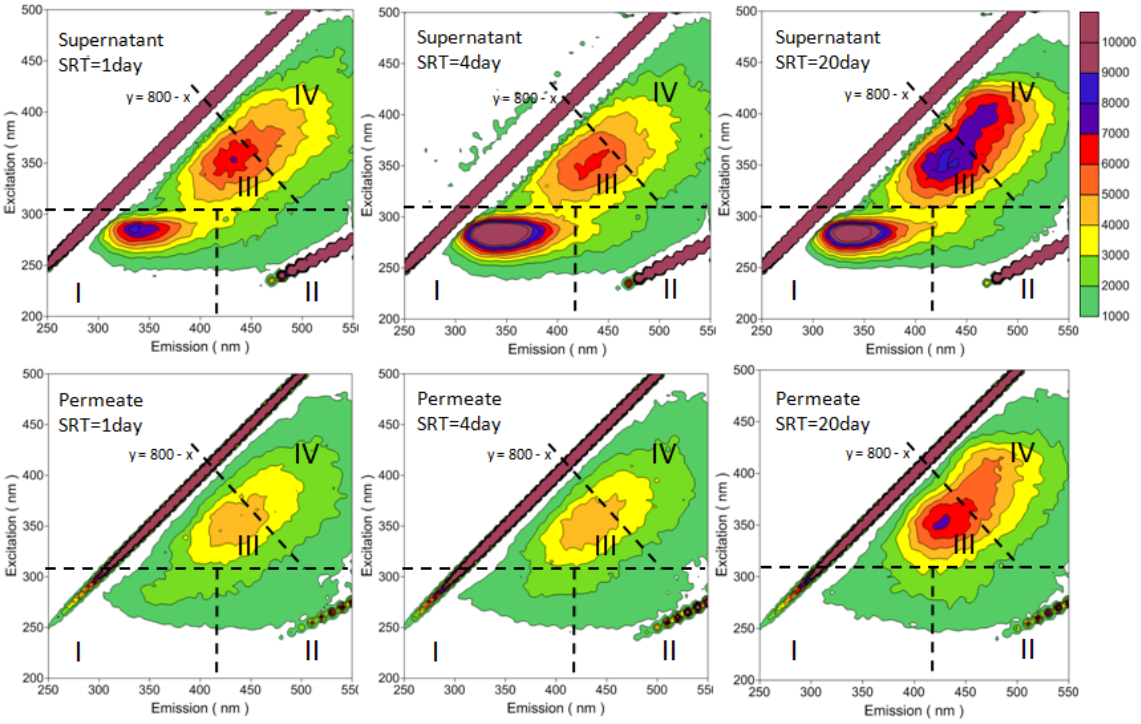


Fig. 7 Excitation-emission spectra of supernatant and permeate from three different SRT

Table 3 Result of Regional integration of EEM spectra

Region	SRT = 1 d			SRT = 4 d			SRT = 20d		
	Superna-tant($x10^6$)	Perme-ate($x10^6$)	Removal %	Superna-tant($x10^6$)	Perme-ate($x10^6$)	Removal %	Superna-tant($x10^6$)	Perme-ate($x10^6$)	Removal %
I	1.28	0.72	44	1.91	0.69	64	1.47	0.67	54
II	0.67	0.55	19	0.73	0.55	25	0.60	0.61	-1
III	1.07	0.77	28	1.13	0.78	31	1.29	1.00	23
IV	2.14	1.57	27	2.00	1.51	25	2.47	2.01	18
Total	5.17	3.61	30	5.76	3.53	39	5.83	4.29	26

shown in Fig. 7, in all supernatant samples, strong peaks are observed at the excitation/emission (Ex/Em) wavelength of 280/340 nm in Region I, which is known as a tryptophan peak (Baker 2001). The weak peak at Ex/Em of 265/475 nm in Region II is known as fulvic-like material's peak (Sierra 2005). The two peaks in Region III (360/430 nm) and Region IV (400/465 nm) are known as humic-like peaks (Coble 1996). The EEM spectra obtained in this study were somewhat different from those of activated sludge effluent in literature (Chen *et al.* 2003, Wang *et al.* 2009, Wu *et al.* 2013). It might be attributable to the different feed compositions, drastically different operating condition especially for DMF1 and DMF4, etc.

According to Fig. 7, it is apparent that the protein-like molecules that contain tryptophan was removed very effectively. Based on the regional integration methods described in section 2.3, removal efficiencies of protein-like material were 44%, 64%, and 54%, respectively, in DMF1,

DMF4, and as summarized in Table. If only the smaller areas near the peak were integrated, removal efficiency could be much greater. The high removal efficiency suggests proteins mainly exist as large conglomerates that can be easily removed by the membrane with 0.04 μm pore size. Overall, this observation agrees roughly with the high protein rejection efficiencies estimated colorimetrically using BCA method as shown in Fig. 5. Removal efficiencies of fulvic-like molecules in Region II were not as high as that of protein-like molecules. Especially in MBR, no removal of fulvic-like molecules was observed. Removal efficiency of humic-like molecules in Region III and Region IV ranged 18-31%. Overall removal efficiencies were 30%, 39%, and 26% in DMF1, DMF4, and MBR, respectively.

3.4 Microscopic observation of mixed liquor

The morphologies of the mixed liquor were observed microscopically. The mixed liquor of DMF1 and DMF4 appeared unique as shown in Fig. 8(a), where only several morphologically different species were present. The dominant species was short rod-shaped bacilli while some motile bacilli, coccus, diplococci, and spirillum with flagella were observed. Appearance of DMF4 sludge was similar to that of DMF1 sludge, but few amoeba-like cells and ciliates without stalk were observed. It was not clear whether such cells were grown in the condition or remaining from the seed sludge. On the contrary, the mixed liquor of MBR grown under 10 times lower F/V ratio at 20-day SRT had much greater microbial diversity as shown in Fig. 8(b). Zoogloea, S. natan, N. limicola, Isosphaera, stalked ciliates, amoeba, etc. were observed just like in typical MBR sludge.

The narrow diversity of microbial community at short SRT is a natural consequence of slowly growing microorganisms being washed out. In addition, the high organic loading at short HRT can encourage fast-growing microorganisms grow much faster than slowly growing counterparts under the condition with no food limitation. Başaran *et al*. (2012) also made a similar observation from the denaturing gradient gel electrophoresis (DGGE) study of the DNA extracted from the mixed liquor taken from a MBR quipped with a side-stream membrane. It was found that the shorter the SRT was the narrower the genetic diversities were.

As mentioned above in section 3.1, it was noticed that microorganisms started to attach on reactor wall at 10^{th}-12^{th} day in DMF1 and DMF4. Simultaneously, unusually large flocs, i.e., 1,000 -2,000 μm, were observed from the two DMFs (picture is not shown since it is vary similar to Fig. 11(a)). Those giant flocs were the detached biofilm from the reactor wall and appeared jelly. Further studies were performed to identify the supporting material as will be discussed in the next section.

3.5 Identification of the support material of biofilm

The biofilm on reactor wall was partially detached by the turbulence and the mechanical scratches during the daily membrane loading and unloading for *ex situ* membrane cleaning. The detached biofilm was mostly collected by hollow fibers after floating in the mixed liquor for some time. Fig. 9(a) shows biofilm debris with jelly appearance on membrane surface. It was not clear how much the biofilm debris affected TMP rise due to the daily membrane cleaning in this study, but they could encourage the deposition of other floating materials in the long run by hampering the membrane movement and by creating low flow zones nearby.

The jelly materials on the membrane bundle were collected and washed to remove microorganisms on the surface. Fig. 9(b) shows the feathery supporting material revealed, but it

appeared too large to be filamentous microorganisms. Since there were no solids in the synthetic feed, the feathery materials were considered synthesized in the mixed liquor. Further studies were performed to identify the chemistry of the materials. The FT-IR spectrum in Fig. 10 strongly indicated that the major component of the sample was secondary amines. The strong single band at ~3280 cm^{-1} was considered as a characteristic N-H stretch band of secondary amine, while the

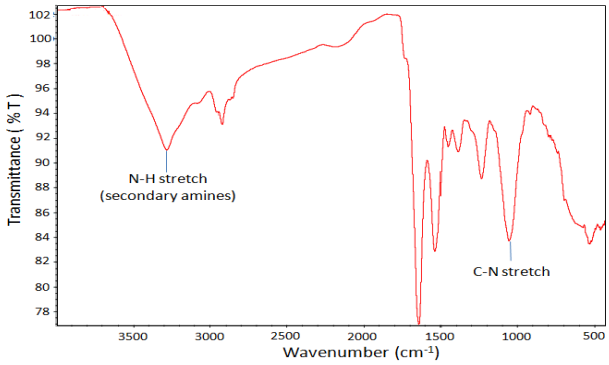
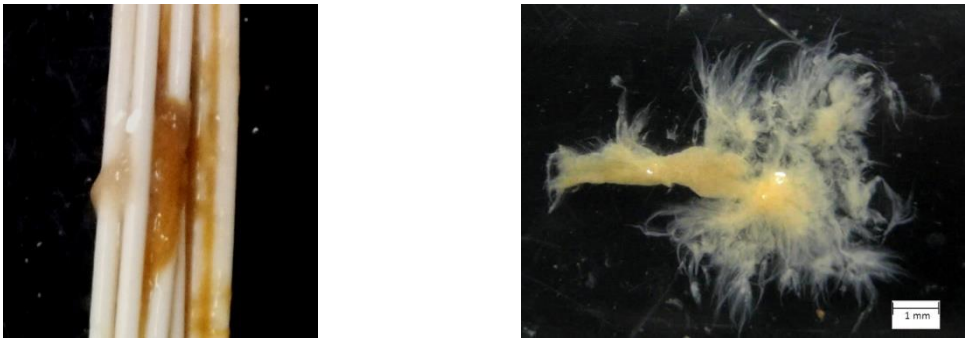


Fig. 8 Excitation-emission spectra of supernatant and permeate from three different SRT



(a) Jelly materials on membrane (b) Washed jelly material in petri dish

Fig. 9 Jelly material attached on hollow fiber membrane and its hairy appearance when washed by deionized water

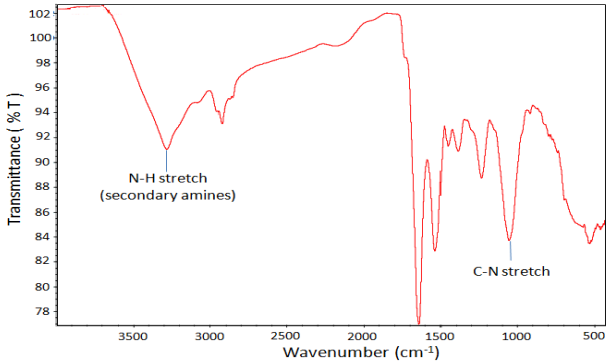


Fig. 10 FTIR spectrum of the fiber-like materials that are somewhat contaminated with microorganisms and their byproducts

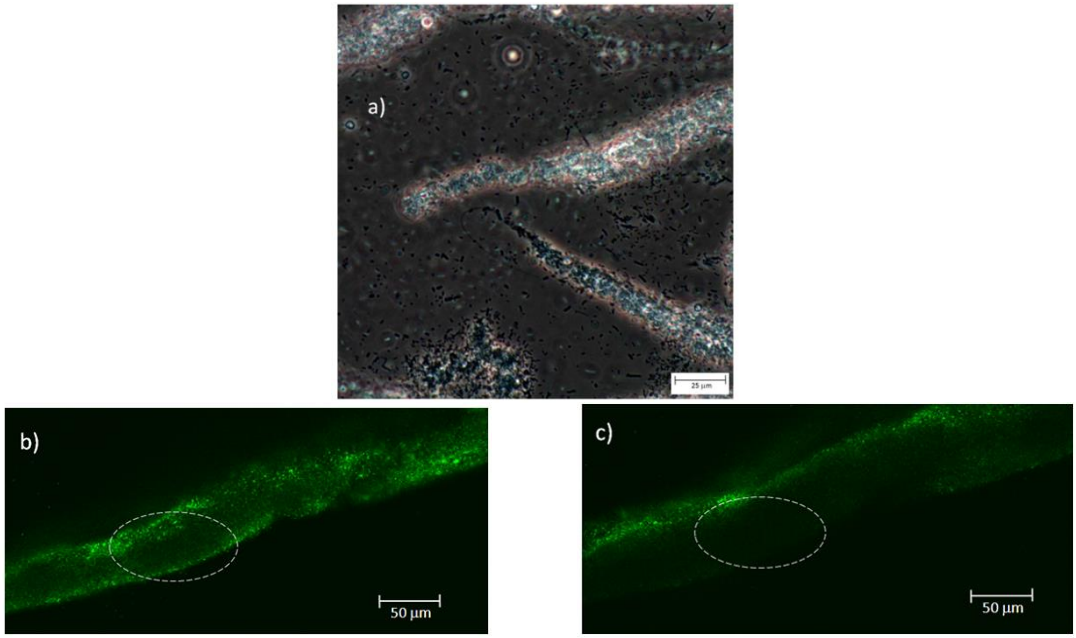


Fig. 11 Microscopic image of fibrous material, (a) Optical image of multiple fiber-like materials, (b) CLSM image of fiber-like material's surface and (c) CLSM image of fiber-like material's internal space (15 micron from the surface). The objects in the optical image and the CLSM images are not identical. In CLSM images, greens spots are the fluorescence signal from microorganisms

band at ~1080cm^{-1} C-N stretch band. It was not clear how such a large amount of feathery structure based on secondary amines was synthesized, but it was not within the scope of this study.

Microscopic observation of a sample taken from hollow fiber membrane bundle revealed the hairy substructure shown in Fig. 11(a), which appeared same as the large flocs floating in mixed liquor as discussed in section 3.4. CLSM was performed to investigate the distribution of microorganisms and polysaccharides in the three dimensional spaces of the hairy substructure. A piece of sample was dyed using SYTO9 and ConA targeting cells and polysaccharides, respectively, under the condition summarized in Table 2. A sizable number of cells were detached from the sample when it was dipped in dye solution, but a good number of cells were still observed on the surface. In Fig. 10(b), green spots indicate fluorescence signals from microorganisms attached on sample surface. However, much less cells were observed in the 15 μm below the sample surface (Fig. 10(b)). These observations suggest that microorganisms reside mainly on the surface of substructure. In the meantime, no strong polysaccharides signals were observed regardless of the depth of the sample (picture not shown). This observation agreed with the FT-IR data that the feathery materials that act as a skeleton of biofilm were mainly secondary amine.

4. Conclusions

The lab scale DMF experiment performed with submerged hollow fiber membranes revealed that 300 mg/L of soluble COD could be successfully reduced to 21 mg/L at very short HRT (30

min) and SRT (1d or 4d). The ammoniacal nitrogen essential for growing plants was well preserved in permeate due to the lack of nitrification. In the meantime, the permeate of the control MBR running at much longer HRT (5hr) and SRT (20d) had much higher COD at 29 mg/L and negligible ammoniacal nitrogen.

SMP concentrations were the highest in MBR in terms of both polysaccharides and proteins while there were little differences in between DMF1 and DMF4 except the slightly higher polysaccharides in DMF4. In MBR, the total EPS concentration including both polysaccharides and proteins was the highest, but normalized EPS to TS was the lowest. Protein contents were somewhat greater than polysaccharides contents in EPS in DMF1 and DMF4, but it was opposite in MBR. Despite the lower SMP and total EPS contents in DMF1 and DMF4 than in MBR, membrane fouling was more significant.

Morphological study revealed the diversity of microorganisms in DMF appeared very narrow. Only five morphologically different species were observed in DMF1 while two more higher life forms were observed in DMF4. On the other hand, highly diverse microbial community was observed in MBR. In addition, microorganisms tended to attach on the wall in DMF. The biofilm formed on reactor wall was partially detached and were collected by hollow fiber membrane bundle. The collected biofilm debris might have caused accelerated membrane fouling, if membrane cleaning was not performed daily.

The structure and the composition of the macrofloc collected from the membrane bundle were analyzed using optical microscope, CLSM and FT-IR. It was found that microorganisms resided on the external surface of a feathery supporting structure. The FT-IR study performed strongly suggested that the supporting materials were secondary amines, perhaps polyamide. Further study is required to elucidate how the material was synthesized and if the synthesis was the consequence of low HRT and SRT.

Acknowledgements

The author would like to thank Dr. Xuejun Wang and Mr. Michael Gutfeldt of Nalco for CLSM analyses and for advices on microbial identifications, respectively.

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