High-Strength N-methyl-2-pyrrolidone-Containing Process Wastewater Treatment Using Sequencing Batch Reactor and Membrane Bioreactor: A Feasibility Study

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Abstract

N-methyl-2-pyrrolidone (NMP) is widely used as a solvent in polymeric membrane fabrication process (at a high concentration > 1000 mg/L), its elimination from the process wastewater prior to discharge is essential because of environmental concern. This study investigated the feasibility of treating high-strength NMP-containing process wastewater in a sequencing batch reactor (SBR; i.e., batch feeding and intermittent aerobic/anoxic condition) and a membrane bioreactor (MBR; i.e., continuous feeding and aeration), respectively. The results showed that the SBR with the acclimated sludge was capable of removing >90% of dissolved organic carbon (DOC) and almost 98% of NMP within 2 h. In contrast, the MBR with the acclimated sludge showed a decreasing NMP removal efficiency from 100% to 40% over 15-day operation. The HPLC and LC-MS/MS analytical results showed that NMP degradation in SBR and MBR could undergo different pathways. This may be attributed to the dissimilar bacterial community compositions in the SBR and MBR as identified by 16s rRNA gene sequencing analysis. Interestingly, the NMP-degrading capability of the activated sludge derived from MBR could be recovered to >98% after they were operated at the SBR mode (batch feeding mode with intermittent aerobic/anoxic condition). This study reveals that SBR is a more feasible process to treat high-strength NMP-containing wastewater, but residual NMP metabolites in the SBR effluent need to be post-treated by an oxidation or adsorption process in order to achieve zero-discharge of toxic chemicals.

Keywords: N-methyl-2-pyrrolidone; Biodegradation; Extracellular polymeric substances; Membrane Bioreactor; Sequencing Batch Reactor

1. Introduction

N-methyl-2-pyrrolidone (NMP, C_5H_9NO) is a nitrogen-containing cyclic organic compound. It has been widely used as a solvent for processing natural and synthetic organic polymers in many industries, such as petroleum industry, pharmaceutical industry, microelectronics industry, due to its high boiling point and polar properties [1-3]. Compared with other volatile and toxic organic solvents such as chlorinated hydrocarbons, NMP has high extraction and recovery yields, as well as relatively low energy requirement and environmental impacts [1,2,4].

Because of these advantages, NMP has also been extensively applied as a solvent in membrane fabrication processes, and plays an important role in determining the ultimate membrane morphology and performance [5]. As a solvent, NMP is generally present at high concentrations in polymer solutions and mixed with other inorganic/organic additives [5]. During the membrane fabrication process, water is used as the coagulant, and thus most NMP diffuses from the nascent membranes into the water. The resultant high-strength NMP-containing process wastewater is not allowed to be directly discharged to the water treatment plants. Especially, the recent studies have revealed the ecotoxicity of NMP in affecting planktonic crustacean *Daphnia magna* (LC_{50} 1.23 mg/L 48 h^{-1}) [6], and marine microorganism *Vibrio fisheri* (EC_{50} 1500 mg/L) [4]. Therefore, to meet discharge standards, elimination and conversion of NMP in the process wastewater to harmless compounds are necessary.

An attractive approach is to oxidize NMP using ozone and hydrogen peroxide, which could reduce organic loading to the industrial wastewater treatment plant [4]. Alternatively, the biodegradation of NMP by activated sludge under the normal conditions of sewage treatment is feasible. Chow and Ng [1] firstly observed that 95% of NMP (100 mg/L) could be

degraded by the activated sludge within 7 days under a semi-continuous condition. Oceguera-Cervantes et al. [7] illustrated that *Alicycliphilus* sp. were capable of utilizing NMP (0.5-25 g/L) as the sole source of carbon and nitrogen in a minimal medium. Lee et al. [8] found that the polyethylene glycol biocarrier-immobilized *Acinetobacter* sp., *Cupravidus* sp., *Paracoccus* sp. and *Pseudomonas* sp. could mineralize 90% of NMP (100-200 mg/L) at a hydraulic retention time (HRT) of 8 h in a continuous reactor. Křížek et al. [2] pointed out that *Pseudomonas*, *Paracoccus*, *Acinetobacter* and *Rhodococcus* genera isolated from activated sludge had capabilities in degrading NMP (300 mg/L) and achieved 74-92% organic carbon removals in 3 days. It was noted that these reported studies mainly focused on the biodegradation of NMP only by pure cultures or air-dried activated sludge, or in batch tests, or at a low concentration. Apparently, there is a lack of studies emphasizing on the treatment of real high-strength NMP-containing process wastewater by activated sludge processes in a continuous operation condition and on identification of NMP degradation metabolites.

The objective of this study is to investigate the feasibility of treating the high-strength NMP-containing wastewater produced from membrane fabrication process using a sequencing batch reactor (SBR) and a membrane bioreactor (MBR), respectively. The reactor performances were compared by evaluating NMP mineralization efficiency. The microbial community structures and NMP degradation metabolites in the SBR and MBR were also identified. This study has shed light on developing a suitable biological treatment process for high-strength NMP-containing process wastewater treatment.

2. Materials and methods

2.1. NMP-containing process wastewater

The NMP-containing process wastewater was produced from a lab-scale hollow fibre membrane fabrication process and collected weekly. The process water mainly contained NMP (1500-1600 mg/L), LiCl (0-150 mg/L) and polyethylene glycol (PEG) (0-800 mg/L), in which NMP accounted for ~90% of organic substances on average. The minerals were supplemented into the feed water, including K_2HPO_4 (0.18 g/L), $Na_2HPO_4 \cdot 12H_2O$ (1.92 g/L), $MgSO_4 \cdot 7H_2O$ (0.1 g/L), $FeSO_4 \cdot 7H_2O$ (0.01 g/L), $CaCl_2$ (0.01 g/L), and NaCl (0.5 g/L) [2]. The chemicals used were from Sigma-Aldrich (USA).

2.2. Operating conditions of SBR and MBR

The SBR and MBR1 were inoculated with activated sludge (3.3 g/L) from the Ulu Pandan Water Reclamation Plant, Singapore. At the end of SBR operation, NMP-acclimated sludge from the SBR (6.2 g/L) was inoculated into the MBR2. The three reactors had the same effective reactor volume of 2.7 L and aeration rate of 2 L/min. In SBR, 2 cycles were performed per day, in which the feeding time and settling time was 5 min and 1 h, respectively, and 1.8 L of the effluent was decanted (i.e., averaged hydraulic retention time (HRT) at 18 h). In the MBR, a polyvinylidene fluoride (PVDF) hollow fibre membrane module (pore size at 0.1 μm, GE, USA) with an area of 0.022 m^2 was submerged into the reactor and the permeate flux was maintained at 6 L/m^2 h (i.e., averaged HRT at 20 h). The permeate suction pressure was recorded via Labview (National Instruments, USA) installed on a computer. When the transmembrane pressure reached 40 kPa, the membrane was taken out from the reactor and chemical cleaning (soaking in 0.5% hypochlorite solution for 2 h) was performed before reuse. No sludge was discharged from the MBR except sampling. The three reactors were operated at a room temperature of 23±1ºC.

2.3. Analytical methods

The dissolved organic carbon (DOC) and total nitrogen (TN) in the feed, effluent, and permeate samples (after centrifuging at 10000×g for 20 min) were monitored using a total organic carbon (TOC)/TN analyzer (Shimadzu, Japan). The pH and conductivity of the feed and effluent samples were examined by a pH/conductivity meter (Mettler Toledo, Switzerland). Mixed liquor suspended solids (MLSS) were determined according to Standard Methods [9].

2.4. NMP and degradation metabolites determination

NMP contents in the feed, effluent, and permeate samples were examined using the Shimadzu Prominence-i LC-2030C High Performance Liquid Chromatography (HPLC) with an Inertsil OD-3 C18 column (150 mm × 1.0 mm, 5 μm) and PDA detector. A mobile phase consisting of 40% methanol and 60% water, with a flow rate of 0.5 mL/min, was used. Column temperature was kept at 40°C. An injection volume of 30 μL was used for HPLC analysis. The retention time of NMP was approximately 5.1 min at a wavelength of 230 nm. NMP concentrations in the samples were calculated based on a calibration curve obtained using NMP solutions (0-1000 mg/L).

In addition, an Agilent 1290 infinity II liquid chromatography (LC) module interfaced via an AJS ESI source with 6460 Series triple quadrupole LC-Mass Spectrometry (MS) detection was employed to identify the individual unknown peaks of the NMP metabolites shown in the chromatograms of HPLC. The LC column and separation conditions were the same as that of HPLC, except injection volume was 2 μL for all the sample analyses. Conditions for the MS were set as follows: drying gas (nitrogen) temperature was 300°C; dry gas flow was 12 L/min; nebulizer pressure was 170 kPa; sheath gas temperature was 300°C; sheath gas flow was 10 L/min; capillary voltage was 3500 V in both positive and negative modes; nozzle voltage was 0 V for positive mode and 1500 V for negative mode. MS analysis was performed with both

positive mode and negative mode in the scan range of m/z 40–200. All the data were processed with Agilent MassHunter Workstation.

2.5. Extracellular polymeric substances (EPS) determination

The solids (pellet) and supernatant were separated from the activated sludge sample (10 mL) by centrifuging at 4000×g for 10 min at 4°C. The bound EPS was extracted from the pellet following a "formaldehyde-NaOH" method described previously [10]. The polysaccharide concentration was determined according to the phenol-sulfuric acid method with glucose as a standard by measuring the absorbance at 490 nm using a spectrometer (Hach, USA) [7], and the protein concentration was determined based on bicinchoninic acid assay protocol with bovine serum albumin as a standard [11]. All the chemicals used in the experiments were from Sigma-Aldrich (USA).

2.6. Adenosine triphosphate (ATP) analysis

Viable cells in the activated sludge were determined by measuring the ATP concentration. In detail, the supernatant of the activated sludge sample was removed after centrifuging at 4000×g for 10 min at 4°C. The settled sludge was washed with sterilized distilled water for three times. The ATP in the settled sludge was measured using the Kikkoman's ATP assay kit and a luminometer (Lumitester C-110, Kikkoman, Japan) according to the manufacturer's guidelines.

2.7. Microbial community analysis

The sludge samples were collected from the SBR and MBR2 at the end of operation. The genomic DNA of the microbial community in the samples was extracted by FastDNA® SPIN kit (MP Biomedicals, USA). The 16S rRNA gene was sequenced by Illumina (Research and Testing Laboratory, USA) using primers 357wF (CCTACGGGNGGCWGCAG) and 785R

(GACTACHVGGGTATCTAATCC). The sequencing results were analysed by Mothur 1.35.1 software [12] using the standard de novo operational taxonomic unit (OTU)-based approach.

3. Results and discussion

3.1. Reactor performance

In this study, the SBR and MBR1 were inoculated with conventional activated sludge (2.7 L with MLSS of 3.3 g/L). At the end of SBR operation, the MLSS concentration reached to 6.2 g/L. The MBR2 was inoculated with the acclimated sludge (derived from SBR after 37-day operation, 2.7 L with MLSS of 6.2 g/L) and the MLSS concentration reached to 26.6 g/L after 15-day operation due to no sludge removal (Figure S1).

Figure 1 shows the pH and conductivity in the SBR and MBRs. In the reactors, the pH varied within 6.95-7.29, comparable to the feed water (~6.89). However, it is noticed that the averaged conductivities in the SBR and MBRs (1.5-2.2 mS/cm) were higher than that of the feed water (~ 0.7 mS/cm). Previous studies indicated that some electrolyte intermediates (such as 2-pyrrolidinone, succinimide) were produced during the oxidation of NMP [4]. When the NMP degradation intermediates could not be fully utilized by the activated sludge, they could result in an increase of conductivity. The increased conductivity may impact microbial activity due to higher osmotic pressures and influence membrane performance due to the change of interaction force between the foulants and membrane surface.

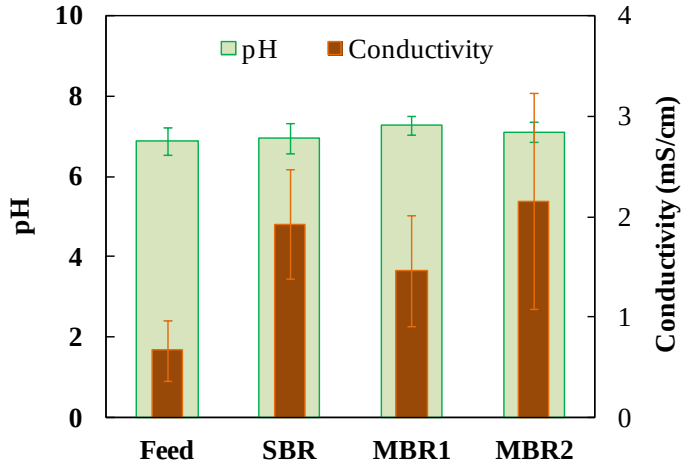

Figure 1. pH and conductivity of the feed and reactor effluent

The reactor performance was evaluated by the DOC and TN removal efficiencies, shown in Figure 2. As the feed, the high-strength NMP-containing process water had 0.74-3.79 g/L of DOC and 0.23-0.75 g/L of TN. In the SBR (Figure 2a), the DOC and TN removal ratios improved from 50% to 92% and from 11% to 43%, respectively, within 18-day operation. After the incremental acclimation to high-strength NMP, the DOC and TN concentrations in the SBR effluent maintained at 40-190 mg/L and 132-171 mg/L respectively, leading to the organic removal efficiency at ~ 92% for DOC and ~43% for TN till the end of operation. Although the effluent quality (equivalent to 107-427 mg/L of chemical oxygen demand, COD) could meet the COD requirements (< 600 mg/L) for discharge of trade effluent into the public sewers in Singapore, the biodegradability of the residual organics in the effluent needs to be further evaluated (see Section 3.3).

In contrast, the DOC and TN removal ratios in the MBR1 decreased from 47 to 23% and from 27 to 0%, respectively, within 12-day operation (Figure 2b). In particular, most of DOC/TN removal was attributed to the biological degradation (80-90%), while membrane separation solely accounted for 10-20% of DOC/TN removal. In a further study, the MBR2 was inoculated with the NMP-acclimated sludge derived from the SBR reactor (at day 37). Unexpectedly, the organic removal capability of the NMP-acclimated sludge in the MBR2

dramatically decreased with the operation time (15 days), showing that the overall DOC and TN removal rates in the MBR2 declined from 81 to 23% and from 28 to 0%, respectively (Figure 2c). These findings indicated the failure of conventional aerobic MBRs in treating high-strength NMP-containing process water.

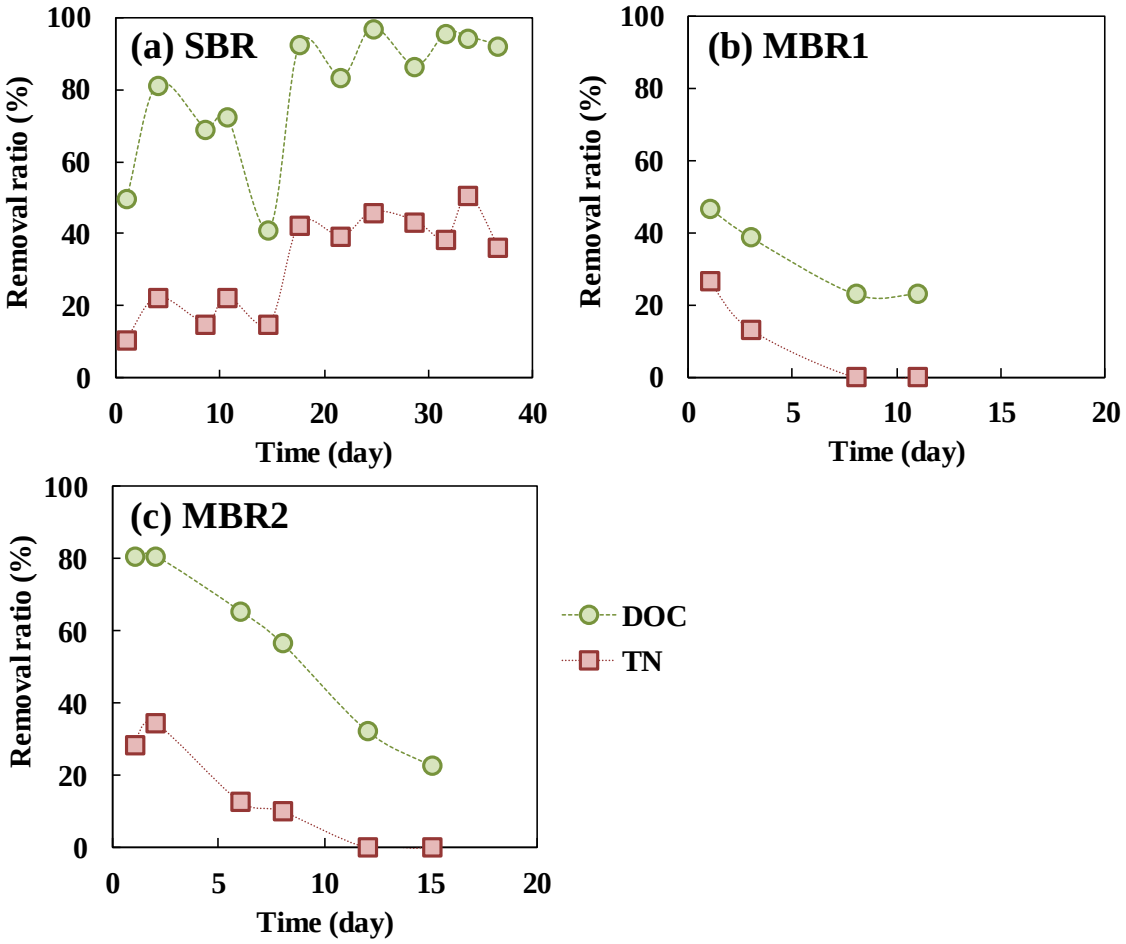

Figure 2. DOC and TN removals in the (a) SBR inoculated with conventional activated sludge; (b) MBR inoculated with conventional activated sludge (MBR1); (c) MBR inoculated with NMP-acclimated sludge (MBR2).

The development of transmembrane pressure (TMP) profiles in MBR1 and MBR2 were recorded respectively as shown in Figure S2. Although the relatively lower permeate flux (6 L/m^2 h) was employed, the membrane fouling was significantly progressive in both MBRs, almost in a two-stage fouling pattern (a slow TMP increase and a sudden TMP jump). In the

MBR1, at the initial stage, the fouling rate reached about 30 kPa/day and then slowly decreased to 5-20 kPa/day. In the MBR2, the membrane fouling rate ranged at 6-11 kPa/day. Nevertheless, these values were greatly higher than those in the conventional activated MBRs treating municipal wastewater [13]. This may be associated with the higher fouling potential of the accumulated organic substances with increased conductivity (Figures 1 and 2) and increased MLSS concentration in the MBRs (Figure S1) [14]. Although NMP and NMP metabolism intermediates have smaller sizes than the membrane pore sizes (0.1 μm), it could be adsorbed on the microbial flocs to change microbial floc properties or adsorbed on membranes (pore blocking or pore narrowing), which led to increased membrane fouling potential.

MBRs have been widely applied in high strength industrial wastewater treatment [15,16]. Compared to SBRs, MBRs experience less shock loading and can retain more biomass that benefit to biodegradation of the organics. However, our findings revealed that SBR was more feasible than MBR to treat the high-strength NMP-containing wastewater. In previous studies, NMP had proven to be readily biodegraded by air-dried activated sludge [1] or pure cultures (such as *Pseudomonas*, *Paracoccus*, *Acinetobacter* and *Rhodococcus*) isolated from the activated sludge [2,8]. Therefore, the high biodegradability of NMP compounds guarantees no shock loading to the SBR in treating high strength NMP-containing wastewater. On the other hand, it is noted that the SBR was operated under an intermittent aerobic/anoxic (during settling period) condition, while the MBR was operated at a continuous aerobic condition. The presence of anoxic situation may lead to developing certain microbial species that can efficiently utilize NMP and NMP biodegradation intermediates.

3.2. NMP degradation

To explore the NMP biodegradation capability of the activated sludge in the SBR and MBR2, the dynamic changes of NMP concentration in both reactors were monitored. Figure 3a indicates that almost 98% of NMP was utilized by the activated sludge (after 37 day-acclimation) in the SBR within 2 h. Accordingly, 84% of DOC and 36% of TN were removed in the SBR. After 3 h, it was found that almost no NMP was detected and around 90% of DOC and 40% of TN were removed. This indicates the feasibility of SBR in treating high-strength NMP-containing process water at a very short HRT. Accordingly, the reduced footprint and improved NMP treatment capacity of SBR could be achieved. It was noted that the residual soluble organics (10% of DOC and 60% of TN) in the SBR effluent could be attributed to NMP degradation metabolites or extracellular polymeric substances excreted by microorganisms, which will be discussed in the next section.

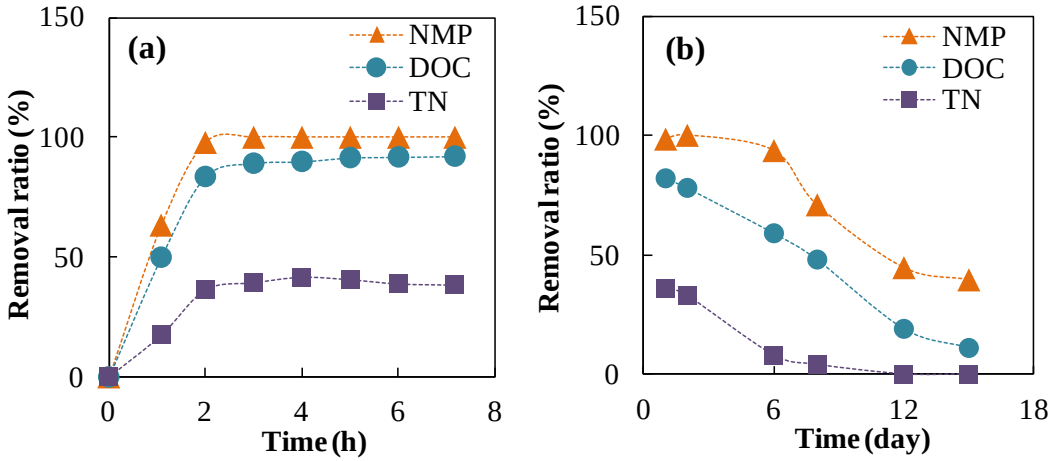

Figure 3. NMP degradation by the activated sludge in (a) SBR (on Day 37) and (b) MBR2.

In the MBR2 (inoculated with acclimated NMP-degrading sludge), NMP was completely degraded in the first two-day, after which the degradation efficiency of NMP decreased with the operation time (Figure 3b). At the end of MBR2 operation (day 15), it was found that only ~40% of NMP was degraded in the reactor, while merely 10% of DOC and almost no TN were removed by the activated sludge in the MBR2. This suggests that the activated sludge in the MBR2 not only exhibited decreasing NMP-degrading capability, but also lacked

of capability in utilizing the accumulated NMP metabolites. The reasons that led to different behaviours of SBR and MBR in treating high-strength NMP-containing wastewater will be illustrated in the following sections.

3.3. NMP degradation pathways and metabolites

It is noted that there was still ~10% of feed DOC remained in the SBR effluent, even when the NMP in the wastewater was completely biodegraded by the NMP-acclimated sludge in the reactor (Figure 3a). To further illustrate the organic composition of SBR effluent, HPLC analysis of the SBR effluent was performed. Figure 4a reveals that the NMP peak (at a retention time of 5.1 min in the chromatogram of HPLC) decreased in height as NMP biodegradation progressed. The intensities of other peaks at shorter retention times (such as at 3.3 min and 3.9 min) were observed when NMP peak almost disappeared (Figures 4a and b).

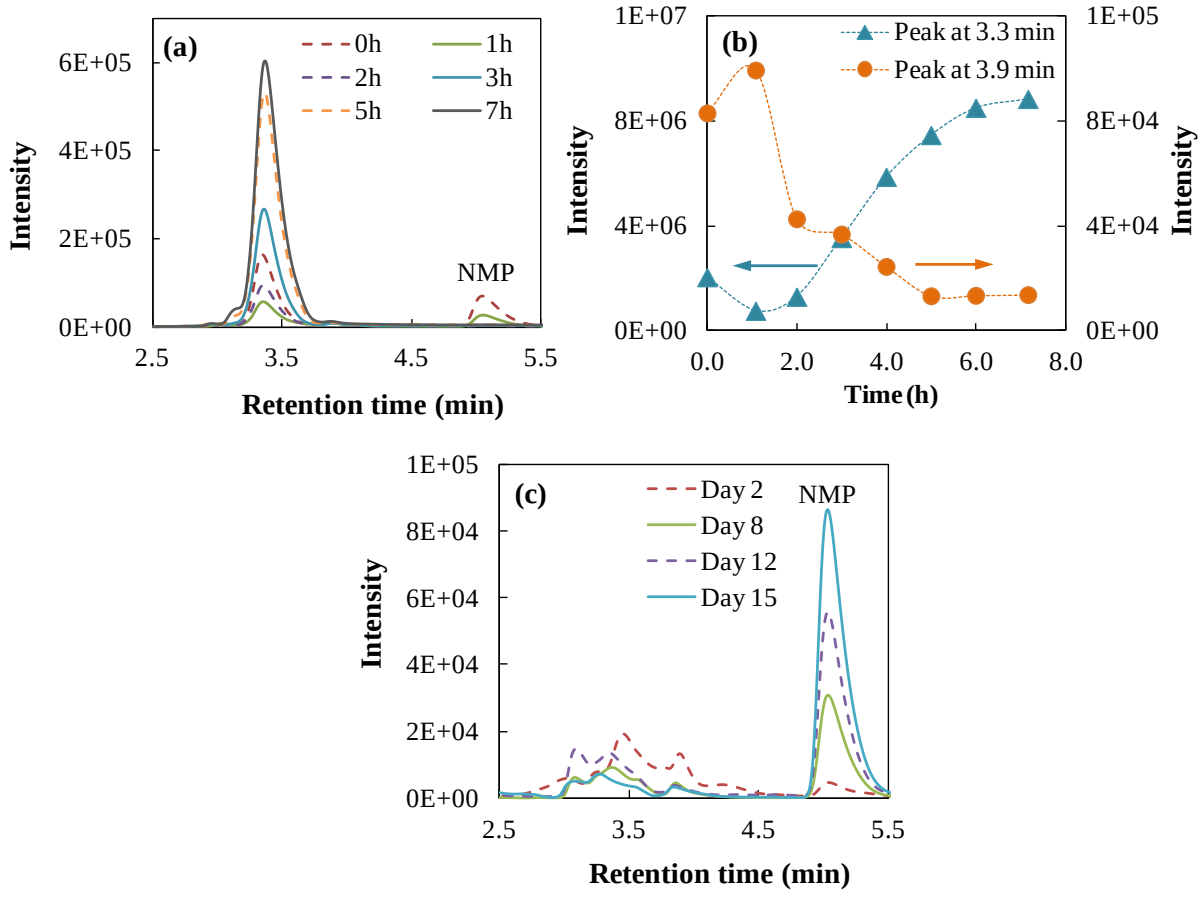

Figure 4. (a) HPLC chromatograms in the SBR (on Day 37), (b) selected HPLC peak profiles in SBR (on Day 37), and (c) HPLC chromatograms in the MBR2.

In the MBR2, within 15-day operation, the NMP removal ratio of the activated sludge dropped from 100% to 40%. Accordingly, merely 11% of DOC and almost no TN were removed by the activated sludge at the end of operation (Figure 3b). The supernatant was separated from the mixed liquor taken from the MBR at different operation periods (Day 2, 8, 12, and 15), and the organic compositions of the supernatant was analyzed by HPLC. The HPLC chromatograms (Figure 4c) reveal that with the accumulation of NMP (peaks at a retention time of 5.1 min) in the reactor, several other components (peaks at a retention time of 3-4 min) shifted significantly and the concentrations of these components (i.e., the intensities of these peaks) varied. For example, at Day 3, when 94% of NMP was removed in the MBR2 (Figure 3b), two obvious peaks occurred at retention times of 3.5 min and 3.9 min in the HPLC chromatogram profile of the supernatant sample. At Day 15, merely 40% of NMP was degraded (Figure 3b), accordingly, the peaks at retention times of 3.0, 3.3, and 3.8 were observed in the HPLC chromatogram profile of the supernatant sample from the MBR2.

To identify the substances associated to the peaks observed from HPLC, further LC-MS/MS analysis was performed, and the detailed mass-mass spectra of peaks (Figures S3-S6) and discussion can be found in Supplementary data. Table 1 lists the detected NMP degradation metabolites in the SBR and MBR2 by LC-MS/MS analysis. The results further verify that the peak at the retention time of 5.1 min detected by HPLC corresponds to NMP (Figure 4). The peaks at the retention times of 3.0, 3.3, 3.4 min, and 3.9 min were likely to be 2-pyrrolidinone (2P), N-methylsuccinimide (NMSc), Succinimide (Sc), and 5-hydoxy-N-methyl-2-pyrrolidone (5-HNMP), respectively.

Table 1. Identification of NMP degradation metabolites by LC-MS/MS analysis

Retention time in LC (min)	Ionization	Molecular mass (m/z)	Identification	Presence
3.0	+ H^+	86	2-pyrrolidinone (2P)	SBR & MBR2
3.3	+ H^+	114	N-methylsuccinimide (NMSc)	SBR & MBR2
	+ Na^+	136		SBR
3.4	+ H^+	100	Succinimide (Sc)	MBR2
3.9	+ H^+	116	5-hydoxy-N-methyl-2-pyrrolidone (5-HNMP)	SBR
5.1	+ H^+	100	N-methylpyrrolidone (NMP)	SBR & MBR2

In the SBR, 2P, NMSc, and 5-HNMP were identified by LC-MS/MS, while in the MBR2, 2P, NMSc, and Sc were identified by LC-MS/MS. The intermediates such as 2P, 5-HNMP and Sc are electrolyte compounds and their presences were thought to be related to the increased conductivity (Figure 1). Based on the present compounds in the SBR and MBR2, we proposed the plausible biodegradation pathways as illustrated in Figure 5. In detail, (1) NMP was degraded to 2P, which was further degraded to Sc; (2) NMP was degraded to NMSc, which was further degraded to Sc; (3) NMP was degraded to 5-HNMP, which was further degraded to NMSc and then to Sc. Sc was an important intermediate compound with easily biodegraded property, which can be converted to other amine products [4]. The proposed pathways are agreeable with those in a previous study involving NMP degradation by photocatalytic degradation [17].

In SBR, these three plausible biodegradation pathways may be involved due to the presence of 2P, NMSc, and 5-HNMP in the SBR effluent. For example, as NMP degradation progressed with time, the intensity of 5-HNMP peak (retention time at 3.9 min) started to increase and reached a maximum at about 1 h. After that, a decreasing pattern with extending

operation time was noticed (Figure 4b). The intensity of NMSc peak (retention time at 3.3 min) started to increase while that of 5-HNMP peak decreased (Figure 4b). The observation clearly revealed that NMP was first degraded to 5-HNMP, followed by conversion to NMSc. In MBR2, the pathways (1) and (2) may occur because MBR2, 2P, NMSc, and Sc were found in the mixed liquor of the MBR2. In this study, as these NMP degradation intermediates were not quantified, the dominant biodegradation pathway in the SBR and MBR2 cannot be identified. However, different NMP degradation behaviors were observed in the SBR and MBR2 (Figures 3 and 4) and the biodegradability of the NMP degradation intermediates were greatly different [3], therefore we hypothesized that the dominant NMP degradation pathway may be dissimilar for SBR and MBR2, which resulted in the accumulation of different intermediate products in the reactors. Further quantification of the NMP degradation intermediates will be conducted in future study.

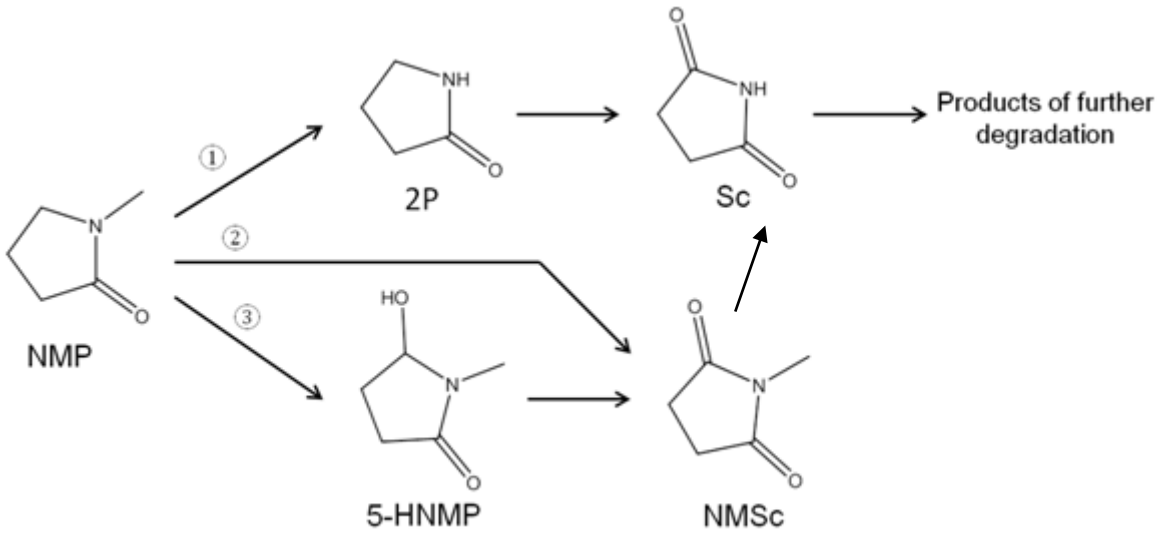

Figure 5. Proposed biodegradation pathways of NMP based on LC-MS/MS analysis.

It is worth noting that the peak intensity of NMSc in the SBR remained at a certain level after 7 h (Figure 4b), indicating that further degradation of NMSc might not be fully complete. This is correspondent with some previous studies that several unidentified metabolites derived from the NMP degradation could not be further biologically degraded [1-3]. These

non-biodegradable intermediates could contribute to the remaining DOC/TN observed in the SBR effluent. To achieve zero discharge of non-biodegradable toxic compounds, it is suggested to remove the remaining DOC by further treating the SBR effluent with oxidation or adsorption techniques.

In the feed, NMP was the sole nitrogen source provider and the C/N mole ratio in NMP is 5 to 1. Such a relatively lower C/N ratio for the microbial growth led to excess nitrogen remaining when the carbon source was almost consumed in the SBR. In addition, the other LC peaks observed in the MBR2, including the peaks at retention times of 2.9, 3.5, 3.6, and 3.8 min, were proposed to be endogenous bacterial metabolites that were not related to NMP degradation metabolites (Figures S4), possibly generated due to the long solid retention time (SRT) of the MBR2.

3.4. Activated sludge characteristics

Since both SBR and MBR2 performed different NMP degradation capabilities, the activated sludge characteristics relating to NMP degradation in the SBR and MBR2 were further analyzed. Figure 6 illustrates the amount of the viable cells per MLSS (presented by the intercellular ATP in MLSS) in the reactors. As SBR supernatant was periodically decanted from the reactor, only easily settled activated sludge tended to remain in the system. The removal of the sludge with a poor settling ability helped to maintain a stable sludge system. This is well supported by the relatively stable viable cells per MLSS in the SBR. In the MBR2, no sludge was periodically removed from the reactor (i.e., SRT of infinity) and increased MLSS was observed (Figure S1). However, the amount of viable cells per MLSS dramatically dropped with operation time, which is correspondent well with NMP degradation and DOC/TN removal profiles (Figure 3). This suggests that the microbes capable of degrading NMP in the SBR did not gain competitive advantages in MBR2 (noted:

the sludge in MBR2 was taken from SBR). Since NMP degradation metabolites had been reported to exhibit some degree of toxicity, the lower NMP-degrading capability of microbes in the MBR2 might be attributed to the accumulation of more toxic NMP metabolites [4].

A comparison of soluble and attached EPS (mainly protein and polysaccharides) in both reactors through the entire reactor operation was performed and the averaged data were shown in Figure 7. The EPS are excreted by many microorganisms, and are normally attached to the cell surface, building up a barrier to protect the cells [18]. Comparable soluble protein and polysaccharides in both reactors were found, while the attached protein and polysaccharides per viable cells in the SBR were 3.7 and 2.6 times of those in the MBR2, respectively. The increased EPS excretion could be considered as metabolic function regulations of the microbial communities in order to adapt to the varied conditions [10]. This result indicates that the activated sludge in the SBR had evolved excellent mechanisms to protect themselves from NMP metabolites while maintaining their normal intracellular functions (i.e., NMP degradation).

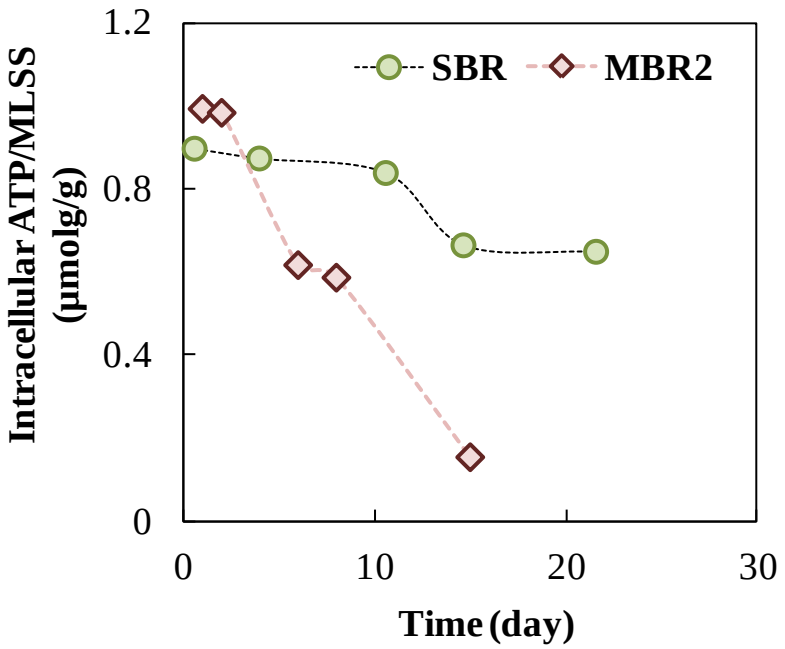

Figure 6. Viable cells per MLSS in the SBR and MBR2 (two measurements for ATP and MLSS; averaged data are presented in the figure).

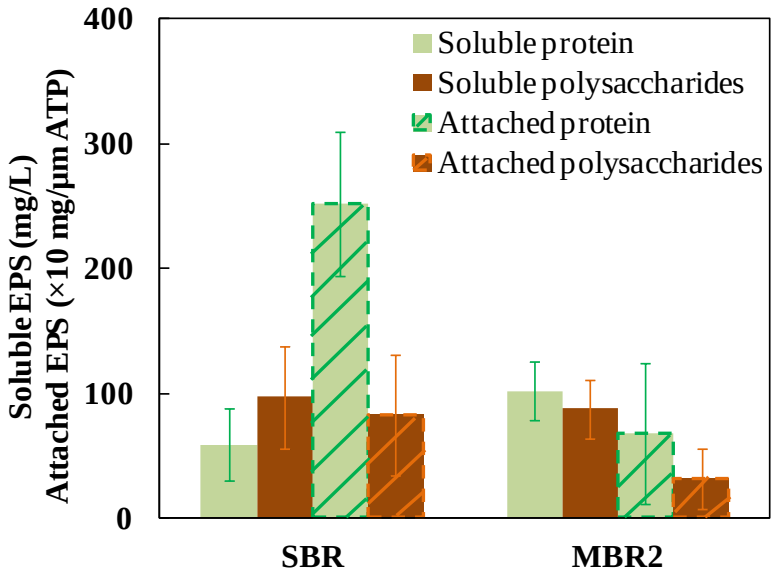

Figure 7. Soluble and attached EPS in the SBR (n=12) and MBR2 (n=6).

Furthermore, the prokaryotic community structures in the SBR and MBR2 were illustrated by sequencing the16s rRNA genes extracted from the activated sludge samples at the end of the reactor operation. The detailed microbial community structures at various taxonomic levels (phylum, class, and genus) are shown in Figures 8a-c. In detail, more than 80% of bacterial sequences were related to proteobacteria and bacteroidetes phyla for both SBR and MBR2, in which proteobacteria phylum was predominant (61% in SBR and 42% in MBR) (Figure 8a). At class level, β-proteobacteria was the most predominant in both SBR (14.4%) and MBR (22.5%), and Sphingobacteria, α-proteobacteria, and γ-proteobacteria also showed their dominance (>10%) in both reactors (Figure 8b). At the genus level, the microbial communities in both reactors were highly diversified and composed of more than 300 OTUs, but in total ~ 100 species were present at abundance higher than 0.1%. The species that were present in the SBR (total 55 species), but present at a lower abundance or absent in the MBR2, were shown in Figure 8c.

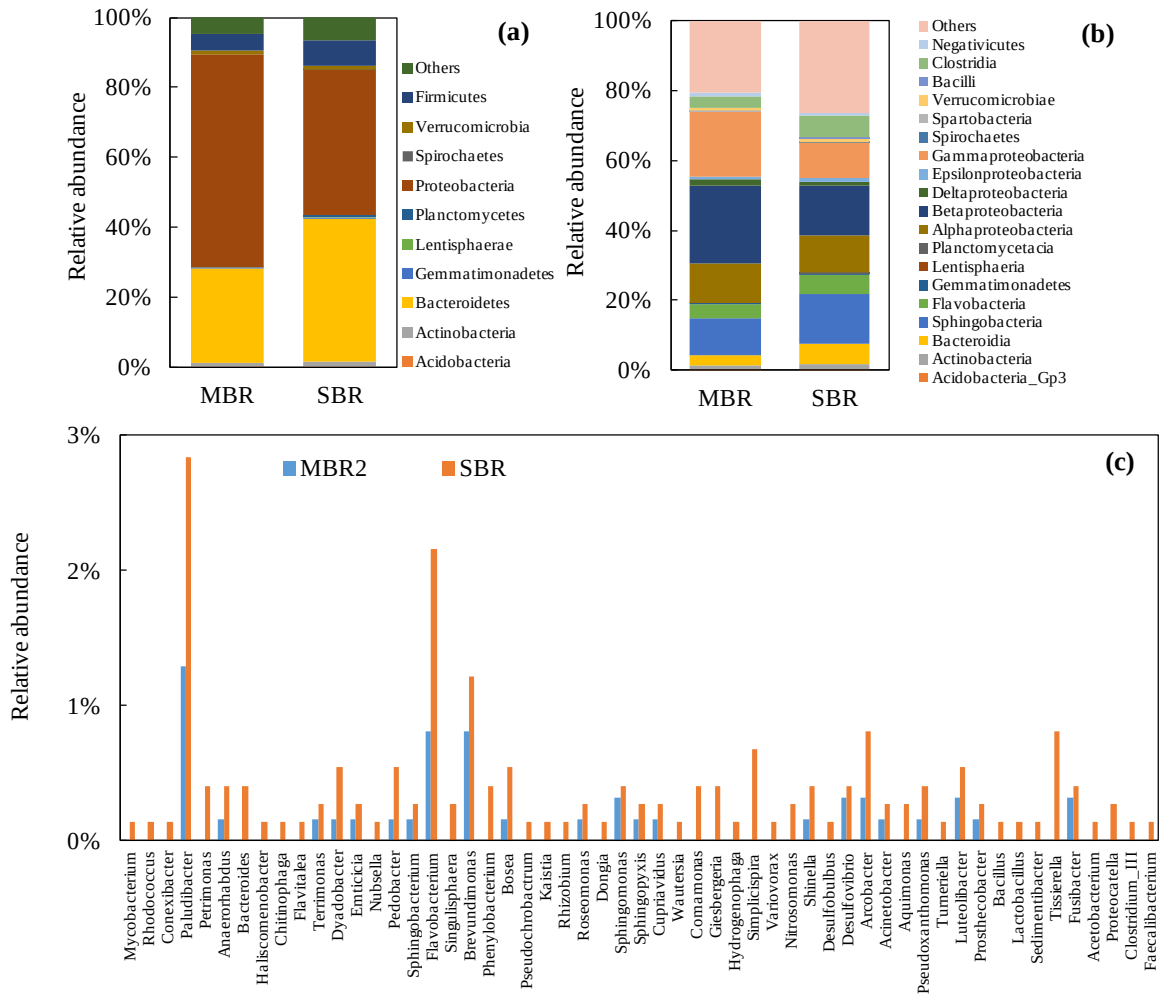

Figure 8. Microbial community structures in the SBR and MBR2. (a) at phylum level; (b) at class level; (c) at genus level.

It had been reported that *Pseudomonas*, *Paracoccus*, *Acinetobacter*, *Cupravidus*, and *Rhodococcus* derived from the activated sludge could mineralize NMP [2,8]. In this study, these species were also found in both reactors. In particular, higher abundances of *Pseudomonas* (0.81% in the MBR2; 0.68% in the SBR) and *Paracoccus* (0.16% in the MBR2; 0.14% in the SBR) in the MBR2 and relatively higher abundances of *Acinetobacter* *Cupravidus*, and *Rhodococcus* in the SBR (Figure 8c) were noticed. Although NMP-degrading species were present in the MBR2, these species did not perform a significant role in NMP degradation. In addition, the differences of bacterial communities between the SBR

and MBR2 might explain the different NMP degradation pathways between the two reactors as revealed in the previous section.

Interestingly, when the activated sludge derived from the MBR2 was operated at a SBR mode (i.e., batch operation mode with an anoxic condition during settling period), almost 100% of NMP and above 90% of DOC could be removed in the reactor after 4-day operation (Figure S7). This finding implies that the NMP degradation capabilities of *Pseudomonas* or other bacterial genus derived from MBR2 could be re-activated in the suitable conditions, such as a batch operation mode with an intermittent aerobic (11 h)/anoxic (1 h) condition. This implies that operation mode, especially oxygen supplier mode, may play a critical role in influencing NMP degradation. Further study on (1) the oxygen requirements for mineralization of NMP and NMP degradation metabolites and (2) the functional enzymes for degradation of NMP and its metabolites will be carried out to further clarify the detailed NMP degradation pathways.

5. Conclusions

In this work, the feasibility of treating the high-strength NMP-containing wastewater from membrane fabrication process by SBR and MBR was investigated. The SBR showed a promising performance with over 90% DOC removal and almost 100% NMP removal at a short HRT of 2 h after 37-day acclimation. However, the MBR had a decreasing DOC and NMP removal efficiencies over time, even when inoculated with NMP-acclimated sludge. It is believed that the different operating modes and oxygen supply conditions in the SBR and MBR could influence bacterial community structures and dominant NMP degradation pathways, which led to their different NMP degradation behaviours. From this study, it was concluded that SBR was significantly more efficient in treating high-strength NMP-containing wastewater (1500–1600 mg/L). The SBR process was suggested to be followed

with an oxidation or adsorption process to further remove the remaining non-biodegradable NMP metabolites (~10% of DOC) in the SBR effluent, so that a zero discharge of toxic compounds could be achieved.

Acknowledgements

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Supplementary data:

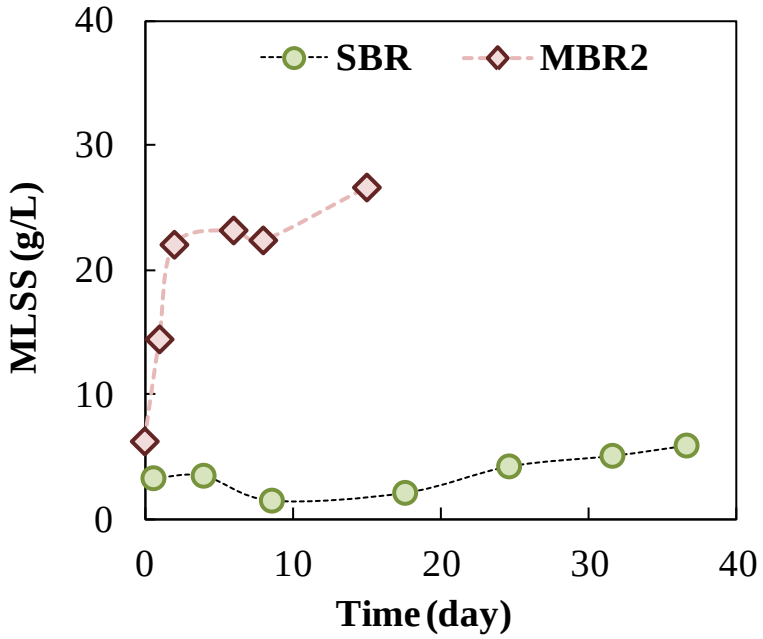

Figure S1. MLSS in SBR and MBR2.

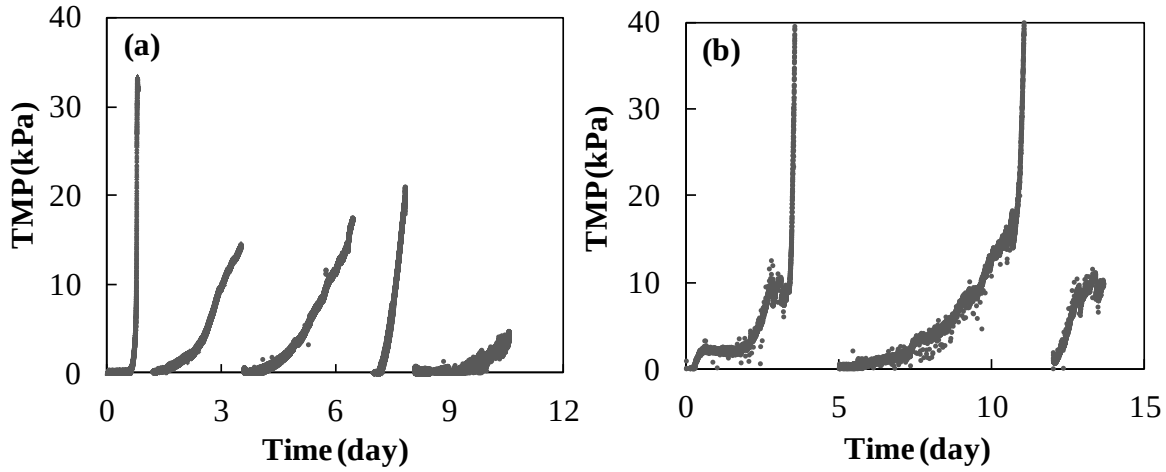

Figure S2. TMP profiles in the (a) MBR1 (inoculated with conventional activated sludge); (b) MBR2 (inoculated with NMP-acclimated sludge).

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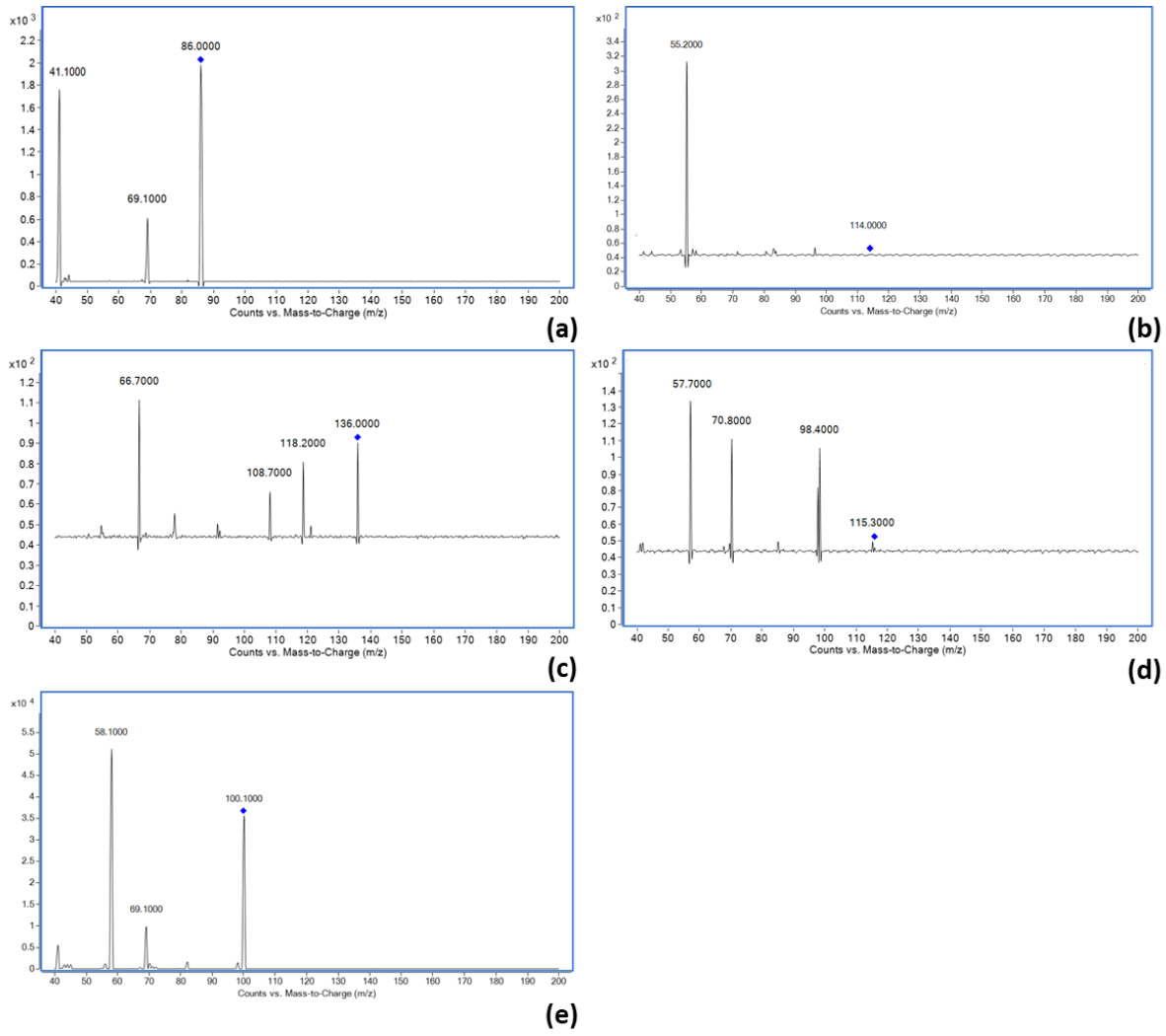

486

487 **Figure S3. Mass-mass spectra of peaks at (a) 3.0 min with precursor ion m/z 86, (b) 3.3**
488 **min with precursor ion m/z 114, (c) 3.3 min with precursor ion m/z 136, (d) 3.9 min with**
489 **precursor ion m/z 116, and (e) 5.1 min with precursor ion m/z 100 for SBR samples in**
490 **positive ion mode.**

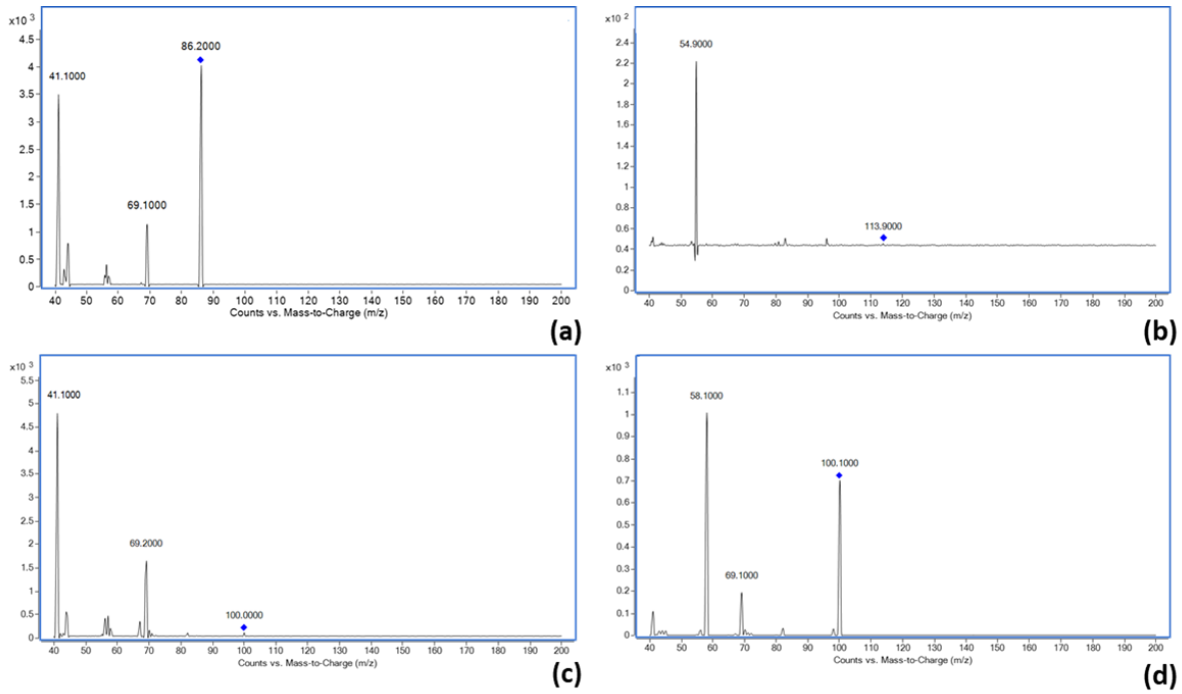

Figure S4. Mass-mass spectra of peaks at (a) 3.0 min with precursor ion m/z 86, (b) 3.3 min with precursor ion m/z 114, (c) 3.4 min with precursor ion m/z 100, and (d) 5.1 min with precursor ion m/z 100 for MBR2 samples in positive ion mode.

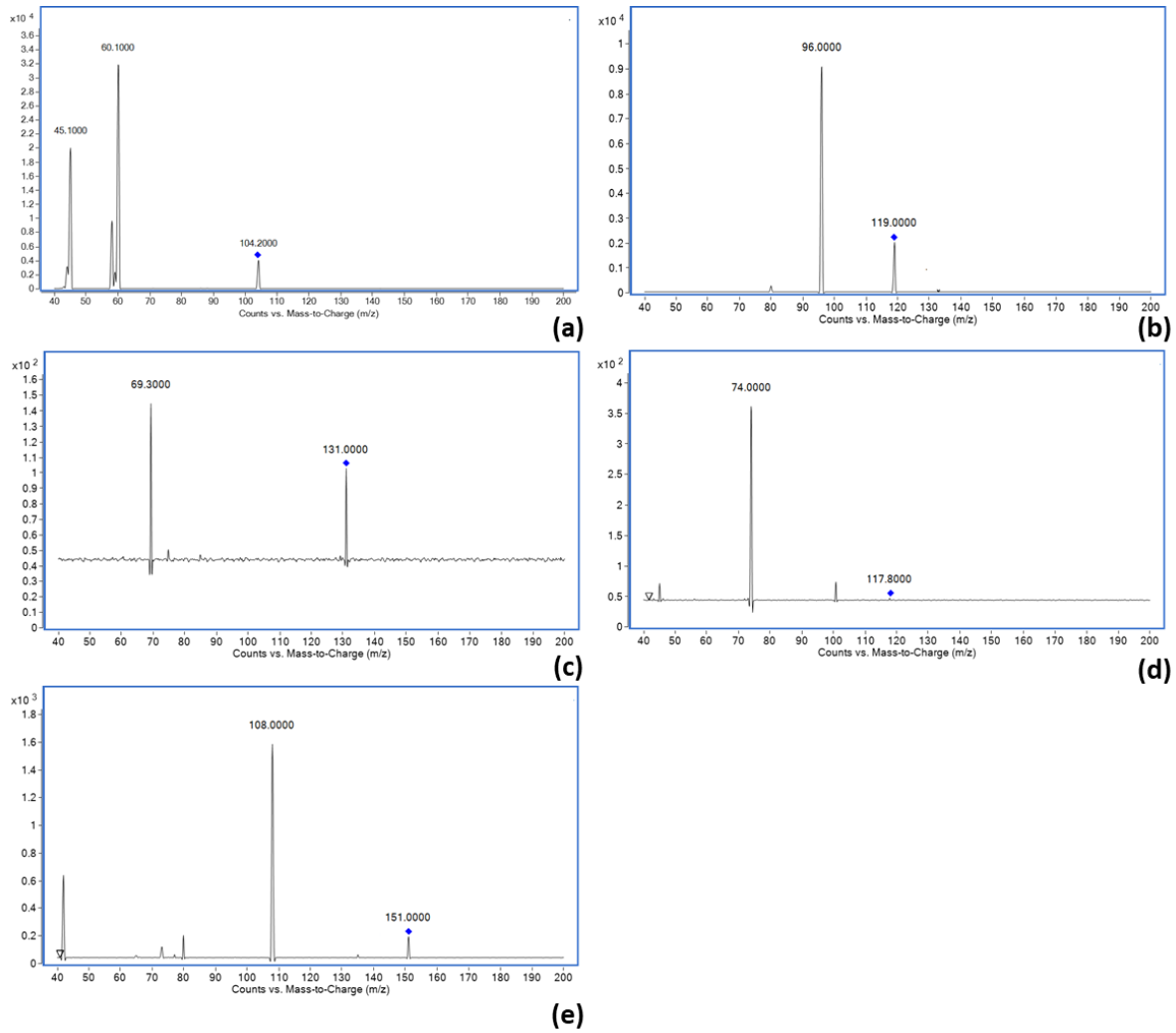

Figure S5. Mass-mass spectra of peaks at (a) 3.1 min with precursor ion m/z 104 in positive ion mode, (b) 2.9 min with precursor ion m/z 119 in negative ion mode, (c) 3.5 min with precursor ion m/z 131 in negative ion mode, (d) 3.6 min with precursor ion m/z 118 in negative ion mode, and (e) 3.8 min with precursor ion m/z 151 in negative ion mode for MBR2 samples.

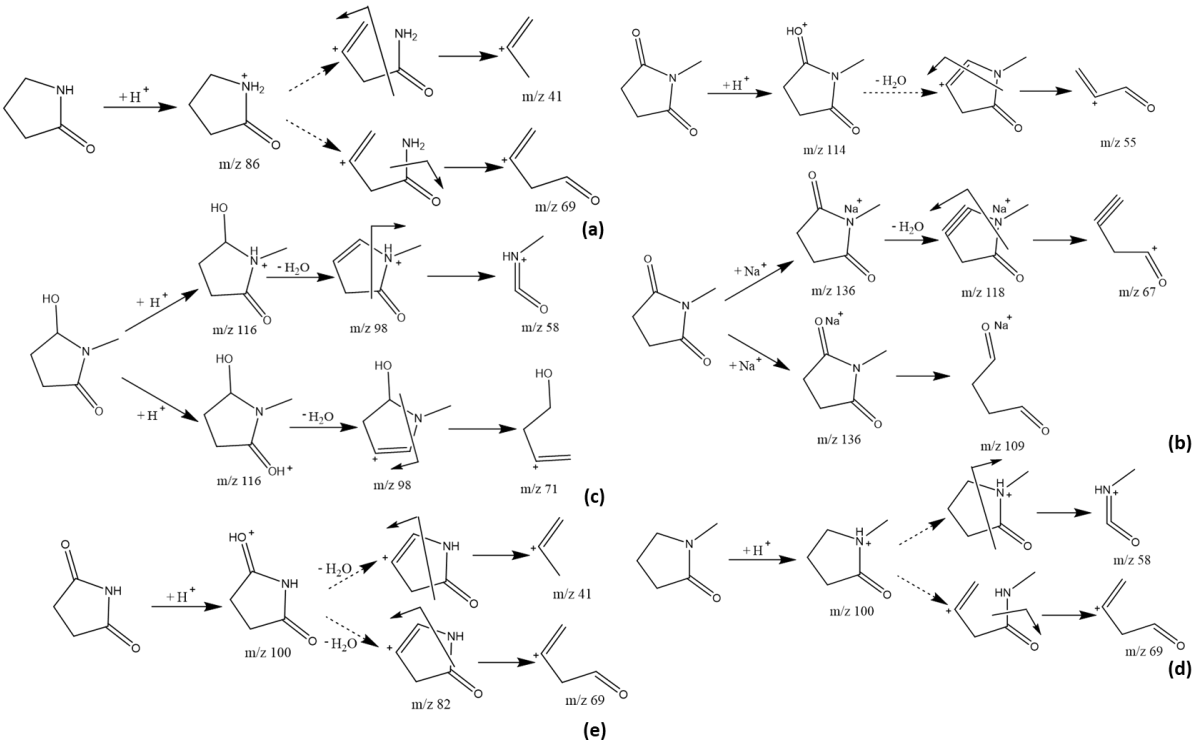

Figure S6. Proposed MS/MS fragmentation pathways of (a) 2-pyrrolidinone; (b) *N*-methylsuccinimide; (c) 5-hydoxy-*N*-methyl-2-pyrrolidone; (d) *N*-methylpyrrolidone; (e) succinimide.

Detailed description for LC-MS/MS analysis

For the SBR samples, the related high abundant precursor ions were identified as m/z 86 for the peak at 3.0 min, m/z 114 and 136 for the peak at 3.3 min, m/z 116 for the peak at 3.9 min and m/z 100 for the peak at 5.1 min by LC-MS in positive mode. To further characterize the chemical structures of the detected compounds, m/z 86, 114, 136, 116 and 100 were selected to serve as precursor ions for LC-MS/MS analysis in positive mode. Figure S3 illustrates MS/MS spectra of different precursor ions for SBR samples. The peak at 3.0 min with the precursor ion of m/z 86 was highly suspected to be 2-pyrrolidinone (2P), in which product ion m/z 69 was matched with literature [19]. Proposed MS/MS fragmentation pathway of 2P (Figure S5a) was used for further supporting the identity of peak at 3.0 min. As m/z 114 and 136 could be the $[M+H]^+$ and $[M+Na]^+$ ions for *N*-methylsuccinimide (NMSc), the peak at 3.3 min was suspected to be NMSc. Both m/z 114 and m/z 136 shown in mass spectrum of

517 the peak at 3.3 min, were used as the precursor ions for further MS/MS experiments. The
518 comprehensive fragmentation pathways of NMSc (Figure S6b) were used for further
519 supporting the identity of peak at 3.3 min. The peak at 3.9 min was highly suspected to be 5-
520 hydoxy-*N*-methyl-2-pyrrolidone (5-HNMP), which was supported by proposed MS/MS
521 fragmentation pathway (Figure S6c). The peak at 5.1 min was confirmed to be NMP, in
522 which both MS/MS fragmentation pattern and retention time were matched with standard.
523 Nevertheless, the MS/MS fragmentation pathway (Figure S6d) was proposed as a reference
524 for some fragments generated by other suspected m/z NMP by-products.

525 Compared to the SBR samples, the MBR2 samples have much more complicated LC-MS
526 profiling. The peak at 2.3 min was suspected to be neutral molecule(s), which has no MS
527 signal in both positive and negative modes. For positive mode, the related high abundant
528 precursor ions were identified as m/z 86 for the peak at 3.0 min, m/z 104 for the peak at 3.1
529 min, m/z 114 for the peak at 3.3 min, m/z 100 for the peak at 3.4 min, and m/z 100 for the
530 peak at 5.1 min by LC-MS. The m/z 86, 104, 114 and 100 were selected to serve as precursor
531 ions for further LC-MS/MS analysis in positive mode for MBR2 samples (Figure S4). Due to
532 similar MS/MS fragmentation patterns as the SBR samples, the peaks at 3.0 min and 3.3 min
533 were suspected to be 2P and NMSc, respectively. However, the related abundance of the
534 suspected 2P in the MBR2 samples was much higher than in the SBR samples. Probably due
535 to the much lower related abundance in MBR2 samples, only m/z 114 was detected as the
536 $[M+H]^+$ ion for the suspected NMSc. Furthermore, the peak at 3.4 min with the precursor ion
537 of m/z 100 was highly suspected to be Sc, which was not detected in SBR samples. Proposed
538 MS/MS fragmentation pathway of Sc (Figure S6e) was used for further supporting the
539 identity of peak at 3.4 min. Meanwhile, the peak at 5.1 min was confirmed to be NMP by
540 both MS/MS fragmentation pattern and retention time. Besides, the peak at 3.1 min with the
541 precursor ion of m/z 104 was suspected to be endogenous bacterial metabolites which are not

542 related to NMP (Figure S5a). Unlike the SBR samples which have no obvious LC-MS signal
543 in negative mode, the related high abundant precursor ions were identified as m/z 119 for the
544 peak at 2.9 min, m/z 131 for the peak at 3.5 min, m/z 118 for the peak at 3.6 min, and m/z
545 151 for the peak at 3.8 min in the MBR2 samples by LC-MS in negative mode. However, all
546 of the peaks detected in negative mode were suspected to be endogenous bacterial
547 metabolites which are not related to NMP (Figure S5b-e).

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550 pyrrolidinone residue: applied to a depletion study of 2-pyrrolidinone in swine liver,
551 Anal. Bioanal. Chem. 399 (2011) 1371-1380.
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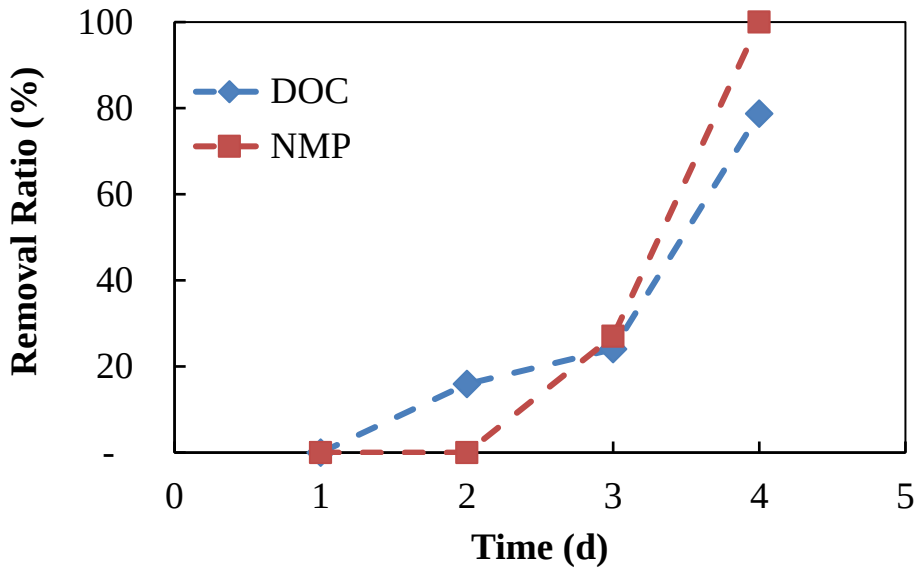

554

555 **Figure S7. DOC and NMP removal ratio in SBR using activated sludge derived from**
556 **MBR2.**

557