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Simultaneous methane production and wastewater reuse by a membrane-based process: Evaluation with raw domestic wastewater

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ABSTRACT

In this study, a membrane-based process was applied to simultaneously reclaim methane and generate reused water from raw domestic wastewater. The system was comprised of up-flow anaerobic sludge fixed bed (UAFB), anoxic sink (AS) and aerobic membrane bioreactor (MBR). The hydraulic retention time of UAFB (HRT_U) was gradually shortened from 8 h to 6 h, 3 h and to 1 h, while the HRT of AS and MBR kept at 8 h. It is found that HRT_U of 3 h was more suitable for the balancing production of biogas and volatile fatty acids (VFAs), and the VFAs served as carbon source for denitrification. The trans-membrane pressure (TMP) of the MBR kept lower than 0.04 MPa without wash or change of membrane sheet, however, the scanning electron microscopy (SEM) analysis indicated that microbes attached to the inner-surface of membrane, causing irreversible fouling after 133-day operation. The denaturing gradient gel electrophoresis (DGGE) profiles of amplified 16S rDNA gene fragments proved that more functional bacteria and higher microbial diversity emerged at HRT_U of 3 h and 1 h. Most bacteria belonged to *Betaproteobacteria* and were responsible for carbon and nitrogen removal.

1. Introduction

For the past decades, a growing number of contaminants have been entering water supplies from human activity, causing prevalence of polluted seas or lakes in both developing and industrialized nations. In China, the most recent National Pollution Census indicated that the municipal and agricultural wastewater have surpassed industrial wastewater in total amount [1]. Considering both municipal and agricultural wastewater is nutrient-rich wastewater in enormous amount, the bio-energy and reuse-potential contained in it are also huge.

Anaerobic process is recognized as a promising technology for energy reclaiming from wastewater [2–4]. Based on the concept of immobilization, an up-flow anaerobic fixed bed (UAFB) has many advantages including compact structure, great sludge retention capability, strong tolerance to hydraulic or quality shock of wastewater, providing satisfactory conditions for microorganisms [5,6], and hence, is a feasible approach for domestic wastewater treatment. In addition, capturing water directly from non-traditional sources such as industrial or municipal wastewaters and restore it to potable quality is predicted to be an overarching goal for the future reclamation and reuse of wastewater [7]. A technology now actively being pursued is membrane bioreactor (MBR) [8–10], which allows high mixed liquor suspended solids (MLSS), enables high removal of organic matter and low production of excess sludge. Novel domestic wastewater treatment approaches that are resource-conserving and environmentally friendly may prevail in the coming decades. Hitherto, most studies and full-scale applications on domestic wastewater treatment aimed at meeting immission standards for receiving aqueous systems, while attempts on simultaneous biogas production and water reuse from domestic wastewater were rarely reported.

The objectives of this study were: (1) to investigate the capability of a membrane-based process to simultaneously produce biogas (i.e. methane), generate reused water and remove nutrient from raw domestic wastewater; (2) to assess the impact of hydraulic retention time of UAFB (HRT_U) on the system, including nutrient (organic matter, ammonia and nitrate) removal rate, methane productivity and volatile fatty acids (VFAs) productivity; (3) to assess the response of the MBR triggered by changing HRT_U, including membrane fouling, sludge reduction and the shift of bacterial community structure.

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Table 1
Raw and synthetic domestic wastewater characteristics.

Parameter	Concentration (average and range)
COD_{tot} (mg/L)	268.5 (110.8–406.8)
COD_{dis} (mg/L)	210.1(63.6–334.8)
BOD (mg/L)	147.9 (63.1–241.7)
Ammonia (mg/L)	45.6 (30.8–62.9)
Alkalinity (mg $CaCO_3$/L)	281.1 (122–350)
pH	7.1 (6.7–7.3)

COD_{tot}, amount of total COD in the tested sample (mg COD/L); COD_{dis}, amount of dissolved COD in the tested sample (mg COD/L).

2. Materials and methods

2.1. Reactor design

The combined system was consisted of a series of reactors: a UAFB, an anoxic sink (AS) and an aerobic MBR (Fig. 1). Cylindrical UAFB (reaction zone diameter 90 mm and height 800 mm) was packed with plastic circular rings (diameter 10 mm), and had an effective volume of 6.0 L. The bottom half of the reactor was designed as a fixed-film section, comprising randomly packed polyethylene ring-shaped matrix pieces. The UAFB was equipped with a temperature sensor and a water heating system to maintain the temperature of 35 °C. Effective volume of the AS was 8.0 L. A mechanical stirrer kept completely mixing the sludge in AS. The aerobic MBR had an effective volume of 8.0 L. The membranes used in the module were polyethylene (PE) hollow fibre sheets (Mitsubishi Rayon Co., Ltd., Tokyo, Japan) with a mean pore size of 0.4 μm. The designed membrane flux was 0.27 m^3/day.

2.2. Operation and start-up

2.2.1. Inoculation

UAFB reactor was inoculated with 4 L of mesophilic anaerobic sludge, with an initial volatile suspended solids (VSS) content of 15 g/L. AS and MBR were both inoculated with activated sludge from a bench-scale anaerobic–anoxic–aerobic (A^2O) reactor treating raw domestic wastewater.

2.2.2. Domestic wastewater

The domestic wastewater was daily harvested from a septic tank located in a community. The value of raw domestic wastewater's COD and ammonia concentration fluctuated every day, dramatically sometimes (Table 1).

Since the effective volume of UAFB was 75% of that of AS and MBR, the amount of UAFB effluent could not match AS/MBR influent at HRT = 8 h. Thus, the UAFB effluent was not directly fed to AS/MBR but pre-mixed with a part of (25%) synthetic domestic wastewater in a 50 L tank (not shown in Fig. 1). The synthetic wastewater contained the following components: sodium acetate, urea, NH_4Cl, K_2HPO_4, $MgSO_4$, $CaCl_2$, and $FeCl_3$). The amount of each component was adjusted to make sure that the COD of mixed domestic wastewater was in the same level as the raw wastewater (see Table 1).

2.2.3. Operating conditions

HRT_U was shortened stepwisely from 10 h (start-up period) to 8 h (for 25 days), 6 h (for 18 days), 3 h (for 59 days) and, finally 1 h (for 29 days).

Reflux ratio of AS and MBR was set at 2.5. MBR effluent pump worked every 3 min followed by 1 min relaxation, controlled by a time-relay. Intensive aeration was applied to the module to delay membrane fouling, leading to dissolved oxygen (DO) in MBR about 6.0 mg/L. Two identical membrane modules were used alternately. There was no excess sludge removed from either UAFB or AS/MBR during the whole experimental trial, except for sampling for MLSS and other chemical analysis.

2.3. Analytical methods

2.3.1. Chemical analysis

COD, ammonia, nitrite, nitrate and MLSS were measured according to the standard methods [11] to evaluate performance of the combined process. Parameters including oxidation reduction potential (ORP) and DO were tested by Handheld Multi-Parameter Instruments (pH/Oxi 340i, WTW, Germany).

2.3.2. VFAs analysis

The concentration of acetic acid, propanoic acid, butyric acid and valeric acid in effluent was determined by gas chromatography (HP7890 Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (GC-FID). The GC was fitted with a capillary column (19095N-123 HP INNOWX). The temperature of column, the injector port and the detector was 70, 250 and 300 °C, respectively. The carrier gas was nitrogen at a flow rate of 10 mL/min and a split flow of 40 mL/min.

2.3.3. Biogas analysis

Biogas content (methane, carbon dioxide and hydrogen) was determined by gas chromatography as described before [12]. For the methane dissolved in effluent, a calculation based on the Henry's law was applied to compensate the underestimation of total methane production, considering methane in effluent was saturated. The solubility constant for each temperature period was dependent on methane content in biogas and experience data obtained from the literature [13].

2.3.4. SEM analysis

Both virgin and fouled membrane sheet were directly mounted on carbon tape and sputter coated in 20 nm gold with an Emitech K550 Sputter Coater. A Hitachi S-4700 SEM (Tokyo, Japan) was used to capture micrographs.

2.3.5. PCR-DGGE and 16S rDNA analysis

The biomass for bacterial population analysis was sampled from MBR at HRT = 6 h, 3 h and 1 h, respectively. Specific bacterial primer GC-338 (primer 338 plus a GC clamp attached at its 5′-end) and a reverse universal primer 518 supplied by Shanghai Songon Biology Engineering Technology & Services Co. Ltd. (China), were used in this study to amplify bacterial 16S rDNA. The nucleotide sequence of the primers was as follows: primer GC-BSF, 5′-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCGCCTACGGGAGGCAGCAG-3′; primer 915, 5′-ASTACCGCGGCTGCTGG-3′. Genomic DNA extraction and PCR conditions were the same as described previously [14] except that the annealing temperature of the touch-down PCR was 65–56 °C. PCR products were verified in 1% agarose gel DGGE analysis of PCR products was performed with a Bio-Rad D-Code System (Bio-Rad Laboratories, Mississauga, Ontario, Canada). PCR samples were concentrated and 300 ng were loaded onto an 8% (w/v) polyacrylamide gel containing a 30–60% gradient of denaturant (80% denaturant correspond to 5.6 M urea and 32% (v/v) deionized formamide). Bands of interest were reamplified, purified and sequenced using the Gel Recovery Purification Kit (Watson Biotechnologies Inc., Shanghai, China) according to the manufacturer's instruction. The DNA sequences were determined using the chain termination method in an ABI 3730 stretch sequencing system by a commercial service (Sangon, China), and submitted for comparison to GenBank database using BLAST algorithms.

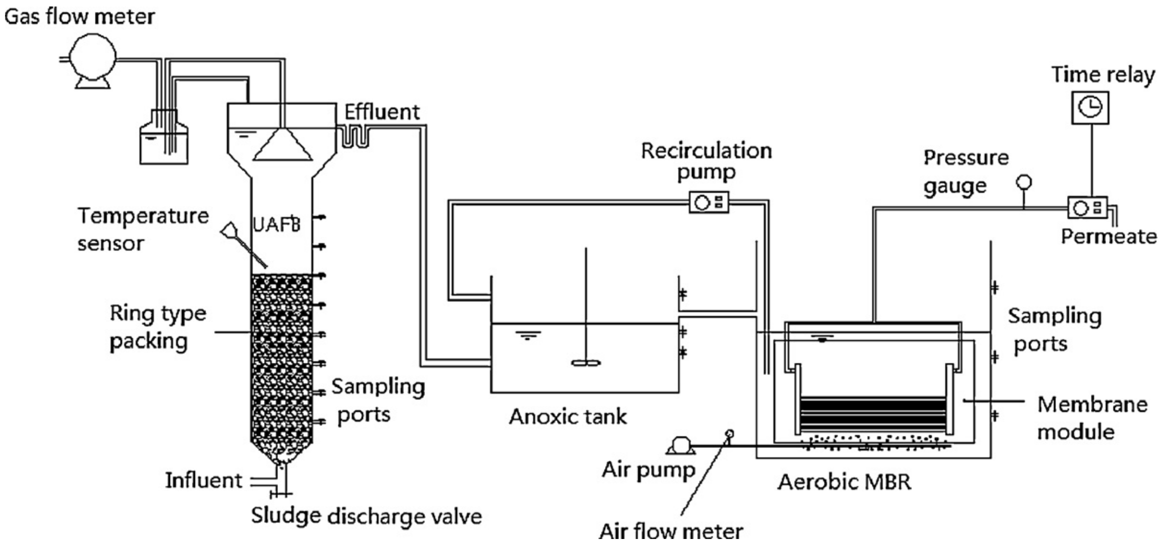

Fig. 1. Schematic diagram of the system.

2.3.6. *Statistical analysis of DGGE profiles*

Shannon diversity index was introduced to analyze the bacterial community diversity (species richness) [15]. The band intensity should be considered in the Shannon diversity index. In present study, each band was considered as a single species and the band intensity as the species abundance. This index was calculated using the following equation: $H' = -(n_i/N)(\log n_i/N)$, where n_i/N is the proportion of community that is made up by species i (brightness of the band i/total brightness of all bands in the lane. The brightness of each band was measured by the Quantity One software.

3. Results and discussion

3.1. *Nutrients removal*

3.1.1. *COD removal*

During the total 177 days' operation, the combined bioreactors performed stably in organic matter removal (Fig. 2). For HRT$_U$ = 8 h, 6 h, 3 h and 1 h, the COD removal rate were 90.5 ± 5.5%, 87.7 ± 4.3%, 90.0 ± 6.9%, and 92.5 ± 4.4%, respectively. This indicated that the system was stable in carbon removal although the influent COD of raw domestic wastewater varied from day to day. It is estimated that about 50% of organic matter contained in raw domestic wastewater was removed in the UAFB, while another 50% was removed in AS/MBR, served as carbon source for denitrifying bacteria (DNB) and other heterotrophic bacteria in the AS/MBR and filtrated by the membrane. Although the influent COD of AS/MBR varied from time to time (80–216 mg/L), the effluent COD of MBR remained stable between 19 and 23 mg/L, indicating high COD removal efficiency of the AS/MBR part.

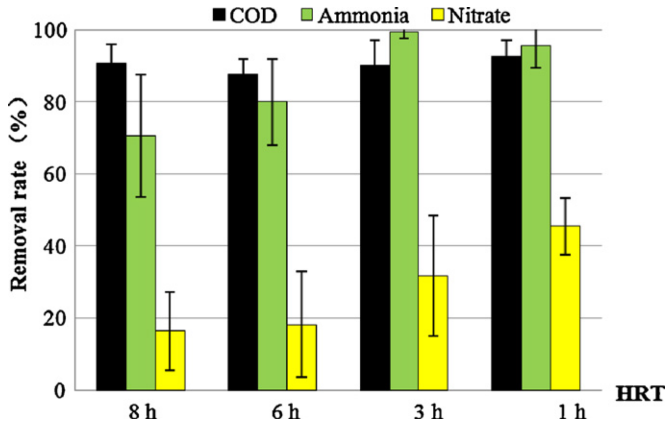

Fig. 2. General performance of the system. In evaluation of the process' performance, concentrations of COD, ammonia and nitrate of raw domestic wastewater and MBR effluent were carefully measured every other day. The error bars represent a bottom-up uncertainty range, partly caused by fluctuation of COD and ammonia in raw domestic wastewater.

3.1.2. *Nitrogen removal*

Both ammonia and nitrate were removed with HRT$_U$ being changed from 8 h to 1 h despite in different efficiencies (Fig. 2). The system reached a higher removal efficiency of ammonia and nitrate when the HRT was set at 3 and 1 h (ammonia removal >95%, nitrate removal >35%), which was similar with the performance of a full-scale conventional A^{2}O process [16]. For each HRTU, the ammonia removal rates were 70.5 ± 16.9%, 79.9 ± 11.9%, 99.4 ± 1.8%, 95.6 ± 6.0%, and the nitrate removal rates were 16.4 ± 10.9%, 18.2 ± 14.6%, 31.6 ± 16.7% and 45.5 ± 7.9%, respectively. Such increase of ammonia and nitrate removal rate can also be observed in Fig. 2.

In our study, both aeration and temperature of the MBR remained the same all the time, hence lack of oxygen at HRT$_U$ of 8 h and 6 h period should not be a reason for low nitrification rate. Corresponding to this, PCR-DGGE analysis identified the emergence and maintenance of two major ammonia oxidizing bacteria (AOB) only at HRT$_U$ of 3 h and/or 1 h (Fig. 3, discussed in Section 3.3.3). This indicated that AOB would not thrive in the MBR until shorter HRT$_U$. It is noticeable that the method measuring ammonia concentration in this study (Nessler's reagent colorimetry) cannot exactly quantify organic nitrogen, for example urea. Such organic nitrogen would be degraded to ammonia and short chain organic molecule in UAFB. Generally, a longer HRT$_U$ is beneficial to the degradation of organic nitrogen, which means higher actual NH$_4^+$-N in UAFB effluent than raw domestic wastewater. Our experimental data strongly indicated such potential deviation (8% on average, 40% maximum), and the longer HRT$_U$, the greater of such divergence. Hence, at long HRT$_U$, the concentration of ammonia in UAFB effluent should be higher than that at short HRT$_U$.

Nitrite concentration in MBR effluent was always below detection limit. A possible reason is that the DO of MBR was maintained high (about 6.0 mg/L) as a result of fierce aeration.

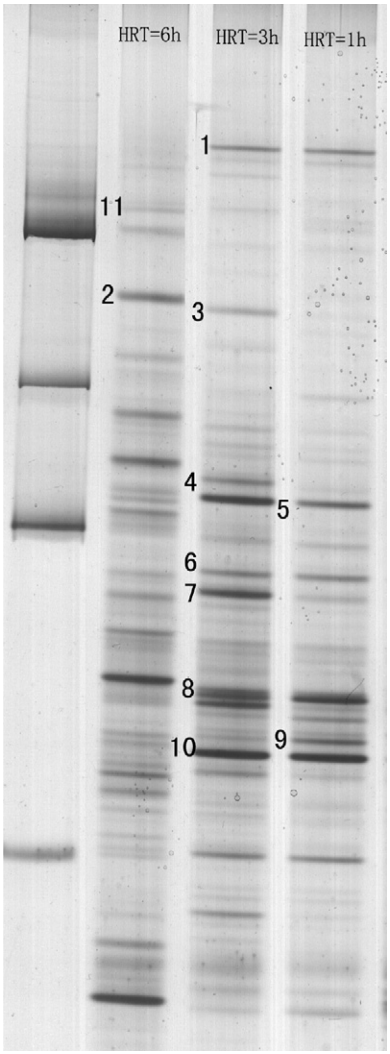

Fig. 3. DGGE profiles of PCR amplified 16S rDNA derived from the bacterial communities in MBR on day 85 (HRT = 6), day 137 (HRT = 3) and day 167 (HRT = 1). The first profile from left is the marker.

Since the HRT_U determined the fermentation process in UAFB, the VFAs, main product of such process, which are most easily utilized by denitrifiers, could be varied along with HRT_U quantitatively. In this case, different nitrate removal rates were reached at different HRT_U. At HRT_U = 1 h, about half of nitrate was removed from the process. However, denitrification was unsatisfactory at other HRT_U. Generally, 3–5 g five-day biochemical oxygen demand (BOD_5) is required for DNB to remove 1 g nitrate-N. The ratio BOD_5/COD of raw domestic wastewater used in this study was about 0.62 (in average), indicating that approximately 240–400 mg/L COD was required to completely remove approximate 50 mg/L nitrate-N (the potential ammonia degraded from organic nitrogen is considered). However, for most of time, the COD concentrations of UAFB effluent were 100–150 mg/L, which can only serve as 25–60% electron donors for DNB in AS. In order to further apply this process, some less-carbon-source-needed nitrogen removal processes should be considered to replace the conventional denitrification process in AS/MBR. For example, shortcut nitrification–denitrification technology can save 25% of oxygen and 40% of organic carbon source compared to full nitrification–denitrification process [17,18]. Furthermore, recent studies demonstrated that aerobic ammonium-oxidizing bacteria (AerAOB) and anoxic (or anaerobic) ammonium-oxidizing bacteria (AnAOB or anammox bacteria) could cooperate in low-organic partial nitritation/anammox systems to remove ammonia from wastewater [19–21]. There was an approximate linear relationship between HRT_U and nitrate removal from the HRT_U of 6 h to 1 h, but such linear trend seemed not suitable for HRT_U of 8 h, because TN removal efficiencies at HRT 8 h and 6 h were close to each other. In fact, 75% UAFB effluent + 25% synthetic domestic wastewater was applied as the influent of AS/MBR when HRT was 8 h. It is likely that the acetate added in the influent played a role as easily-utilising substrate for denitrifying bacteria which is also reported before [22].

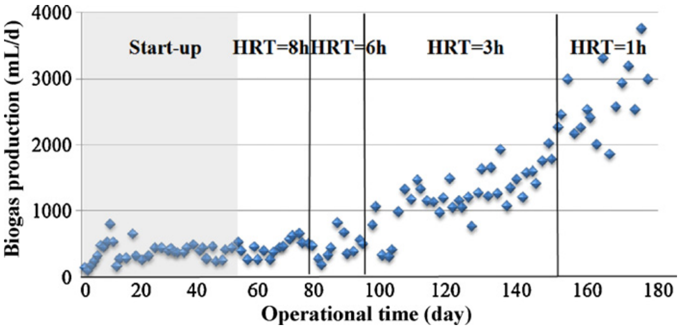

Fig. 4. Biogas production of UAFB at different HRTs.

3.2. Biogas and VFAs production

Methane and VFAs production were measured to evaluate anaerobic fermentation of the UAFB. During the whole experimental trial, the ORP of the reactor effluent always remained −360 to −390 mV, indicating satisfactory anaerobic condition.

3.2.1. Biogas production

Biogas production changed with decreasing HRT_U. While the production of biogas increased with HRT_U changing from 8 h to 1 h (Fig. 4), the composition ratio of methane, carbon dioxide and hydrogen stayed steady: methane took the most part of biogas (61.8–80.6%), and the proportion of carbon dioxide and hydrogen were 0.9–12.3% and 2.0–15.6%, respectively. The increase of biogas production was actually a result of organic loading augment for UAFB. In fact, methane productivity decreased from 187.8 to 115.0 mL $CH_4/gCOD_{removed}$ with HRT_U being shortened (Table 2). At short HRT_U, the production of methane was restrained as a result of incomplete methanogenesis.

3.2.2. VFAs accumulation

The concentrations of acetic acid, propanoic acid, butyric acid and valeric acid at different HRT_U were shown in Table 2. The VFAs accumulated more with the decrease of HRT_U, and the total VFAs production for each HRT_U is 0.5 ± 0.03, 1.2 ± 0.07, 3.3 ± 0.27, and 16.8 ± 3.78 g/day. Acetic acid was most sensitive to the change of HRT_U, whose concentration at HRT_U = 1 h was nearly fivefold higher than that at 8 h. For HRT_U from 8 h to 1 h, acetic acid took up 66%, 74%, 74% and 80% of the total VFAs in UAFB effluent. The complete hydrolysis, acidification and methanogenesis were only achieved at HRT_U of 8 h. When the HRT were shortened, insufficient time for methanogen to convert acetic acid, formic acid, methanol into methane led to the accumulation of VFAs. Since higher VFAs concentration in the UAFB effluent was beneficial to the following denitrification process, a relatively short HRT could be a compromised choice to achieve a balance between methane production and nitrogen removal.

Table 2
Performance of UAFB at different HRTU.

	HRT=8 h	HRT=6 h	HRT=3 h	HRT=1 h
COD of RDW (mg/L)	238.6 ± 26.0	262.9 ± 43.1	266.9 ± 32.6	285.4 ± 45.1
Effluent COD (mg/L)	113.16 ± 16.9	111.1 ± 15.0	107.7 ± 19.7	155.5 ± 18.0
Average VFAs concentration in UAFB effluent				
Acetic acid (mg/L)	19.7 ± 2.9	39.4 ± 1.8	52.2 ± 6.7	94.6 ± 14.4
Propanoic acid (mg/L)	3.4 ± 0.29	5.7 ± 0.53	8.1 ± 1.53	8.5 ± 2.02
Butyric acid (mg/L)	3.7 ± 0.27	4.0 ± 0.15	4.6 ± 1.32	8.4 ± 2.26
Valeric acid (mg/L)	3.1 ± 0.26	3.8 ± 0.12	5.8 ± 1.35	6.6 ± 1.50
Average biogas production (mL/day)	417 ± 34	483 ± 31	1050 ± 102	2277 ± 137
Average methane productivity (mL methane/g removed COD)	187.8 ± 12.8	143.8 ± 21.2	139.7 ± 17.6	115.0 ± 8.7

3.2.3. Pathway of organic matter in UAFB

To find the most suitable HRT value benefitting to both reclaiming methane and providing carbon source for the following nitrogen removal, a direct approach is to investigate the pathway of organic matter in UAFB at different HRTs. The input and output organic mass balance (in form of COD) of UAFB can be expressed as:

$$\mathrm{COD}_{\mathrm{influent}} = \mathrm{COD}_{\mathrm{VFAs}} + \mathrm{COD}_{\mathrm{methane}} + \mathrm{COD}_{\mathrm{CO_2}} + \mathrm{COD}_{\mathrm{biomass}} + \mathrm{COD}_{\mathrm{others}}$$

In above equation, $\mathrm{COD}_{\mathrm{influent}}$ and $\mathrm{COD}_{\mathrm{VFAs}}$ are the COD concentrations of raw domestic wastewater and effluent VFAs, respectively. $\mathrm{COD}_{\mathrm{biomass}}$ represents the organic matter contributing to biomass formation (i.e. heterotrophic and mixotrophic bacteria). $\mathrm{COD}_{\mathrm{methane}}$ and $\mathrm{COD}_{\mathrm{CO_2}}$ represent the part of organic matter that lost from UAFB in the form of methane and carbon dioxide. $\mathrm{COD}_{\mathrm{others}}$ included organic and inorganic compounds, for example, complex or long-chain organic matter that is unbiodegradable but can be measured as a part of COD, or inorganic matter such as sulfide that could also be chemically measured as COD.

The pathway of organic matter in UAFB obviously changed with $\mathrm{HRT_U}$ (Fig. 5). At long $\mathrm{HRT_U}$ (i.e. 8 and 6 h), more organic matter of raw domestic wastewater was transformed into methane than VFAs, while on the contrary, more proportion of VFAs were generated at short $\mathrm{HRT_U}$ (3 and 1 h). The ability of reclaiming energy from raw domestic wastewater was weakened at short $\mathrm{HRT_U}$, and 3 h was a balanceable HRT value for both methane and VFAs production. Hence, 3 h appeared to be the most suitable HRT value.

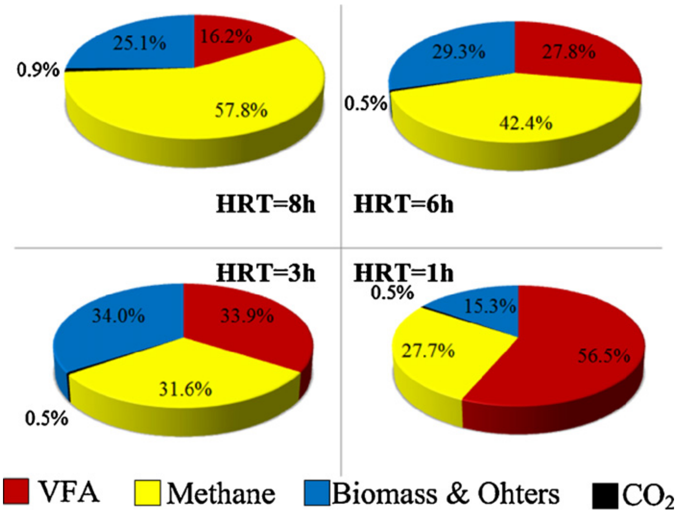

Fig. 5. Pathway of organic matter in UAFB. Ratios in each cake were calculated from COD data (in average).

3.3. MBR performance at different $\mathrm{HRT_U}$

3.3.1. Sludge reduction

Compared to other conventional aerated bioreactors, MBRs have low sludge yields [23]. Extremely low or even zero sludge production could be achieved when the sludge loading rate became low enough. This phenomenon well accorded with the goal of sludge reduction.

In our study, the F:M ratio of the MBR is only 55–80 mg COD/(g VSS day), which is about 30–50% lower than that of other MBRs fed with domestic wastewater [24,25]. Although nearly all sludge was retained in MBR, MLSS of our MBR kept around 2000 mg/L despite of different $\mathrm{HRT_U}$. Under such a low F:M circumstance, the bacteria utilize organic matter for maintenance purpose more than growth purpose. In addition, since our MBR was operated with complete sludge retention, higher organisms such as protozoa and metazoan began to be observed since the $\mathrm{HRT_U}$ was shortened to 6 h (day 89), which might also help to maintain stable MLSS of MBR [26,27].

3.3.2. Membrane fouling

Based on experience from full-scale MBR plants, capital and energy costs brought by membrane fouling and membrane replacement are two of the most significant components of MBR expenditure [28–30]. In this study, low sludge concentration efficiently delayed membrane fouling, resulting in 133-day stable operation with only slight membrane fouling. During this period, the TMP kept lower than 0.05 MPa, and we did not change or wash the membrane sheet.

The membrane pores were clearly seen in SEM images of the virgin membrane inner- and outer-surface (Fig. 6a and b). Fig. 6c and d shows the surface of fouled membrane sampled from day 76. A thick cake layer and an agglomerate of coccus were clearly seen on the outer-surface (Fig. 6d). It is surprising to find that a small quantity of microbes squeezed across the outer-surface and attached to the inner-surface (Fig. 6c). This type of fouling cannot be solved by tap-water-cleaning or backwash cleaning, also may be a reason causing irreversible or permanent fouling of the membrane.

3.3.3. Microbial community structure in MBR

Monitoring bacterial community shifts is a diagnosis of bioprocess. In this study, molecular tools were used in order to analyse the relationship between $\mathrm{HRT_U}$ and microbial community structure in the MBR. DGGE analysis of 16S rDNA genes revealed both a high diversity and a changing community in MBR. Different $\mathrm{HRT_U}$ led to different compositions and concentrations of UAFB effluent, which strongly impacted the microbial community structure in MBR (Fig. 3). Since fed with raw domestic wastewater, a lot of uncultured bacteria were found in this study and it was hard to know their genus and function, so only affirmatory and typical populations were presented here.

Band 1 *Dechloromonas*, band 4 *Nitrosovibrio*, band 5 *Variovorax* and band 8 *Nitrospira*, responsible for nitrogen removal, mainly

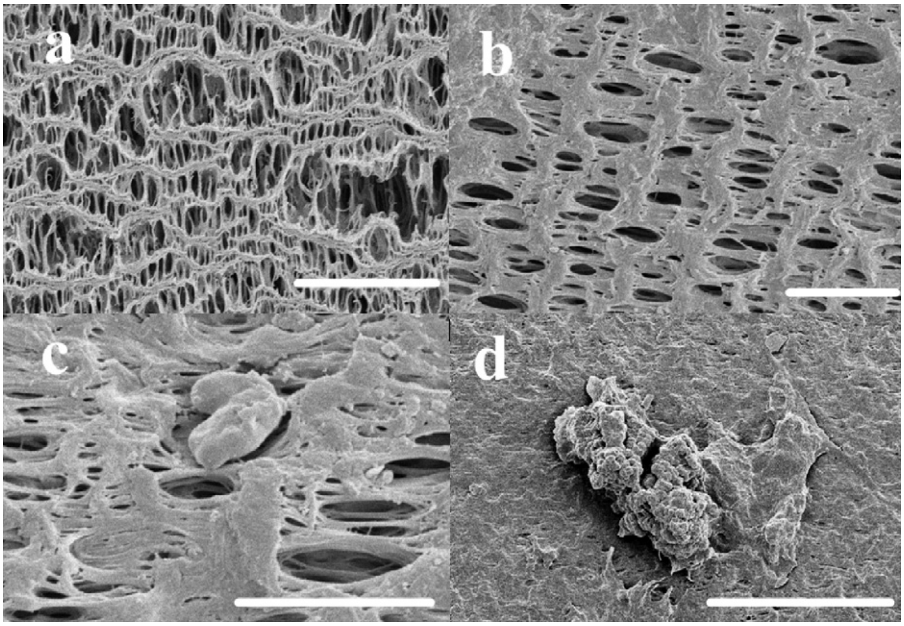

Fig. 6. SEM images of virgin and fouled membrane. (a and b) SEM images of inner- and outer-surface of the virgin membrane; (c) SEM images of inner-surface of the fouled membrane; (d) SEM images of cake layer and agglomerate of coccus attached on the outer-surface of membrane. The bar scale corresponds to (a) 10 μm, (b) 5 μm, (c) 5 μm, and (d) 30 μm.

existed at short HRT_U of 3 h and 1 h. The band sequence closely related to *Betaproteobacteria* (band 10), capable of organic compound removal, existed consistently from HRT of 3 h to 1 h. All these implied that with the decreasing of HRT_U, the microbial community structure in MBR was fit for nitrogen and COD removal. Some bands related to phosphate accumulating organisms, such as band 6 *candidate division TM7* and band 7 *Saprospiraceae*, always existed during all HRT_U, which proved the potential of phosphate removal for this combined system.

It is surprising that band 9 *Azospira*, a perchlorate/chlorate-dependent Fe(II)-oxidizing bacteria that is commonly found in anoxic conditions [31,32], existed all the time in MBR and became one of the dominant populations at HRT of 1 h. In addition, band 2 *Thiothrix* disappeared since HRT of 3 h, which is a kind of filamentous bacteria, and it is believed to be associated with operational problems in domestic wastewater treatment plants, such as sludge bulking [33]. In addition, we also found a part of populations belonged to *Proteobacteria* class, including *Beta-Proteobacteria*, *Gamma-Proteobacteria* and *Delta-Proteobacteria*. This result was also found by Huang et al. in their submerged MBRs [34].

Statistical analysis of the DGGE profiles showed that the quantity and abundance of the bacterial populations varied with HRT_U changing. Shannon index were 0.67, 0.95 and 0.76 at HRT of 6 h, 3 h and 1 h, respectively. This indicated that at HRT = 3 h, the diversity of MBR populations was higher than the others. This result was coincident with previous discussion that 3 h was the most suitable HRT parameter for this membrane-based process, also indicating that high diversity is a necessary condition for a robust and efficient reactor.

4. Conclusions

The study demonstrated that the membrane-based process was efficient and suitable for raw domestic wastewater treatment, and can achieve simultaneous production of biogas and reused wastewater under appropriate operation conditions. More specific outcomes of the study were as follows:

(1) For UAFB, 3 h is a balanceable and suitable HRT_U value for the process to simultaneously reclaim energy and generate reused water from raw domestic wastewater. Methane productivity decreased when the HRT_U was shortened, while VFAs accumulated with the decrease of HRT_U.
(2) For AS/MBR, removal of ammonia and nitrate obviously increased when HRT_U was shortened from 8 h to 1 h. The MBR had advantage on sludge reduction, which resulted from extremely low organic loading rate and/or the emergence of protozoa and metazoan. Low sludge concentration also efficiently delayed membrane fouling.
(3) DGGE analysis of 16S rDNA gene sequences revealed both a high diversity and a changing microbial community in MBR. Highest Shannon index of 0.95 at 3 h HRT_U was coincident with the best performance of AS/MBR at that HRT_U.

Clearly, there are still many open questions and problems in scale-up application of this process, for example, insufficient self-supply of carbon source for DNB must be solved to reduce operating expenditure; in addition, the ability of phosphor and sulfur removal still warrants further exploration. However, our research may open a door for treatment of vast domestic wastewater facing the next decades' demand, and also provides a new way in mitigating the worldwide concern about energy and water crisis.

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