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Improving volatile fatty acid yield from sludge anaerobic fermentation through self-forming dynamic membrane separation

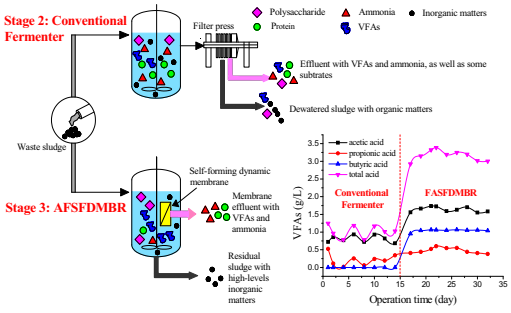
Hongbo Liu [a,b,c], Yuanyuan Wang [a], Bo Yin [a], Yanfang Zhu [a], Bo Fu [a,b,c], He Liu [a,b,c,*]

[a] School of Environmental and Civil Engineering, Jiangnan University, Wuxi 214122, Jiangsu Province, PR China
[b] Jiangsu Collaborative Innovation Center of Technology and Material of Water Treatment, Suzhou 215011, China
[c] Jiangsu Key Laboratory of Anaerobic Biotechnology, Wuxi 214122, Jiangsu Province, PR China

HIGHLIGHTS

- VFA yield from sludge anaerobic fermentation is improved by SDM separation.
- Stable SDM operation is maintained in separating high-TSS fermentation sludge.
- Timely substrates retention and product discharge are achieved by SDM separation.
- Enzymatic activities in fermentation sludge are enhanced by SDM separation.
- Functional bacteria are enriched by SDM separation.

GRAPHICAL ABSTRACT



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ABSTRACT

Self-forming dynamic membrane (SFDM) separation was applied to the conventional sludge fermenter for improving VFA yields. Results indicated SFDM presented good performance in transferring products, retaining substrates, and enriching useful bacteria. The retention ratios of suspended solids, soluble COD, proteins, and polysaccharides reached 99%, 30%, 70%, and 40%, respectively, and more than 90% of the VFAs and ammonia could be transferred in a timely manner. The structure of the microbial community was optimized, which led to enhanced releases of hydrolytic enzymes and accelerated enrichments of functional bacteria. Protease and β-glucosidase activities increased from 1.0 to 5.0 U/mL and 15.0 to 23.0 μmol/L·h, respectively. VFA yield and sludge conversion ratio increased by 233.3% and 227.9%, respectively. Moreover, SFDM had good operation stability, including a short formation time, a long operation period, and a low transmembrane pressure. These results show VFA yield from sludge fermentation can be greatly improved by SFDM separation.

1. Introduction

Activated sludge systems have been widely adopted in municipal wastewater treatment plants (WWTPs) for wastewater treatment, and a large amount of waste sludge (WS) is generated in China (National Bureau of Statistics of China, 2014). Using conventional sludge treatment processes, WS disposal has led to 50–60% of the total operation cost of the WWTPs (Xu et al., 2011). Anaerobic fermentation for volatile fatty acid (VFA) production is a promising technology in WS treatment and reutilization, in which the carbon resources in WS are converted into useful VFAs, instead of carbon dioxide and methane, and it simultaneously treats WS, generates valuable biochemical products, and biologically fixes carbon (Lee et al., 2014; Yang et al., 2014; Singhania et al., 2013; Huang

* Corresponding author at: School of Environmental and Civil Engineering, Jiangnan University, Wuxi 214122, Jiangsu Province, PR China.
E-mail address: liuhe@jiangnan.edu.cn (H. Liu).

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et al., 2014). Therefore, it has attracted increasing attentions from researchers.

However, recent studies and applications demonstrate there are several key problems with this technology. For example, the high concentrations of acids and ammonia produced in fermentation have been proven to greatly lower the VFA yield (Argun and Kargi, 2009; Mohapatra et al., 2010; Chang et al., 2011; Uma Rani et al., 2012). Lee (2014) and Liu (2016) reported that the reaction driving force in conventional fermentation systems is not strong enough to accelerate the conversion of organic matter that exhibits poor biodegradability. Moreover, the long reaction time of the fermentation process requires a large reactor volume, which results in high energy consumption and cost (Wang et al., 2009; Jeong et al., 2007).

With many virtues such as small reactor, efficient functional bacteria retention and acceleration of refractory organic matter degradation, membrane separation technology has been proven feasible for the separation of effluent and activated sludge during wastewater treatment (Wang et al., 2009; Jeong et al., 2007; Zhang et al., 2009). Therefore, membrane separation technology might be also feasible to solve the aforementioned problems in conventional sludge fermentation processes. For example, Xu et al. (2011) successfully utilized a membrane bioreactor (MBR) for sludge digestion, and efficiently controlled membrane fouling by implementing online ultrasonic equipment. However, our previous study (Zhu et al., 2015) indicated that compared with activated sludge, fermented sludge had a higher suspended solids (SS) concentration, smaller particle size, higher viscosity, and larger filtration resistance. This leads to membrane fouling, which is a bottleneck that hinders the stable operation of MBRs for sludge anaerobic fermentation (AFMBR).

A self-forming dynamic membrane (SFDM) is a separation layer that is formed on a porous support, such as a silk screen or steel mesh, by the precipitation of microorganisms and their metabolites during the filtration of activated sludge. Compared with conventional separation membranes, an SFDM possesses several distinct features that make it more competitive in the solid-liquid separation of fermented sludge. Firstly, an SFDM has strong anti-pollution potential, the membrane fouling can be easily cleaned, and the membrane flux can be completely restored. Secondly, the membrane flux of an SFDM is as high as 40–100 $L/h \cdot m^2$, which is 1–4 folds higher than that of conventional membranes. Thirdly, an SFDM has low filtration pressure, and effluent can flow out with the aid of gravity. Finally, an SFDM uses cheap supports, rather than expensive membranes, which greatly reduces investment costs. Therefore, it is technically and economically feasible to substitute a conventional membrane in an AFMBR with an SFDM. Additionally, this novel reactor has obvious advantages, especially in preventing membrane pollution.

Based on the above discussion, the objectives of this study were to: (1) develop a novel MBR for sludge fermentation and acid production (AFSFDMBR) that solves the aforementioned problems associated with conventional sludge fermentation processes; (2) investigate the performance and stability of the AFSFDMBR; and (3) clarify the mechanisms of SFDM separation in enhancing VFA production, including the retention efficiencies of substrates, the diffusion ratios of VFAs and ammonia, the distributions of microbial communities, and mass balance.

2. Experimental

2.1. Self-forming dynamic membrane bioreactor for sludge fermentation and VFA production

2.1.1. Reactor setup

The AFSFDMBR was a column reactor with an oval bottom and an elliptical head, an internal diameter of 26 cm, a height of 40 cm, and a working volume of 14 L, the configuration of which was similar to conventional fermenters. The influent flow of the WS was 2.6 L/d, and the total hydraulic reaction time was approximately 5.4 d.

As shown in Fig. 1, WS was first pumped into the fermenter at the upper part of the AFSFDMBR, in which organic matter could be degraded and VFAs could be produced. Then, solid particles and macromolecular organic matter, such as proteins and polysaccharides, could be retained in the fermenter by the dynamic membrane for further solubilization and degradation. Low-molecular-weight matter, such as VFAs and ammonia, could be discharged along with the permeated water. Under gravity, the permeated water could seep through the surface of the dynamic membrane, enter the cavity inside the membrane subassemblies, and finally flow out from the AFSFDMBR along the gathering pipe and the hollow propeller of the agitator. Using the online monitoring system, the pH was controlled at approximately 10.0 to inhibit methanogenesis (Liu et al., 2012), and temperature was maintained at approximately 35 °C. An agitator with a partially hollow propeller was used to mix the fermented sludge and provide a cross-flow on the surface of the dynamic membrane to control membrane

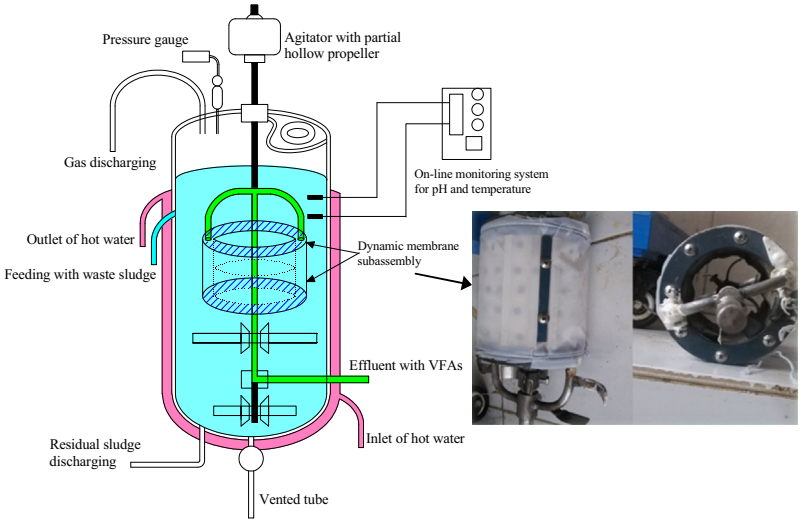

Fig. 1. Diagram of the AFSFDMBR.

fouling. A sandwich structure was adopted in the fermenter, and fermented sludge was heated by the cycling of hot water.

2.1.2. Dynamic membrane in AFSFDMBR

In the AFSFDMBR, the dynamic membrane subassembly was similar to a traditional tubular membrane, in which silk with an aperture of approximately 0.1 mm was used as the separation layer. The effective area of the dynamic membrane was 3.14 dm^2. As shown in Fig. 1, the dynamic membrane subassembly was fixed on the propeller and rotated along with it. In view of the poor filterability of fermented sludge, several strategies were implemented to alleviate membrane fouling. (1) Rapid rotation of the membrane subassembly without effluent was adopted to clean the membrane when the membrane resistance was low and controllable. However, when fouling was severe, the membrane subassembly would be taken out and flushed with tap water, which could completely restore the membrane flux. (2) The dynamic membrane was operated under a very low membrane flux, approximately 1.0–2.0 $L/m^2 \cdot h$. Compared with wastewater treatment, the hydraulic reaction time for sludge anaerobic fermentation is much longer, approximately 5–10 d, which indicates that the flow of its effluent could be maintained at a very low level. Therefore, in this experiment, membrane fouling was greatly alleviated by adopting a low membrane flux. (3) Increasing transmembrane pressure was implemented to extend the operation period of the dynamic membrane. It is known that the filtration pressure of the dynamic membrane is very low, 1/10–1/100 of that of a microfiltration membrane, which indicates that the transmembrane pressure of the dynamic membrane can be regulated over a much larger range.

2.2. Substrates

WS that used as the substrate was obtained from the secondary sedimentation tank of an urban wastewater plant in Wuxi, China. Fresh WS was first concentrated by allowing it to settle for 4.0 h. Then, it was filtered through a metal sieve with a 0.7-mm aperture and finally stored at 4.0 °C for later use. The WS had a pH of 6.0–7.5, a total chemical oxygen demand (TCOD) of 12.0–15.0 g/L, a soluble COD (SCOD) of 0.8–1.0 g/L, a total suspended solids (TSS) concentration of 12.0–15.0 g/L, a volatile suspended solids (VSS) concentration of 7.0–8.0 g/L, a soluble protein concentration of 80–100 mg/L, and a soluble reducing sugar concentration of 40–50 mg/L.

2.3. Seeding sludge for anaerobic fermentation

Anaerobic sludge from an up-flow anaerobic sludge bed (UASB) reactor for brewery wastewater treatment was collected as the seeding sludge. To accumulate acetogenic bacteria and kill methanogens, the anaerobic sludge was pretreated before use according to a previously published method (Liu et al., 2016).

2.4. Anaerobic fermentation

According to the operation modes, the total sludge anaerobic fermentation process could be divided into three stages, namely the batch operation stage (stage 1), the continuous operation stage (stage 2), and the continuous operation stage with dynamic membrane separation (stage 3). Indeed, stage 1 could be regard as the startup phase of stage 2, and stage 1 and 2 could be regard as the startup phase of stage 3. In stage 1, the fermenter was filled with WS and seeding sludge at mass ratio of 9.0. Then, TSS was maintained at approximately 15–20 g/L. Dissolved oxygen in the WS and the gas in the headspace of the flasks were removed by sparging gaseous nitrogen for approximately 30 min to maintain a strictly anaerobic condition. As the experiment proceeded, the concentration of VFAs in the fermenter increased. When the VFA concentration stabilized, stage 1 ended and stage 2 began. In stage 2, the operation parameters of TSS, temperature, pH, substrate reaction time and stirring intensity, were the same as those in stage 1, but the feeding and discharging of the fermenter were shifted to be continuous. Additionally, when the VFA concentration stabilized, stage 2 ended and stage 3 began. In stage 3, the operation parameters of TSS, temperature, pH, substrate reaction time and stirring intensity, were the same as those in stage 2. WS was continuously fed into the fermenter, but the liquor containing VFAs and ammonia was continuously discharged through the dynamic membrane as the effluent. In the total fermentation process, the rotation speed of the agitator was maintained at approximately 60 rpm, the temperature was maintained at approximately 35 °C, and the pH was maintained at 10.0. Samples were removed from fermenter at certain intervals and analyzed. While calculating the VFA production and final VFA concentration, the average of the values obtained at the stable period were used in order to avoid the influence of the residual VFA from last stage. All of the experiments were conducted independently in triplicate.

2.5. Analytical methods

2.5.1. Measurements of conventional indexes

Conventional indices, including pH, NH_4^+-N, SS, COD, VSS, and TSS, were analyzed according to the standard methods issued by the Ministry of the Environmental Protection Agency of China (CEPB, 1989; A.P.H.A., 1998). The soluble carbohydrate concentration was measured by the phenol-sulfuric method using glucose as the standard (Dubois et al., 1956). The soluble protein concentration was determined by the Lowry–Folin method using bovine serum albumin as the standard (Lowry et al., 1951). VFAs were measured by a gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan) that was equipped with an auto-injector (AOC-20i, Shimadzu) (Liu et al., 2016).

2.5.2. Measurements of enzymatic activities

β-Glucosidase (β-GLC) activity, representing the capability of polysaccharide degradation, was measured spectrophotometrically (Mapada UV-1600, Shanghai, China) in 5-cm cuvettes according to the procedure published by Li and Chróst (2006). Protease activity was determined by a standard method (SB/T, 1988).

2.5.3. Microbial community analysis

Microbial communities in the sludge at different periods and locations were analyzed when the concentration of total acid reached stable in each stage, that is, samples were taken from the reactor at stage 1 on d 10 (B), stage 2 on d 34 (S), stage 3 on d 44 (A1), stage 3 on d 54 (A2), as well as from the surface of the membrane at stage 3 on d 44 (M1) and on d 54 (M2). First, the collected samples were centrifuged. Then, microbial detection and analysis were performed, including DNA extraction, polymerase chain reaction amplification, and processing of MiSeq (Illumina, San Diego, CA, USA) sequencing data, according to the procedures published by Yuan et al. (2015). Each sample was analyzed in triplicate, and the standard deviations of all analyses were always less than 5%.

2.6. Calculation methods

The VFA yield ratio from SCOD (Y_{SCOD}) was computed using Eq. (1), and the VFA yield ratio from VSS (Y_{VSS}) was computed using Eq. (2). The purity of VFAs in the effluent (P_{VFAs}) was computed using Eq. (3). COD recovery (R_{COD}) was calculated using Eq. (4), and the loss ratio of VSS (L_{vss}) was calculated using Eq. (5).

$$Y_{SCOD} = (VFA_t - VFA_0)/SCOD_t, \quad (1)$$

$$Y_{VSS} = (VFA_t - VFA_0)/VSS_0, \quad (2)$$

$$P_{VFAs} = 100\% \times VFA_t/SCOD_t, \quad (3)$$

$$R_{COD} = (TCOD_t/TCOD_0) * 100\%, \quad (4)$$

$$L_{VSS} = (VSS_0 - VSS_t)/VSS_0 * 100\%, \quad (5)$$

where $SCOD_t$ was the SCOD concentration in the fermented sludge at the end stage. $TCOD_0$ and $TCOD_t$ were the sums of the measured COD in the liquid, in the solid particles, and in the gas phase at the beginning and end of the fermentation process, respectively. VFA_t and VFA_0 were the concentrations of VFAs in the fermented sludge at the initial and end stages of the fermentation process, respectively. VSS_0 and VSS_t were the concentrations of VSS at the beginning and end of the fermentation process, respectively.

3. Results and discussion

3.1. Performance of the AFSFDMBR in sludge fermentation and VFA production

3.1.1. Enhancement of the VFA yield from sludge fermentation

Obviously, stages 1 and 2 represent traditional batch and continuous fermentation processes, respectively, and stage 3 indicates the performance of the AFSFDMBR. As shown in Fig. 2, the VFA yield in stage 3 was 26.5% greater than that in stage 1, and 233.3% greater than that in stage 2. In stage 1, VFA concentrations quickly increased in the first 8 d, and reached the maximum concentration (approximately 2.53 g/L) on the 15th day. In stage 2, VFA concentrations decreased rapidly during the first 5 d because of the shift in the operation mode, and finally stabilized at approximately 0.96 g/L. In stage 3, the VFA concentrations increased rapidly during the first 6 d and finally stabilized at approximately 3.2 g/L. Therefore, the results indicate that VFA yields from sludge anaerobic fermentation could be greatly enhanced by SFDM separation.

Moreover, as shown in Fig. 2, the application of dynamic membrane separation increased the butyric acid yield, while continuous operation increased the propionic acid yield. In stage 1, acetic acid was the dominant component, accounting for approximately 96% of the total VFAs, which might have resulted from anaerobic fermentation at pH values of approximately 10 (Yuan et al., 2015). In stage 2, although both the total VFA and acetic acid concentrations decreased greatly, which might be attributed to the insufficient degradation of organic matter under continuous operation, the proportion of propionic acid in the total VFAs increased from 4.18 to 33.4% and remained stable in the next stage. Interestingly, in stage 3, in addition to the increases of the acetic and propionic acid concentrations, the butyric acid concentration also greatly increased, reaching as high as 1.05 g/L and accounting for 32.8% of the total VFAs. The results indicate that the application of membrane separation might stimulate shifts in the dominant microorganisms in the fermenter, thereby resulting in changes in the degradation pathways of organic matter.

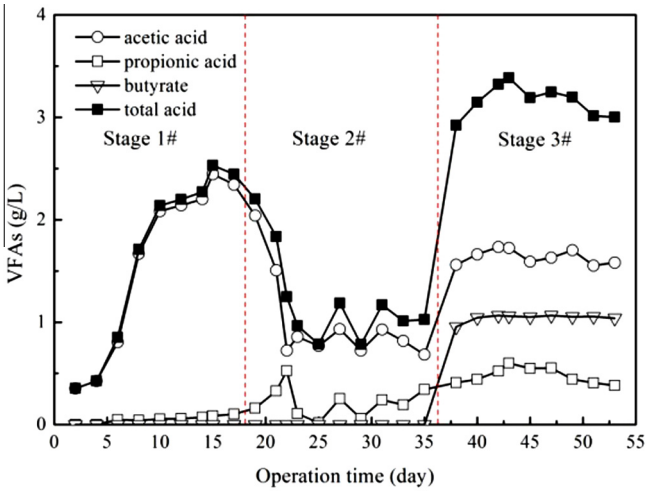

Fig. 2. Performance of the AFSFDMBR in sludge fermentation for VFA production.

3.1.2. Sludge conversion ratio

The performances of sludge fermentation in the different stages were systematically investigated through VFA yield ratios (Y_{SCOD} and Y_{VSS}) and sludge loss ratios (L_{VSS}). As shown in Table 1, dynamic membrane separation accelerated the conversion of sludge into VFAs. The yield ratio of VFAs from SCOD (Y_{SCOD}) in stage 3 reached 734.16 mg VFAs/g SCOD, which was 61.21% higher than that in stage 1 and 227.87% higher than that in stage 2. The yield ratio of VFAs from VSS (Y_{VSS}) in stage 3 reached 529.60 mg VFAs/g VSS, which was 50.60% higher than that in stage 1 and 250.4% higher than that in stage 2. The extended degradation time of soluble organic matter and suspended solids should be the main factors responsible for these increases. Although the total sludge retention times in stages 1, 2, and 3 were the same, SS and high-molecular-weight soluble substrates were retained in stage 3. The accumulation of bacteria that effectively degrade refractory organic matter and hydrolyze solids should be another key contributor. Previous studies indicated that in conventional anaerobic fermentation processes, a large amount of substrates, such as proteins and polysaccharides, still exist in the residual sludge (Yin et al., 2016). In the AFSFDMBR, although bacteria that are essential for the degradation of refractory organic matter exhibit a low growth ratio, they could be retained by the dynamic membrane and, thus, accumulated.

3.2. Stability of the dynamic membrane in the SFDMBR

3.2.1. Variation of the dynamic membrane flux under constant transmembrane pressure

Fermentation sludge, which has a high solid concentration and a high viscosity, is a non-Newtonian fluid (Zhu et al., 2015). The formation and stable operation of the dynamic membrane are the key problems in separating fermentation sludge. Fig. 3(A) shows the variation of the dynamic membrane flux during one operation period when the MLSS was 15 g/L, the transmembrane pressure was constantly maintained at 1.3 kPa, and the rotational speed of the dynamic membrane was 50 rpm. The total filtration process could be divided into three phases. The first phase was from 0 to 0.5 h, in which the membrane flux and the SS concentration in the effluent fluctuated greatly. The second phase was from 0.5 to 1.5 h, in which the membrane flux kept decreasing while the SS concentrations in the effluent stabilized at low levels. The third phase was from 1.5 to 350 h, in which the membrane flux and SS concentration in the effluent remained stable.

Table 1
Acids concentrations in effluent and conversion rates in different stages.

Parameter	Stage 1#	Stage 2#	Stage 3#
Total VFAs concentration (g/L)*	2.30	0.99	3.22
Acetate concentration (g/L)*	2.21	0.82	1.66
Propionate concentration (g/L)*	0.09	0.17	0.50
Butyrate concentration (g/L)*	0.00	0.00	1.06
Y_{SCOD} (mg VFAs/g SCOD)	455.41	223.92	734.16
Y_{VSS} (mg VFAs/g VSS)	351.65	151.14	529.60

* Indicates the data are the average values of the samples obtained during stable operation.

Previous reports showed that a dynamic membrane could completely form within 10 min during wastewater treatment (Liu et al., 2009). Although slightly slower than that in wastewater disposal, a dynamic membrane formed rapidly (within 30 min) during the separation of the fermentation sludge (the first phase), which might be due to the smaller particle size of the fermented sludge (Zhu et al., 2015). Moreover, the results indicate that the dynamic membrane could be stably operated more than 14 d, and that the transmembrane pressure was controlled at a low level to ensure that the effluent could flow out only with the aid of gravity. Furthermore, the period of stable operation could be further prolonged by increasing the transmembrane pressure and the rotational speed.

3.2.2. Variation of the transmembrane pressure under a constant membrane flux

The variations of the transmembrane pressure under a constant membrane flux are shown in Fig. 3(B). When the MLSS was 15 g/L, the rotational speed of the dynamic membrane was 50 rpm, the limiting transmembrane pressure was set to 6.0 kPa, and the membrane fluxes were constantly maintained at different levels (3.5, 2.3, and 1.2 L/m^2·h), the dynamic membrane could be stably operated for more than 5, 7, and 12 d, respectively.

The results indicate that the variation of the transmembrane pressure was greatly influenced by the membrane flux. A small membrane flux could result in a slow increase of the membrane resistance during wastewater treatment (Liu et al., 2009). However, during the separation of fermentation sludge, the variations of the membrane resistance are different from those in wastewater treatment, as they can be easily divided into several phases, including cake filtration, complete blocking, intermediate blocking, and standard blocking (Liu et al., 2009). However, as shown in Fig. 3 (B), the transmembrane pressure increased linearly as the operation proceeded, and obvious phases were not observed. The results indicate that the filtration mechanisms of the dynamic membrane differ between the separations of activated sludge and fermented sludge.

3.3. Efficiencies of retention and selective diffusion by the dynamic membrane

3.3.1. Substrate retention efficiencies

High retention of substrates, namely SS, SCOD, polysaccharides, and proteins, by the dynamic membrane was one of the key contributors to the increased VFA production by the AFSFDMBR. As shown in Fig. 4(A), the dynamic membrane exhibited good SS retention. The SS concentration in the effluent was less than 150 mg/L, which translates to a greater than 99% retention ratio. Moreover, the SS retention efficiency stabilized rapidly within 3.0 h. However, as shown in Fig. 4(B), compared with SS, the SCOD retention efficiency was much lower (less than 30%), and the dynamic membrane required more than 6 d to achieve stable SCOD retention. The main reason for this is that the SCOD in the effluent results from the presence of soluble, low-molecular-weight substrates, such as VFAs, ammonia, and polysaccharides, the retention of which requires the formation of a much more compact separation layer. As shown in Fig. 4(C) and (D), the dynamic membrane exhibited a high retention of proteins, but a slightly lower retention of polysaccharides. The dynamic membrane exhibited stable protein retention within 3 d, and the protein retention ratio was greater than 70%. However, although the dynamic membrane also only needed 3 d to stably retain polysaccharides, the polysaccharide retention ratio was only 40%. Proteins and polysaccharides are the main components in sludge, with proteins accounting for greater than 60% of the total nutritional substrates (Liu et al., 2012). Therefore, the results indicate that the dynamic membrane could retain most of the substrates in sludge, thereby increasing VFA production and sludge conversion.

3.3.2. Diffusion efficiencies of VFAs and ammonia

Online discharging of products, such as VFAs and ammonia, from the sludge fermentation system was another key contributor to the increased VFA yield of the AFSFDMBR. VFAs and ammonia were widely considered as the main toxicants to anaerobic digestion (Pratt et al., 2012; Chen et al., 2014). As shown in Fig. 4 (E) and (F), the concentrations of VFAs in the effluent and in the reactor were very similar, and the removal rate was less than 10%. Moreover, the removal rate of ammonia by the dynamic membrane was also very low, approximately 10%, which was mainly due to the adsorption and degradation of the microorganisms on the surface of the dynamic membrane. Therefore, the results indicate that the products of sludge fermentation, VFAs and ammonia, could be discharged in a timely manner through the dynamic membrane, thereby avoiding their inhibitory effects on VFA production.

3.3.3. Enhanced enzymatic activities in the AFSFDMBR

The activities of proteases and β-GLC were investigated to evaluate the capability of anaerobic sludge during substrate degradation and VFA production. As shown in Fig. 5, dynamic membrane separation enhanced the enzymatic activities of the sludge in the

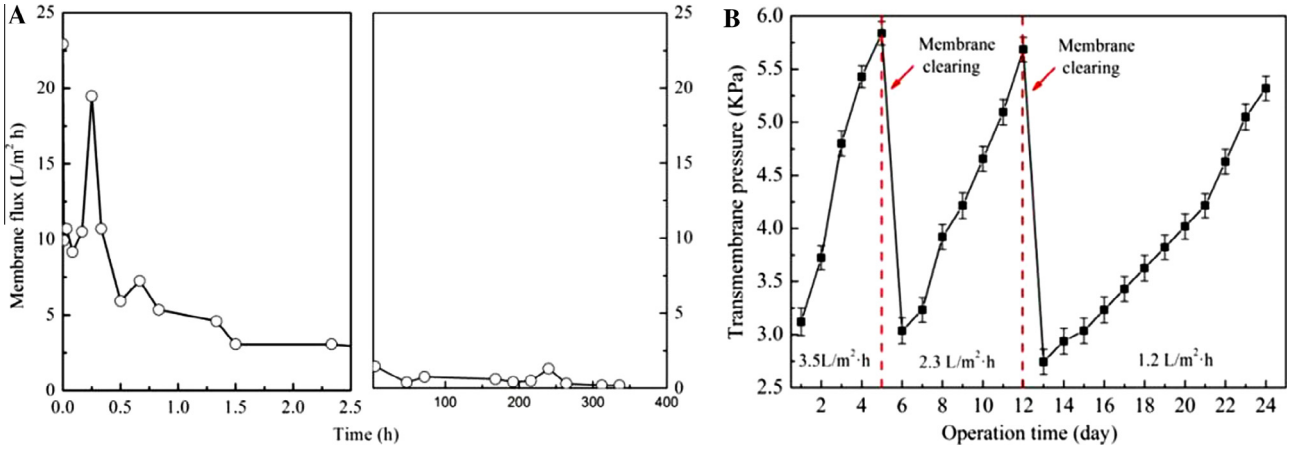

Fig. 3. Variation of the membrane flux under constant membrane resistance during one operation period (A), and variations of membrane resistance under a constant membrane flux (B).

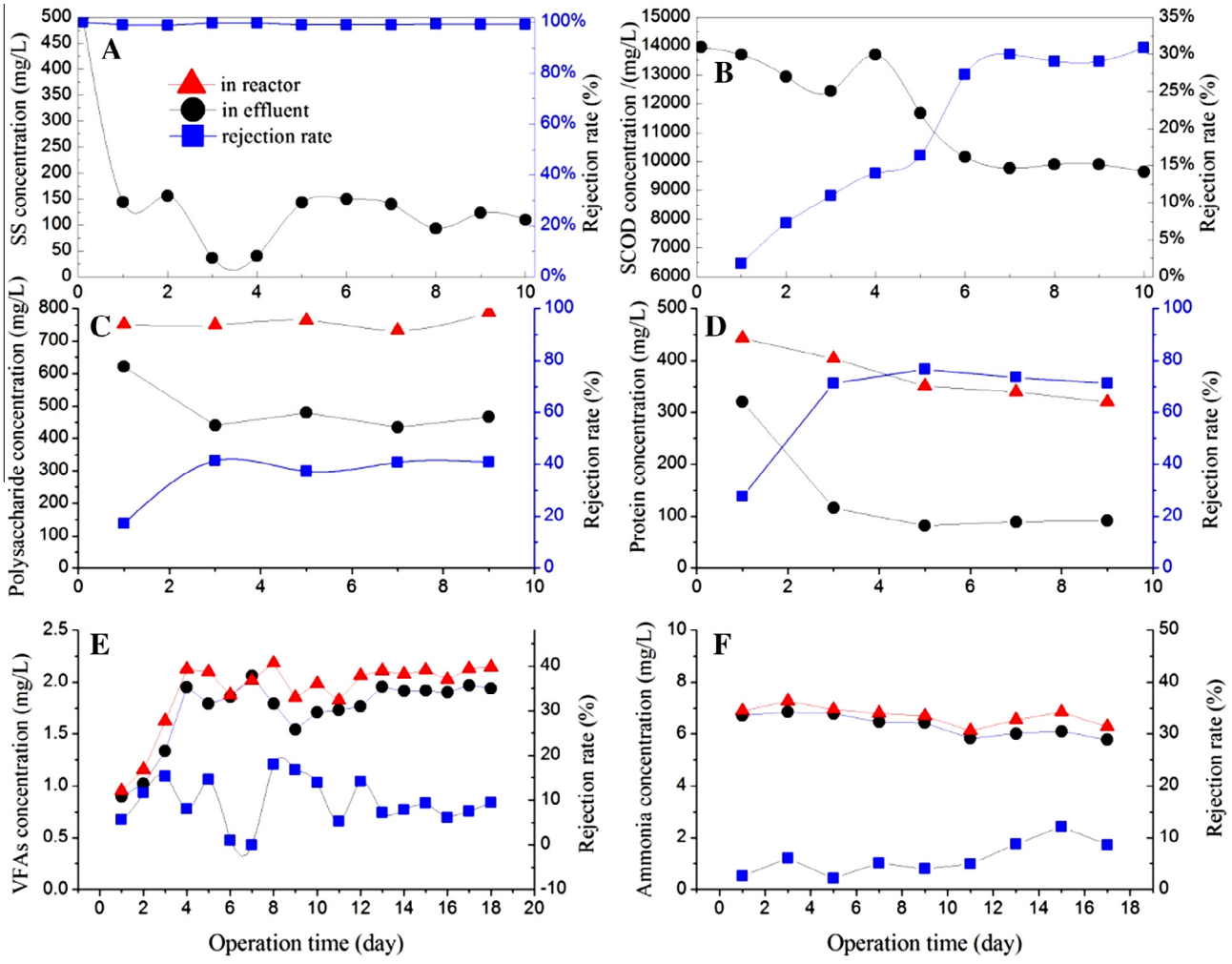

Fig. 4. Retention efficiencies of SS (A), SCOD (B), polysaccharides (C), proteins (D), VFAs (E), and ammonia (F) by the dynamic membrane.

AFSFDMBR. During stage 2 without the dynamic membrane, the protease and β-GLC activities were very low, less than 1.0 U/mL and 15 μmol/L·h, respectively. During stage 3 with dynamic membrane separation, anaerobic sludge exhibited higher protease and β-GLC activities of 5 U/mL and 23 μmol/L·h, respectively. Efficient retention of biological enzymes by the dynamic membrane should be one of the main reasons for the enhanced enzymatic activities. Biological enzymes in anaerobic sludge can be divided into exoenzymes and endoenzymes. The former exist extracellularly, and the latter exist in microbial cells. Both of them could be rejected by the dynamic membrane and accumulate in the AFSFDMBR. Increasing the accumulation of organic matter for microorganisms should be another key reason for the increased enzymatic activities. With the help of the dynamic membrane, substrates could be retained, which led to their accumulation, thereby resulting in a rich-nutrient environment for microorganisms. Thus, the enzymatic activities increased, which promoted the degradation of the abundant substrates in the SFDMBR.

3.4. Microbial communities in the SFDMBR

3.4.1. Diversity indices

To investigate the influences of the SFDM separation on microorganisms during sludge fermentation, microbial communities were analyzed in different sludge samples, namely B, S, A1, A2, M1, and M2. As shown in Table 2, there were significant differences ($P < 0.05$) in the Shannon and Simpson diversity indices among the different samples. First, the application of the SFDM greatly reduced bacterial richness and evenness in the fermentation sludge, resulting in a sharp decrease of the Shannon index from 3.87 to 1.97, and an increase in the Simpson index from 0.04 to 0.31; however, these changes could be partly reversed by increasing the operation time. Moreover, the habitat on the surface of the dynamic membrane placed strong selective pressure on the microbial community. As a result, the bacterial richness and evenness of the sludge on the surface of the SFDM were lower than those in the reactor, and this situation could not be reversed by increasing the operation time. Finally, the two operation modes, the batch and continuous operations, had no obvious influence on bacterial richness and evenness, which resulted in similar diversity indices of the B and S samples. Thus, the application of the SFDM in a conventional fermenter accelerated the accumulation of effective bacteria, thereby resulting in reductions of bacterial richness and evenness in fermented sludge.

3.4.2. Genus-level taxonomic distribution

To further confirm the community functions, we performed a phylogenetic classification of the 16S rRNA gene sequences at the genus level. After removing genera with low relative abundances

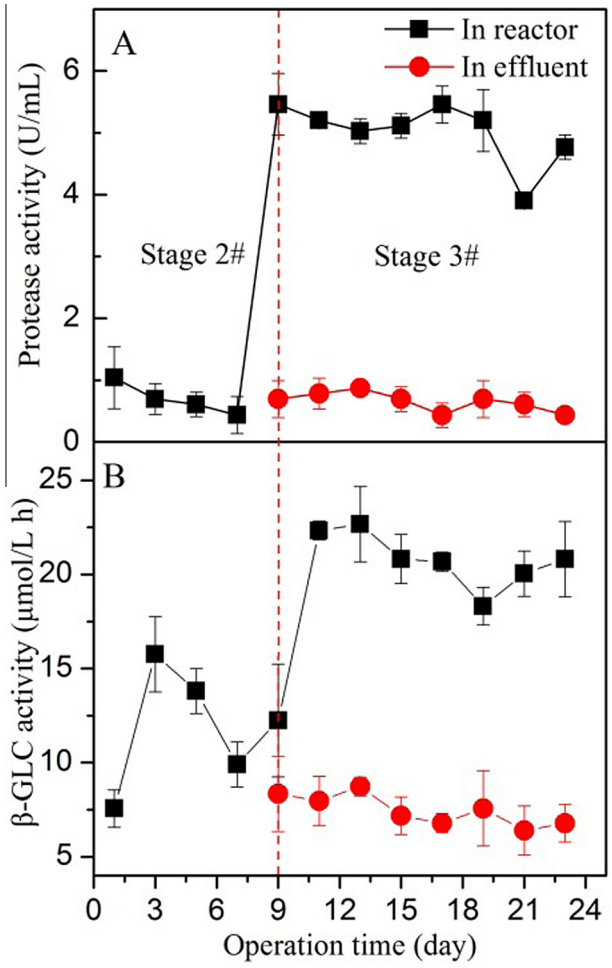

Fig. 5. Influence of the dynamic membrane separation on enzymatic activities.

Table 2
Diversity indices used in this study.[a]

Samples	B	S	A1	M1	A2	M2
OTU	238	241	165	157	171	186
Shannon	3.78	3.87	1.97	1.67	3.04	1.43
Simpson	0.05	0.04	0.31	0.45	0.10	0.51

[a] An asterisk indicates values that are significantly different ($P < 0.05$). The significant diversity is greater than 0.97. OTU, operational taxonomic units.

(<1%), 23 genera were categorized as the dominant genera in the six samples (Table 3).

Alkaliphilus and *SRB2 no rank* dominated in samples B and S, with 11% of the total population. Takai et al. (2001) reported alkaliphilic microorganisms was a strictly anaerobic chemoorganotroph capable of utilizing proteinaceous substrates in alkaline conditions. Therefore, their accumulations could improve protein hydrolysis. *Anaerobranca* had a higher relative abundance in sample S (14%) than in sample B (3%), which might be due to the greater abundance of substrates in sample S, since Gorlenko et al. (2004) ever reported it was the major bacterial genus capable of converting proteins and carbohydrates to acetate. Many microbial genera, such as *Alcaligenes*, *Petrimonas*, *Enterococcus*, *Proteiniclasticum*, and *Garciella*, only existed in samples B and S. By introducing the SFDM into a conventional fermenter, the relative abundance of *Alkaliphilus* increased from 11% (in sample S) to 22% (in sample A1), and the abundance of *Corynebacterium* increased from 6% (in sample S) to 51% (in sample A1). It has been reported that *Corynebacterium* could produce abundant hydrolytic enzymes (Lee et al., 1985), which should be the main contributors to the enhanced VFAs production during stage 3. *OPB54 no rank* (8%), the vadinBC27 wastewater-sludge group (14%), and *Bacillus* (2%) had higher relative abundances in sample A2 than sample A1. *Acinetobacter*, a fermentative, aromatic-degrading microorganism (Antunes et al., 2011), was also detected and largely enriched in sample A2.

Overall, the application of the SFDM has a great influence on the microbial structure of sludge fermenters. Dynamic membrane separation could accelerate the enrichment of bacteria, such as *Alkaliphilus*, *OPB54 no rank*, *Acinetobacter*, and *Corynebacterium*, that are capable of hydrolyzing substrates and that are either absent or have lower relative abundances in conventional fermenters. However, many bacteria that exist in conventional fermenters would disappear in the AFSFDMBR, thereby resulting in low bacterial richness in fermented sludge. Moreover, the dominant genus would shift with the operation time of the AFSFDMBR, which might be stimulated by the accumulation of refractory organic matter. For example, compared with sample A1, *Acinetobacter* appeared and the proportion of *Corynebacterium* decreased in sample A2. In addition, the living environment on the surface of the dynamic membrane constituted a strictly selective environment on which only a few bacterial genera, such as the vadinBC27 wastewater-sludge group (relative abundances of 66% in sample M1 and 70% in sample M2), could survive. Therefore, these results show that in the fermenter with the SFDM, the retention of substrates, including both refractory and easily degraded organic matter, not only induced the release of hydrolytic enzymes, but also accelerated the enrichment of bacteria with special functions, which enabled the dynamic membrane separation to enhance the VFA yield from sludge anaerobic fermentation.

3.5. Mass balance and WS reduction

Organic matters entering into AFSFDMBR system included the organic matters in the liquor and solid of WS. Organic matters flowing out from AFSFDMBR system include the organic matters in the membrane effluent, contained in the residual sludge and emitted as the gas. Using COD concentration to express the content of organic matters, the consumption approaches of organic matters in AFSFDMBR system was shown in Fig. 6. There was a difference of the total COD between that entering into and flowing out from AFSFDMBR system, and the ratio of the latter to the former is called COD recovery. A COD recovery that is close to 100% in a system shows a good carbon balance of the process (Arslan et al., 2012). In this study, the COD recovery determined by Eq. (4) was 94.26%, which indicated that this analysis of the carbon balance had high accuracy. This analysis showed that the carbon sources entering into the AFSFDMBR, calculated as the COD, were mainly released from the AFSFDMBR as the membrane effluent and residual sludge, which accounted for 68.4% and 25.8% of the COD, respectively. The amount of the carbon sources that was discharged as a gas was negligible because of the strict inhibition of methanogens by the alkaline conditions. Most of the solids in the influent were liquidized, and the VSS reduction ratio reached 82.5%, compared with 50% in conventional sludge fermentation processes (Liu et al., 2012). Moreover, the obtained VFAs exhibited high purity, and VFAs accounted for 73.4% of the total soluble organic matter, compared with 30–60% in conventional sludge fermentation processes (Liu et al., 2012). Therefore, the results indicate that both the yield and quality of the VFAs

Table 3
Abundance of bacterial genera in the different samples.

Taxonomic level (phylum, genus)		Relative abundance of 16S rRNA gene sequences (%)[a]					
		Batch	Continuous	Day 10		Day 20	
		B	S	A1	M1	A2	M2
Firmicutes	***Alkaliphilus***	**11**	**11**	**22**	**8**	**3**	**3**
	SRB2 norank	**11**	**11**	**4**	**2**	**9**	**12**
	Natronincola	6	8	1	–	2	–
	Enterococcus	5	3	–	1	–	–
	Proteiniclasticum	5	3	–	–	–	–
	Garciella	4	3	–	–	–	–
	Anaerobranca	**3**	**14**	**5**	**10**	**5**	**6**
	Proteiniphilum	3	1	–	–	–	–
	Tissierella	2	1	–	–	–	–
	Peptostreptococcaceae incertae sedis	2	2	–	–	–	–
	Sedimentibacter	2	1	–	–	–	–
	Clostridium	1	2	–	–	–	–
	OPB54 norank	1	3	5	5	8	1
	Caldicoprobacter	1	–	–	–	–	–
	Lachnospiraceae unclassified	1	–	–	–	–	–
	Selenomonadales norank	–	–	1	–	1	–
	Bacillus	–	–	–	–	2	–
	Ruminococcaceae incertae sedis	–	–	–	–	1	–
	Ruminococcaceae unclassified	–	–	–	–	–	1
Bacteroidetes	*Petrimonas*	6	7	–	–	–	–
	vadinBC27 wastewater-sludge group	3	4	3	66	14	70
	vadinHA17 no rank	1	–	–	–	–	–
Proteobacteria	*Alcaligenes*	5	3	–	–	–	–
	Acinetobacter	–	–	–	–	20	–
	Halomonas	–	–	–	–	1	–
RF3	*No rank*	1	3	–	1	–	–
Actinobacteria	***Corynebacterium***	9	6	51	–	22	–

Bold values: bacterial genera exisiting significant differences.
[a] –: Not detected.

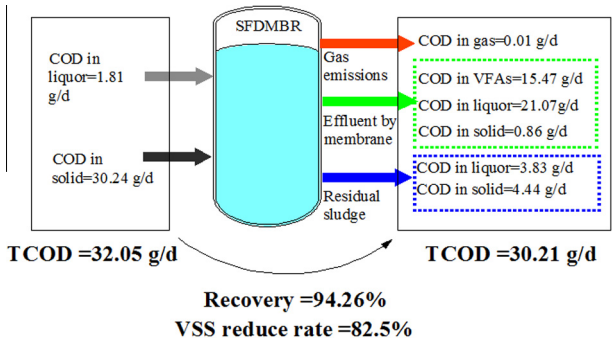

Fig. 6. COD balance during stage 3 in the AFSFDMBR.

could be greatly enhanced by applying SFDM separation in conventional sludge anaerobic fermentation.

4. Conclusions

Both yield and quality of the VFAs obtained from sludge were greatly improved by introducing SFDM into conventional anaerobic fermenter, and SFDM presented high stability. Efficient retentions of substrates, 70% of the proteins and 40% of the polysaccharides, and online releases of products, 90% of the ammonia and VFAs, were achieved in this innovative reactor. Additionally, the application of SFDM increased enzymatic activities, optimized the microbial community structure, and enriched functional bacteria, all of which contributed to the enhanced VFA yield, which was 61.21% higher than that of traditional batch fermentation and 227.87% higher than that of traditional continuous fermentation.

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