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Effects of temperature and temperature shock on the performance and microbial community structure of a submerged anaerobic membrane bioreactor

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ABSTRACT

Effects of temperature and temperature shock on the performance and microbial community structure of a submerged anaerobic membrane bioreactor (SAnMBR) treating thermomechanical pulping pressate were studied for 416 days. The results showed that the SAnMBR system were highly resilient to temperature variations in terms of chemical oxygen demand (COD) removal. The residual COD in treated effluent was slightly higher at 55 °C than that at 37 and 45 °C. There were no significant changes in biogas production rate and biogas composition. However, temperature shocks resulted in an increase in biogas production temporarily. The SAnMBR could tolerate the 5 and 10 °C temperature shocks at 37 °C and the temperature variations from 37 to 45 °C. The temperature shock of 5 and 10 °C at 45 °C led to slight and significant disturbance of the performance, respectively. Temperature affected the richness and diversity of microbial populations.

1. Introduction

Submerged anaerobic membrane bioreactor (SAnMBR) has attracted much interest due to its high-treatment efficiency, high biomass concentrations, low sludge production, excellent effluent quality, small footprint and net energy production (Liao et al., 2006). It has been successfully studied for a wide range of wastewater treatment in recent years, such as domestic wastewater, pulp and paper wastewaters (Xie et al., 2010), saline effluent, municipal solid waste leachate, and so on. However, the stability and efficiency of anaerobic treatment processes is greatly influenced by many factors (Chen et al., 2008), including wastewater specificity, hydraulic retention time (HRT), solid retention time (SRT), organic loading rate (OLR) (Wijekoon et al., 2011), pH (Gao et al., 2010), temperature (Choorit and Wisarnwan, 2007) and nutrient availability, etc. Among these factors, temperature is commonly believed to play a significant role in the biological wastewater treatment performance and stability.

Anaerobic digestion can be conducted in psychrophilic (<25 °C), mesophilic (25–40 °C), and thermophilic (>45 °C) temperature ranges (El-Mashad et al., 2004). Earlier studies investigating effect of temperature on the anaerobic digestion process have mainly focused on the comparison of the steady state performance at two or more fixed operating temperatures (Kim et al., 2002; Ndegwa et al., 2008; Yilmaz et al., 2008). The effect of temperature fluctuations on anaerobic treatment efficiency at certain temperature ranges has been also studied. A temporary decrease in the temperature (between 10 and 20 °C) of psychrophilic anaerobic reactors treating swine manure only have temporary effects on the performance and stability of the process (Massé et al., 2003). The effects of digestion temperature and temperature shock on the biogas yields, temperature shocks of the mesophilic anaerobic digestion were recently studied (Chae et al., 2008). Some of the studies were only conducted in the thermophilic range because thermophilic process is characterized to be more susceptible to the environmental and operational conditions than mesophilic process (Ahring et al., 2001; Iranpour et al., 2005). El-Mashad et al. (2004) found that the imposed daily upward temperature fluctuation affected the maximum specific methanogenesis activity more severely than daily downward temperature fluctuations in completely stirred tank reactors (CSTRs).

There are also a few reports of the influence of temperature variation from mesophilic to the thermophilic temperature ranges. Both temperature increases and temperature drops will affect the performance of the anaerobic digestion (Ahn and Forster, 2002).

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Bouskova et al. (2005) found that one-step temperature increase (from 37 to 55 °C) is better than step-wise increase in changing from mesophilic to thermophilic operation in anaerobic digestion.

The effects of temperature variations have been linked with poor sludge settling that brings high effluent suspended solids, effluent turbidity and biomass washout (Ahn and Forster, 2002). However, these problems caused by temperature variation can be alleviated in an SAnMBR with the incorporation of the membrane filtration technology. The employment of membrane in the anaerobic process can achieve complete solid–liquid separation, so that biomass can be fully retained in the reactor. Obviously, SAnMBR is more resistant to temperature variation than conventional anaerobic digestions do. To our knowledge, the effect of temperature variation and temperature shock on the performance and microbial community of an SAnMBR has not been investigated. Thus, the effect deserves to be investigated in detail.

Some causes of temperature variations can be controlled (operational conditions) or predicted (environmental conditions in a region) so that the system can be adjusted to accommodate to the new conditions, whereas sudden transient changes can lead to deterioration of the reactor's performance. In the pulp and paper industry, wastewaters are discharged at high strength and wide temperature (20–70 °C) range (Jahren and Rintala, 1997; Morgan-Sagastume and Allen, 2003). In this case, temperature variations can be caused by frequent temperature transits of wastewater streams or the accordant junction of them. Failure to control temperature (unexpected cooling and heating problems) will also introduce temperature fluctuations. It is important for a system to deal with these situations in industrial applications. The treatments of several types of pulp and paper effluents have been successfully achieved by mesophilic and thermophilic SAnMBRs in our previous studies (Gao et al., 2010; Xie et al., 2010). However, little is known about the tolerance of an SAnMBR to changes of operating temperature and temperature shocks. In addition, temperature has primary impact on microbial activity and community composition in a wastewater treatment reactor. Although microbial community has been analyzed in both anaerobic and aerobic membrane bioreactors (Calderón et al., 2011; Chang et al., 2011), there is insufficient understanding concerning the effect of temperature variations (from mesophilic to thermophilic temperatures ranges) and temperature shocks on microbial community structures in an SAnMBR.

Consequently, the purpose of this study was to investigate the feasibility of using an SAnMBR for thermomechanical pulping pressate treatment and to assess the influence of temperature and temperature shocks on the performance of the SAnMBR with respect to process stability, process recovery and microbial community in the reactor.

2. Methods

2.1. Experimental set-up

The lab-scale SAnMBR (10 L) used in this study was schematically presented by Xie et al. (2010). The system was equipped with a 0.03 m^2 of flat sheet ultrafiltration membrane module (Shanghai SINAP Membrane Science & Technology Co. Ltd., China). Nominal molecular weight cut off (MWCO) and material of the membrane were 70,000 Daltons and polyvinylidene fluoride (PVDF), respectively. The physical configuration of the SAnMBR was described previously (Xie et al., 2010), although the biogas sparging rate was increased from 0.75 to 1.5 L/min to mix the biomass continuously and to control solid deposition over the membrane surface. The SAnMBR was inoculated with seeded sludge developed from a previous study for kraft evaporator condensate treatment (Xie et al., 2010). During the operation of the reactor, no sludge was discharged except for sludge sampling. This corresponded to a sludge age of approximately 350 days. The pH value was maintained at 7.0 ± 0.2 by an automatic pH regulation pump and a pH electrode (Thermo Scientific, Beverly, MA).

Thermomechanical pulping pressate collected from a local pulp and paper mill (Abitibi-Bowater Inc., Canada) was auto matically fed into the reactor by a feeding pump which was controlled by a level sensor (Madison Co., USA) and controller (Flowline, USA). The characteristics of the thermomechanical pulping pressate are listed as follows: pH: 3.89–4.43; total sus pended solids (TSS): 170–400 mg/L; total COD: 2120–3600 mg/L; soluble COD: 1220–2000 mg/L; total phosphorus: 0.82–1.28 mg/L; total nitrogen: <0.03 mg/L; aluminum: 0.363–0.450 mg/L; Arsenic: 0.021–0.024 mg/L; barium: 0.489–0.577 mg/L; calcium: 33.67–34.18 mg/L; chromium: 0.003–0.004 mg/L; copper: 0.014–0.030 mg/L; iron: 0.304–0.384 mg/L; potassium: 31.8–34.2 mg/L; magnesium: 6.15–6.51 mg/L; manganese: 2.223–2.424 mg/L; sodium: 53.91–56.04 mg/L; lead: <0.015 mg/L; total sulfur: 59.03–61.29 mg/L; strontium: 0.127–0.134 mg/L; titanium: 0.010–0.014 mg/L; zinc: 0.302–0.367 mg/L. A trace element solution (Xie et al., 2010) was supplemented to the influent to prevent trace metal limitations of the methanogens. In order to sustain the nutrient concentrations required for biomass growth in an anaerobic environment, nitrogen (NH_4Cl) and phosphorus (KH_2PO_4) were fed as macro-nutrients in a proportion of COD:N:P of 100:2.6:0.4 (Xie et al., 2010). The trans-membrane pressure (TMP) was measured by a vacuum gauge connecting the bioreactor and the suction pump. Membrane flux was controlled by adjusting the speed of a peristaltic pump with intermittent suction of a 4-min run and 1-min pause cycle. Before starting the reactor, nitrogen (99.998%) was bubbled for 5 min to remove air in the system. The biogas production was monitored with a water displacement gas collector at ambient temperature (23–25 °C). The biogas yield values were corrected to standard conditions (1 atm, 0 °C).

2.2. Temperature variations

The operating temperature was controlled by circulating water through the water jacket. The main temperature variations were described as follows.

In phase 1, the wastewater was initially treated at a mesophilic temperature of 37 ± 1 °C. The SAnMBR was then subjected to periodic temperature shocks: 5 °C shocks (from 37 to 42 °C) and 10 °C shocks (from 37 to 47 °C). Three temperature shocks were conducted at each temperature fluctuation. The temperature shocks were simulated by increasing suddenly from 37 to 42 or 47 °C and lasted approximately 12 h from the point at which the temperature started to rise until the point at which the temperature began to drop. It was adjusted back to the normal operating temperature quickly (within 1 h) right after the shock. The next shock started after the system stayed at 37 °C for 36 h.

In phase 2, the operating temperature of the SAnMBR was increased slowly from 37 to 45 °C (0.5–1 °C/day) over a 10-day interval. Similarly, the SAnMBR was subjected to 5 °C shocks (from 45 to 50 °C) and 10 °C shocks (from 45 to 55 °C) after steady-state operation was achieved at 45 °C. Finally, the temperature was gradually increased to a thermophilic temperature of 55 °C (0.5–1 °C/day) over a period of 13 days (phase 3). Each operating temperature and temperature shock was delayed until the system performed as well as it was prior to the temperature change, except in phase 3. Two new pieces of membrane (one on each side of the module) were used for each operating temperature.

Samples of influent, effluent and mixed liquor were taken from the system every 2–3 days during the steady state of each operating temperature, while specific samples were collected right before and after the temperature shocks were imposed.

2.3. Particle size distribution

The particle size distribution (PSD) of mixed liquor was measured by a Malvern Mastersizer 2000 instrument (Worcestershire, UK) with a detection range of 0.02–2000 μm. The scattered light is detected by means of a detector that converts the signal to a size distribution based on volume or number. Each sample was measured 3 times. The PSD measurements were conducted routinely every week.

2.4. Analytical methods

The mixed liquor samples were centrifuged at 18,700 times of gravitational acceleration for 15 min to obtain supernatant for chemical oxygen demand (COD) measurement. COD and mixed liquor suspended solids (MLSS) were determined in duplicate according to standard methods (APHA, 2005).

Biogas samples were taken from the headspace of the reactor by a syringe. Composition of biogas (methane, nitrogen, and carbon dioxide) was measured by gas chromatography (Shimazu, GC-2014) equipped with a thermal conductivity detector and a silica gel packed column. Helium was used as the carrier gas at a flow rate of 30 mL/min.

2.5. Microbial community analysis

For microbial community analysis, duplicate samples of each mixed liquor sample were collected at the steady state of three operating temperatures. The mixed liquor samples before and after the temperature shocks were also collected. Genomic deoxyribonucleic acid (DNA) was extracted in duplicates from 0.25 g of mixed liquor samples respectively with a Fecal DNA isolation kit (MoBio Laboratories, Solana Beach, USA) according to the manufacturer's instructions.

Polymerase chain reaction (PCR) amplification was performed in 50 μL reaction volume on a Hybaid Thermocycler (Thermo Electron Corp., USA). The primer set, 341f-GC (5′-GC-clamp-CCTAGGGAGGCAGCAG-3′) and 534r (5′-ATTACCGCGGCTGCTGG-3′) were used. PCR cycling was consisted of an initial denaturation at 94 °C for 5 min followed by 35 cycles consisting of denaturation at 94 °C for 1 min, primer annealing at 56 °C for 1 min, and DNA extension at 72 °C for 1 min. A final extension step was conducted at 72 °C for 5 min prior to cooling at 4 °C. PCR products were purified using a DNA purification kit (Fermentas Life Sciences, Burlington, ON, Canada) in accordance with the manufacturer's recommendations prior to use in denaturing gradient gel electrophoresis (DGGE) analysis. Polyacrylamide gels (10% polyacrylamide, 25–65%) were cast using a gradient maker (Bio-Rad, USA). The gels were run at 30 V for 18 h at 60 °C. After electrophoresis, the polyacrylamide gel was stained in 150 mL TAE buffer containing 15 μL 10,000× concentrated SYBR Green I stain (Fermentas Life Sciences) for 1 h, and then photographed with a CCD camera (SynGene a division of Synoptics Ltd., UK) to acquire the DGGE band image. Duplicate measurements were performed for each sample.

Cluster analysis of the DGGE band patterns was performed with Fingerprinting II Informatix Software Program v.3.00 (Bio-Rad, USA) by using the Jaccard coefficient and the unweighted-pair group method with arithmetic mean (UPGMA). A position tolerance of 1.00% was used.

3. Results and discussion

3.1. Effect on COD removal

Throughout the 416 days of operation, the pH, OLR, membrane flux and gas sparging rate were controlled approximately constant to separate the influences of the temperature variation on system performance from other factors. MLSS concentration was maintained at 10.9 ± 0.5 g/L (Fig. 1). Although the growth/decay rates are different at different temperatures, MLSS concentration was not correlated to temperature variations, which will be discussed in later sections. The SAnMBR was operated at an average OLR of 2.59 ± 0.53 kg COD/m^3 day (Fig. 1). The influent COD concentrations fluctuated from 2120 to 3600 mg/L. The first 28 days were considered to be the initial start-up period during which the permeate COD value fell from 1220 to 470 mg/L to allow the acclimation of the biomass (Fig. 2).

The membrane flux was first maintained at 6.89 ± 0.56 L/m^2 h. After the operating temperature rose to 55 °C, the flux dropped to 5.68 ± 0.43 L/m^2 h (Fig. 1). It is known that PVDF is a hydrophobic and chemically inert fluoropolymer which has a very low glass transition temperature (Tg) of −40 °C and a relatively low melting point of around 177 °C, making it quite flexible for membrane application and tolerate with a wide temperature range (−30 and 140 °C) for various applications (Nunes and Peinemann, 2006). The suggested maximum operating temperature of PVDF membrane is 40 °C from a number of membrane manufacturers. In spite of this, the properties of the PVDF membrane may change with increasing temperature. The temperature usually enhances flux as the sludge viscosity becomes lower and the membrane pore size

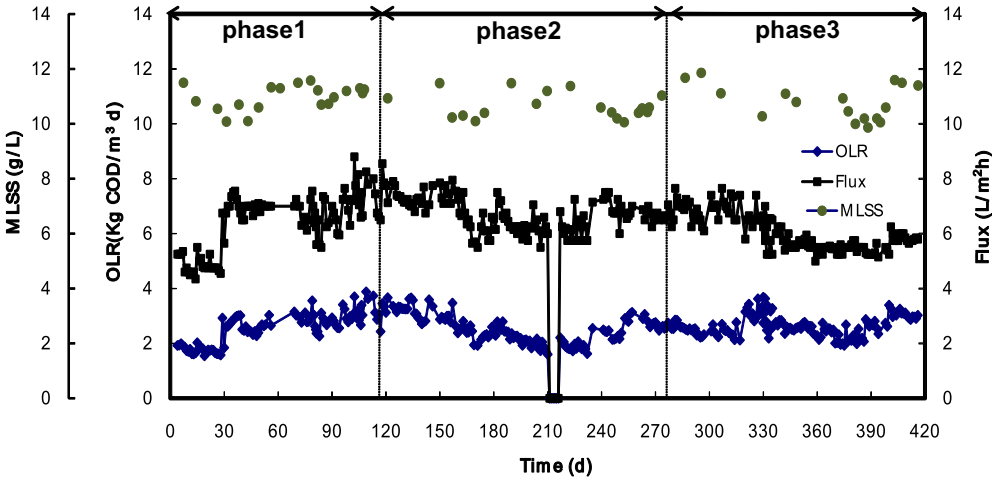

Fig. 1. Variation of organic loading rate and membrane flux during operation.

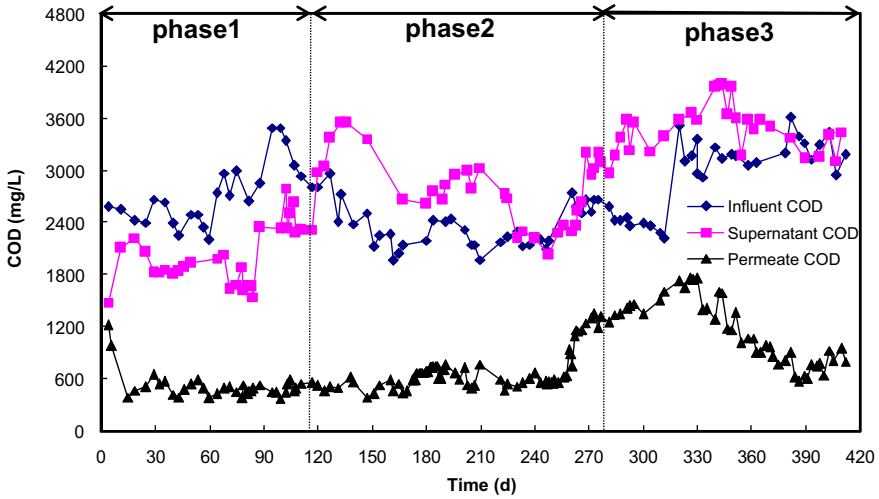

Fig. 2. Variation of the influent, supernatant and permeate COD.

may increase at a higher temperature. However, no such influence was observed with increasing temperature. Instead, it is noted that a higher temperature (55 °C) resulted in a lower membrane flux in this study. The impact of temperature on membrane flux was considered to be largely offset by a significant increase in membrane fouling extent. Potential impact of temperature on membrane properties needs to be further studied in future studies.

The stability and efficiency of the reactor is usually evaluated in terms of the COD removal, gas production and methane content. At phase 1, the permeate COD value changed between 380 and 530 mg/L during the steady state at 37 °C. The 5 and 10 °C temperature shocks were conducted as described above at day 78–83 and day 102–107, respectively. As shown in Fig. 2, there is no changes in permeate COD value during and after the temperature shocks. Although the higher COD value in the supernatant during the 10 °C shocks period can be observed, it decreased as soon as the temperature was restored to normal condition. These results indicated that the system can tolerate the 5 and 10 °C shocks at the operating temperature of 37 °C.

The reactor processes were reasonably stable while slowly increasing the temperature from 37 to 45 °C at phase 2. It even remained stable after short period (day 211–216) of shut down for repair of bioreactor. The permeate COD value remained between 500 and 720 mg/L until the 10 °C shock at 45 °C resulting in a deteriorating COD removal efficiency (from 80.6% to 53.3%). Meanwhile, the supernatant COD value went higher than the influent COD from then on probably because of improved dissolution of organic compounds and increased cell lysis at higher temperatures (Fig. 2). The SAnMBR seemed not to be affected by 5 °C temperature shocks (day 245–250), while the 10 °C temperature shocks (day 259–264) exerted significantly negative impacts on COD removal. This is supported by experiments on temperature shifts (Bouskova et al., 2005; Choorit and Wisarnwan, 2007) showed a loss of stability and change in the performance of the reactor when an operating temperature was raised over 45 °C. Because 45 °C is at the edge between mesophilic and thermophilic temperature ranges, the SAnMBR was more sensitive to the temperature shocks when operated at 45 °C than at 37 °C.

At phase 3, starting from day 278, the operating temperature gradually shifted from 45 to 55 °C. The permeate COD value subsequently went up to 1760 mg/L. It took approximately 94 days for the COD removal efficiency to restore to steady values exceeding 75%. The average level of permeate COD at 55 °C steady state was around 600–810 mg/L. The SAnMBR produced lower quality effluent during the 55 °C steady state compared with that during the 37 and 45 °C steady state in some sort. These results are consistent with previous findings (Harris and Dague, 1993; Song et al., 2004) that the mesophilic anaerobic digestion was superior in effluent quality to the thermophilic digestion. The poorer performances at thermophilic conditions and the above mentioned MLSS observations could be caused by the combined action of the temperature and the substrate limitation. In a thermophilic aerobic process, Abeynayaka and Visvanathan (2011) observed that although the reaction rates as well as the growth/decay rates are higher at thermophilic conditions than that at mesophilic conditions, achievement of maximum growth rate was simultaneously affected by the substrate available in the reactor. Limited substrate could enhance decay rate and thus induce low net biomass production. The by product of cell lysis and decay may cause COD increment both in supernatant and permeate. The poorer performances at 55 °C might also be attributed to that the community are less adept at utilizing the same range of substrates at a thermophilic temperature, which was found by LaPara et al. (2000) during an aerobic process. The high COD value of supernatant might indicate the presence of a great many of colloidal particle, soluble microbial products (SMPs), and volatile fatty acids in sludge suspension due to high temperature. Konopka et al. (1999) found that in aerobic biological wastewater treatment systems, thermophilic microbes might have more difficulty in maintaining cell integrity than mesophilic microbes. The release of cell lysate and other microbial products might be responsible for the high COD values in the supernatant. Further studies regarding the high supernatant COD value are needed.

3.2. Effect on gas production

Fig. 3A shows the biogas production rate converted to standard values (1 atm, 0 °C) during the whole operating time. At 37, 45 and 55 °C steady state, the biogas production rates were 0.21 ± 0.03, 0.20 ± 0.03 and 0.21 ± 0.02 L/g COD removed, respectively, indicating a similar biogas production rate at three operating temperatures. It may attribute to the similar species of microorganisms developed in the same reactor all along. The short period of shut down did not affect the biogas production which rose back to the previous level within 24 h. The observed biogas production rate

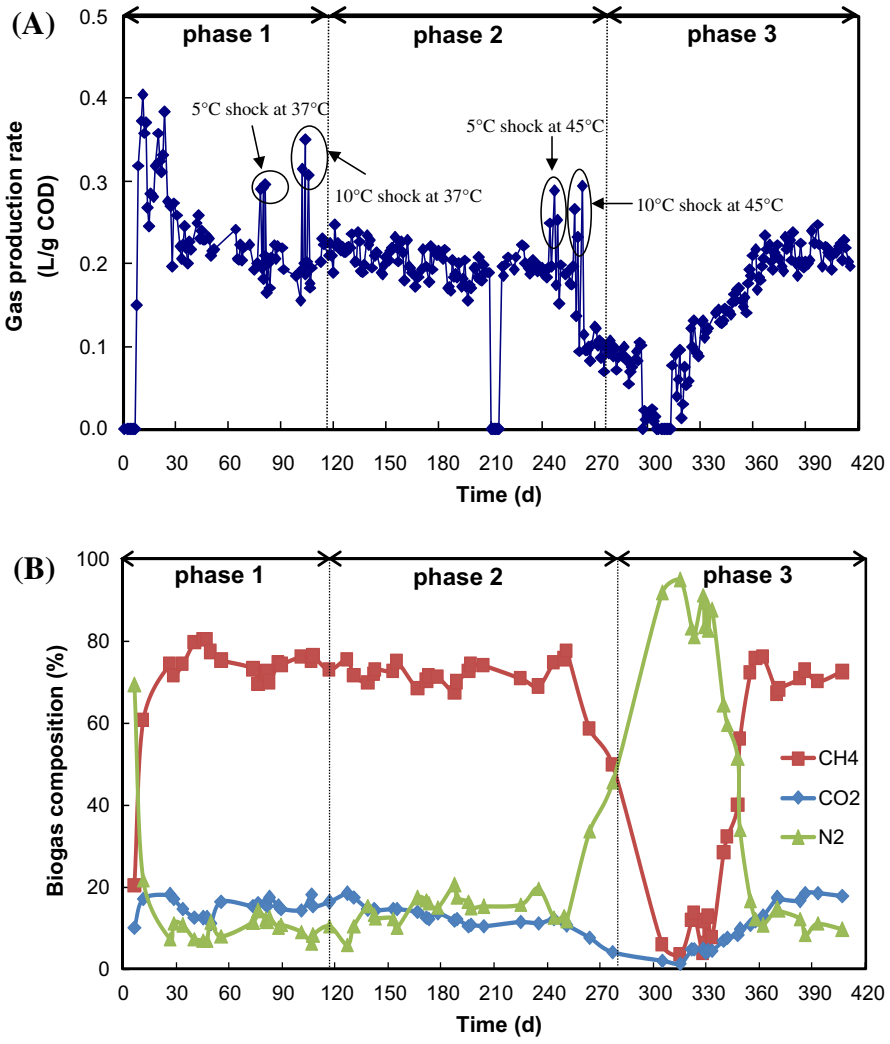

Fig. 3. Biogas production (A) variation of biogas production rate (B) biogas composition and concentration.

in this study could be less (up to 10%) than the theoretical values because of the loss of a small part of CO_2 in the water displacement arrangement by the dissolution of CO_2 in water.

An increase in biogas production rate was observed during each temperature shocks. Increasing temperature is known to lead to an increase in the maximum specific growth and substrate utilization rates (Chen and Hashimoto, 1980). Therefore, the temperature shock could temporarily result in much faster chemical and biological reactions (Ratkowsky et al., 1982). Both 5 and 10 °C shocks at 37 °C did not show any detrimental effect on biogas production rate and composition (Fig. 3B) after the shocks. Minor instabilities of the process were observed after the 5 °C shocks at 45 °C, although steady-state conditions were re-established within 24 h. However, temperature shocks of 10 °C at 45 °C gave a serious drop in the treatment efficiency. It took 16 days for the SAnMBR system to reproduce methane after the temperature variation from 45 to 55 °C in phase 3. The growth and activity rates of microorganisms as a function of temperature are considered to increase in certain temperature ranges. Further increases in temperature cause a decline in these rates until zero. Therefore, the extent of influences was determined by the magnitude of temperature shocks and the tolerance of the microorganisms in the sludge liquor, which is in agreement with the finding of El-Mashad et al. (2004). Barr et al. (1996) also found that in an activated sludge system, transient upsets in treatment efficiency were proportional to the magnitude of the rapid temperature decreases.

The first indication of a loss of stability of the process was biogas production rate. It occurred right after the 10 °C shocks (at 45 °C) were imposed, indicating a significant change in the balance among the microbial groups involved in the system as the various groups of bacteria respond the change in a different manner (Peck et al., 1986). For a complex anaerobic microbial community, the biogas production depends on the activities of various groups of bacteria during the four steps (hydrolysis, fermentation, acetogenesis and methanogenesis) of the anaerobic digestion. When the system reached a steady state at certain operating temperature, these groups of bacteria were compatible. When the temperature was beyond the durability of bacteria, their death rate would exceed growth rate and consequently result in a loss of the performance of the SAnMBR in terms of the removal efficiency and biogas production (Visser et al., 1993). Slower yield of methanogens due to high temperature (Kim et al., 2002) may be responsible for the long recovery period. Although the anaerobic microorganisms in the SAnMBR could not quickly adapt to the imposed high temperature variation, they finally thrived at the new living condition and had a tolerance for a fairly wide range of temperatures.

3.3. Effect on particle size distributions

Fig. 4 shows the comparison of particle size distributions (PSDs) before and after each temperature shocks. Fig. 4A shows that the large flocs shrinked after the 5 °C shocks at 37 °C. Floc strength

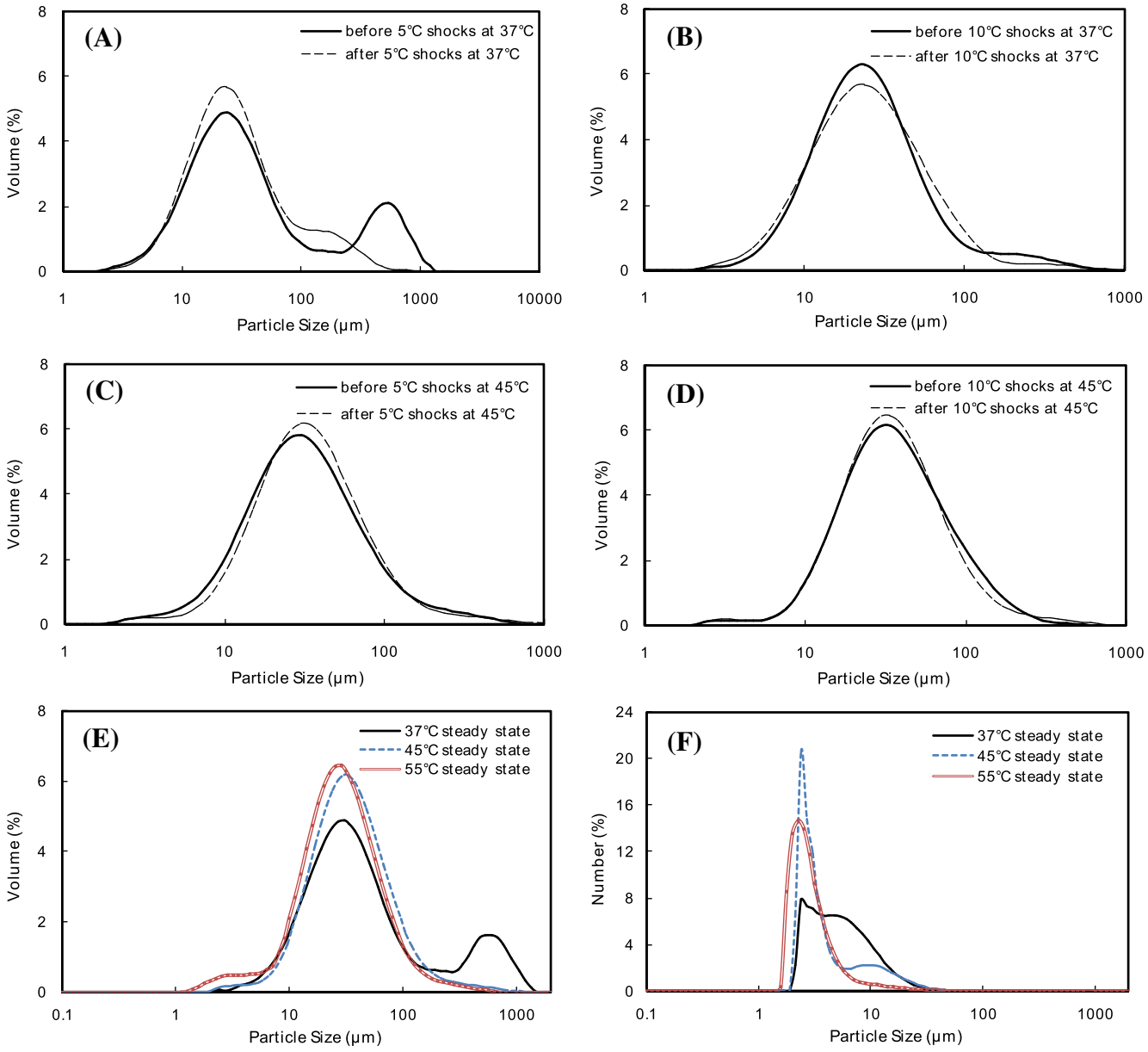

Fig. 4. Particle size distribution of sludge suspension (A) before and after 5 °C shocks at 37 °C (B) before and after 10 °C shocks at 37 °C (C) before and after 5 °C shocks at 45 °C (D) before and after 10 °C shocks at 45 °C (E) at 37 °C, 45 °C and 55 °C steady state based on volume (F) at 37 °C, 45 °C and 55 °C steady state based on number.

has been reported to decrease with increasing temperature (Jarvis et al., 2005), so higher temperature during the shocks induced the breakage of large flocs. There is no obvious difference between the two curves in Fig. 4B–D indicating 10 °C shocks at 37 °C, 5 and 10 °C shocks at 45 °C had no significant impact on PSDs.

The PSDs of sludge suspension in the SAnMBR at steady state of three operating temperatures are presented in Fig. 4. Two distinct peaks of the PSD of 37 °C steady state ranging from 2 to 1500 μm are showed in Fig. 4E. One is in the range of 2–200 μm with a mean size of 28–36 μm, and the other is in the range of 200–1400 μm with a mean size of 564–632 μm. Although the mean sizes of sludge flocs at 45 and 55 °C steady state are similar, we can see a small portion in the range of 1.5–2 μm at 55 °C. Much larger differences can be noticed in the PSD based on number (Fig. 4F). At the 55 °C steady state, 92.2% of sludge flocs were smaller than 5 μm. This percentage decreased by 75.8% when the operating temperature was 45 °C. Only 48.5% of flocs were in that size range during 37 °C steady state, and there were a large part of flocs in the range of 5–20 μm. The results suggest that operating temperature had a significant impact on the floc size in the SAnMBR. Long-term exposure at high temperatures can induce the deflocculation of the sludge flocs with large sizes (Morgan-Sagastume and Allen, 2005). In spite of this, the MLSS and the effluent quality were not affected by the deflocculation because of the efficient separation by membrane filtration, demonstrating that SAnMBR have the ability to alleviate the effect of temperature variations on the performance of the system in some extent.

3.4. Microbial community

The DGGE fingerprints of mixed liquor samples collected at three operating temperatures and after the temperature shocks were presented in Fig. 5A. It was evident that not only the diversity

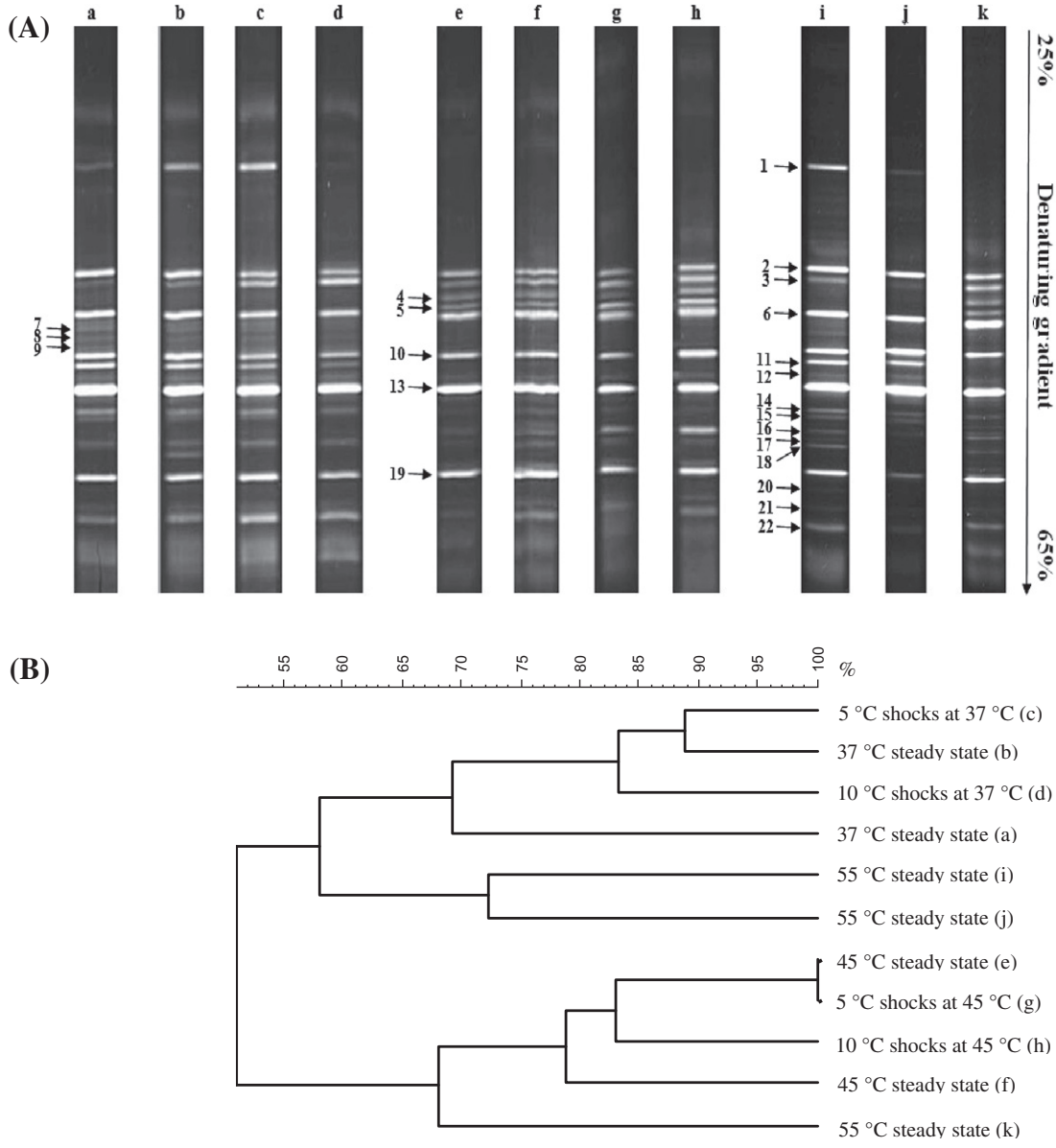

Fig. 5. PCR-DGGE fingerprints of 16S rDNA gene fragments amplified from DNA extracted from bulk sludge (A) and cluster analysis of the DGGE band patterns (B). (a) 37 °C steady state (day 53); (b) 37 °C steady state (day 68); (c) 5 °C shock at 37 °C; (d) 10 °C shock at 37 °C; (e) 45 °C steady state (day 207); (f) 45 °C steady state (day 245); (g) 5 °C shock at 45 °C; (h)10 °C shock at 45 °C; (i) 55 °C steady state (day 400); (j) 55 °C steady state (day 412); (k) 55 °C steady state (day 416).

but also the species richness of the microbial populations was affected by temperature variations.

It appears that sudden temperature increases had little impact on the microbial community. Temperature shocks at 37 °C did not affect the microbial community, except that there was a missing of band 1 after 10 °C shocks at 37 °C. For temperature shocks at 45 °C, only some changes in intensity of band 14, 15, 16, 17 and 18 were observed. The dendrogram generated by cluster analysis also reflects similarities of DGGE band patterns between the temperature shocks and steady states accordingly (Fig. 5B). There are 3 major clusters with similarity at approximately 70%. In addition, the 5 and 10 °C shocks at 37 °C and 37 °C steady state were located in one cluster, and so were the 5 and 10 °C shocks at 45 °C and 45 °C steady state. These results further confirmed the results of the performance of the SAnMBR that temperature shocks didn't affect the COD removal ability of the reactor.

Comparing the DGGE patterns of the mixed liquor samples at 37, 45 and 55 °C steady state, it is clear that there are some differences in species diversity among the three operating temperatures. For example, band 11 which appeared both at 37 and 55 °C steady state were missing at 45 °C. Band 4 and 5 were detected at 45 °C steady state while they were not detectable at 37 °C steady state. Moreover, in the dendrogram (Fig. 5B), three main clusters clearly separated the patterns of the three operating temperatures except that lane k was more close to lane f. On the other hand, there were populations (bands 2, 6, 10, 13 and 19) presented in all the samples. These species may be highly temperature tolerant and play a significant role in maintaining the stability of the SAnMBR. This might be the reason of the similar biogas production rate at 37, 45 and 55 °C steady state. The similarity of the microbial community at three operating temperatures may be due to the development of thermotolerant mesophiles rather than true thermophiles when the temperature was raised from the mesophilic range to the thermophilic range (Iranpour et al., 2002).

Some temporal changes in the DGGE patterns at 55 °C steady state within 16 days of operation can be noticed (Fig. 5A, lanes i,

j, k). Cluster analysis also illustrates that similarity of microbial community between 55 °C steady state was the lowest among that between the steady states of three operating temperatures, and k seems to be more like the 45 °C than the other two 55 °C populations (Fig. 5B). After the temperature was lifted from the mesophilic range to the thermophilic range, some microorganisms adapted to the new living conditions quickly. In contrast, some other populations which could not acclimatize themselves to the new condition decreased sharply or even disappeared in the system, while a minor part of them got the chance to live because of their high temperature tolerance. This part of microorganisms would grow up and become abundant again.

4. Conclusions

It is feasible to treat thermomechanical pulping pressate by using an SAnMBR at the operating temperatures of 37, 45 and 55 °C and achieve a COD removal efficiency of 76–83%. Temperature shocks led to a temporary increase in biogas production rate. Larger magnitudes (10 °C) of temperature shock had more severe impact on the performance of the SAnMBR. Increasing the operating temperature caused the deflocculation of the large sludge flocs. Temperature shocks had little impact on the microbial community structure, while the diversity and species richness would be affected by temperature variations.

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