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Microbial community analysis of anaerobic granules in phenol-degrading UASB by next generation sequencing

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ABSTRACT

The objective of this study was to investigate microbial communities in a continuous anaerobic phenol-degrading system using a next generation sequencing tool. The anaerobic granules adapted to phenol were first obtained by repeated-batch operation, which were then inoculated in an up-flow anaerobic sludge blanket reactor (UASB) operated at various organic loading rates (OLRs). Lag periods for both phenol degradation and CH_4 production decreased as batch fermentation was repeated, indicating a progressive adaptation of the granules to phenol. In the UASB operation, the highest OLR handled was 6 kg COD/m^3/d, in which the attained biogas production rate, phenol degradation, and CH_4 contents were 2.1 m^3/m^3/d, 79.0%, and 75.3%, respectively. *Syntrophorhabdus* and *Clostridium* were found to be the dominant bacteria, whose sum occupied around 60% of total bacterial sequences. In particular, there was a significant increase in *Syntrophorhabdus* (39.2% of total bacterial sequences), known to degrade phenol to benzoate and subsequently to acetate and hydrogen in syntrophic association with a hydrogenotrophic methanogen. In terms of archaea, *Methanosaeta* (42.1% of total archaeal sequences), and *Methanobacterium* (24.5% of total archaeal sequences) became dominant as operation continued, which were negligible in the inoculum.

1. Introduction

Anaerobic digestion (AD) is a biological treatment process that stabilizes organic wastes with the subsequent conversion to biogas, comprised of methane (CH_4) and carbon dioxide. The produced biogas can then be utilized for heat and electricity generation, or directly supplied to households and vehicles after upgrading, which can substitute natural gas [1]. The main feedstock of AD has been agricultural by-products, sludge, organic fraction of municipal solid wastes, and manure; however, there also have been a few attempt to treat toxic compounds such as phenol by AD [2,3].

Phenol is a wide spread contaminant found in different industrial wastewaters generated from petroleum refineries, coal gasification, coking, and pharmaceutical plants. As a toxic and potentially carcinogenic chemical, the release of phenol into the environment is of great concern. To survive in the presence of phenol and degrade it, easily biodegradable organics such as glucose and sucrose were sometimes supplemented with phenol [4,5]. However, numerous reports have shown that anaerobic microorganisms can tolerate and utilize phenol as the sole carbon and energy source [2]. With the support of enzymes and intermediates detected, it was reported that phenol can be degraded by two pathways: 4-hydroxybenzoate into benzoyl-CoA and caproate to acetate [6–8].

In the reactor configuration being applied for phenol degradation, the early study focused on simple batch and completely stirred tank reactor (CSTR) operation. However, a high-rate reactor configuration such as an up-flow anaerobic sludge blanket reactor (UASB) also has been used to handle high organic loading [3,9]. The inoculated granules were generally obtained from anaerobic digester plants treating various wastewater sources such as dairy wastewater [10], brewery wastewater [9], and tobacco wastewater [11]. Because these seeding sources have never been exposed to toxic chemicals, a change in the microbial community

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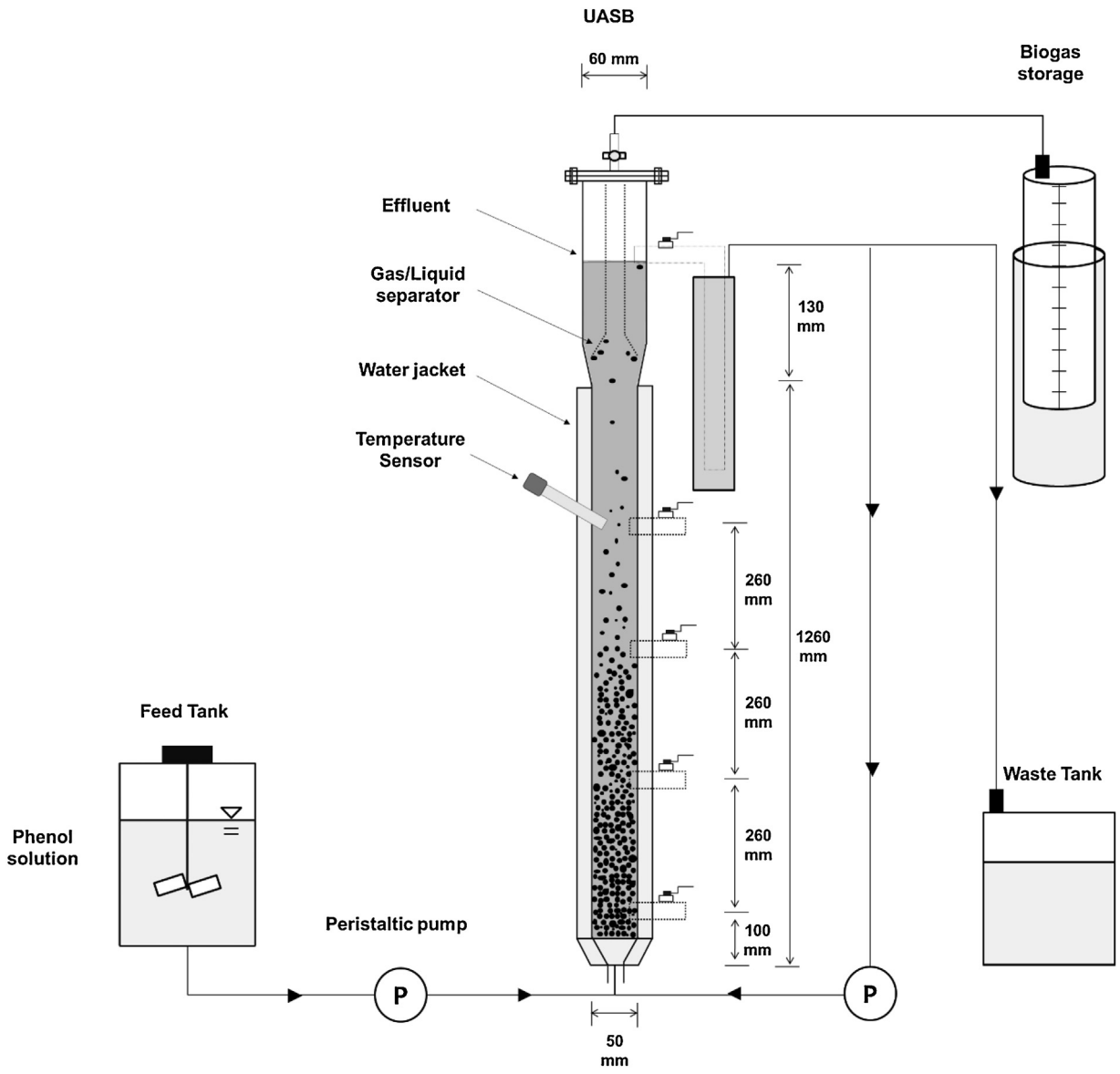

Fig. 1. A schematic of phenol-degrading up-flow anaerobic sludge blanket reactor.

can easily be expected during continuous operation. Knowledge on these population changes is helpful to elucidate the complex phenol-degrading pathway and provides basic information to further enhance the performance. However, there have been limited molecular-based studies on microbial communities in continuous anaerobic phenol-degrading system [11,12].

The tools previously used in treatment of phenol were fluorescence in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), and 16S rRNA gene clone library analysis, which are laborious, expensive, and have a low sensitivity when using a large number of samples [6,11,13]. On the other hand, a recent development of high-throughput sequencing technology called next generation sequencing (NGS) has made a huge number of sequences available within acceptable costs to explore microbial structure with higher resolution and clearly revealing complicated interactions among different microbial populations [14]. The number of studies using NGS investigating microbial communities in various habitats is rapidly increasing but never have been applied to phenol-degrading microbial consortia.

In the present work, anaerobic granules adapted to phenol were first obtained by repeated-batch operation, which were then inoculated in an UASB operated at various OLRs. Biogas production and intermediates were monitored during the continuous and batch operations. Finally, the microbial communities of seeding source and phenol-degrading UASB were investigated with an NGS tool. The obtained knowledge on bacterial and archaeal information was linked to the degradation pathway of phenol.

2. Material and methods

2.1. Inoculum preparation

The granulated sludge obtained from a full-scale anaerobic plant treating brewery wastewater was used as a seeding source. The pH, alkalinity, volatile suspended solids (VSS) concentration and total solids concentration (TSS) were 7.5, 6.7 g $CaCO_3$/L, 102.6 g VSS/L and 157.3 g TSS/L, respectively. Prior to use, the inoculum was acclimated under mesophilic condition (35 °C) until biogas production was no longer observed.

2.2. Adaptation of anaerobic granules

To adapt anaerobic granules to phenol, a batch operation was repeated three times. In a 1.5 L cylindrical fermenter (working volume of 1.0 L; 191 mm high with a 100 mm ID), 500 mL of granules and 500 mL of phenol-containing medium were added. The phenol concentration in the medium was 1 g/L on chemical oxygen demand (COD) basis, and NH_4Cl, KH_2PO_4, and $FeCl_2{\cdot}4H_2O$ were supplied at a COD:N:P:Fe ratio of 100:5:1:0.33. The medium also contained the following nutrients (in mg/L): $NaHCO_3$ 2000; $MgCl_2{\cdot}6H_2O$ 200; $CaCl_2{\cdot}2H_2O$ 150; $Na_2MoO_4{\cdot}4H_2O$ 0.02; H_3BO_3 0.1; $MnCl_2{\cdot}4H_2O$ 1.0; $ZnCl_2$ 0.1; $Cucl_2$ 0.06; $NiCl_2{\cdot}6H_2O$ 0.1; $CoCl_2{\cdot}2H_2O$ 1.0; and Na_2SeO_3 0.1 [15]. Before starting fermentation, N_2 gas was purged for 10 min to establish an anaerobic condition. The agitation speed was set at 10 rpm to avoid breaking the granules. When biogas production ceased, the agitation was stopped for 30 min, and 500 mL of supernatant was decanted, which was replaced by 500 mL of new medium. The reactor was placed in a temperature controlled room at 35 °C. Biogas production, residual phenol, and intermediates were measured at interval of 1–5 d. The batch operation was conducted in triplicate and the results were averaged.

2.3. UASB operation

As the seed biomass, 1 L of the adapted granules obtained from the repeated-batch operation was transferred to an UASB (working volume 2.5 L, Fig. 1). An effluent equivalent to five times the hydraulic loading was recycled to reduce the phenol toxicity in the UASB [6,9]. The OLR was gradually increased from 0.5 to 5 kg COD/m^3/d by increasing the phenol concentration from 0.25 to 2.5 g COD/L at a fixed HRT of 12 h. As performance failure was observed at 2.5 g COD/L, the phenol concentration was decreased to 2.0 g COD/L, after which the HRT was gradually shortened, up to 6 h. At each phase, the reactor was operated for at least 10 days at more than 20 times of the HRT to establish steady-state conditions, determined by the metabolic products. The temperature of UASB was controlled using a water bath circulator and a built-in water jacket at 35 °C.

2.4. Microbial community analysis

DNA in the mixed samples from the reactor was extracted and purified using an Ultraclean Soil DNA Kit and Microbial DNA Isolation Kit (Mo Bio Laboratory Inc., CA, USA). Then libraries prepared using PCR products according to the GS FLX titanium library prep guide were quantified using Picogreen assay (Victor 3). The emPCR, corresponding to clonal amplification of the purified library, was carried out using a GS-FLX titanium emPCR Kit (454 Life Sciences CT, USA). A 20 ng aliquot of each sample DNS was used for a 50 μl PCR reaction. The 16S universal primers 27F (5′ GAGTTTGATCMTGGCTCAG 3′) and 800R (5′ TACCAGGGTATCTAATCC 3′) for bacteria, and Arch349-F (5′GYGCASCAGKCGMGA AW 3′) and Arch1017R (5′ GGC CAT GCA CCW CCT CTC 3′) for archaea, were used for amplifying the 16s rRNA genes [16–18]. A Fast Start High Fidelity PCR System (Roche) was used for PCR as previously described [17]. After the PCR reaction, products were purified using AMPure beads (Beckman coulter). Sequencing was then performed using a 454 pyrosequencing Genome Sequencer FLX Titanium (Life Sciences, CT, USA), according to the manufacturer's instructions, by a commercial sequencing facility (Macrogen, Seoul, Korea).

The sequences generated from the pyrosequencing were mainly analyzed with the software MOTHUR for pre-processing (quality-adjustment, barcode split), identification of operational taxonomic units (OTUs), taxonomic assignment, community comparison, and statistical analysis. The methods for sequence filtration and trimming were done as previously described [17]. The sequences spanning the same region were then realigned with the NCBI BLAST database (www.ncbi.nlm.nih.gov). In the database screening with the BLAST program, the threshold E-value to include a sequence in the next iteration was 0.001.

2.5. Chemical analysis

The volume of produced biogas in the batch operation was measured by the displacement of a glass syringe piston, while it was measured by water-displacement method using a gas storage tank in the UASB operation. The amount of produced gas was converted to a value at standard temperature and pressure (STP) conditions. Biogas at the head space in the reactors was collected using a gas-tight micro syringe, and the biogas composition was analyzed by a gas chromatograph (Gow-Mac Series 580, USA) equipped with a thermal conductivity detector and a stainless steel column. The temperatures of the injector, column, and detector were kept at 50, 80, and 90 °C, respectively. The carrier gas was N_2 and the flow rate was 30 mL/min.

Organic acids including volatile fatty acids (VFAs, C2-C6) and lactate were analyzed by a high performance liquid chromatograph (HPLC) (Finnigan Spectra SYSTEM LC, Thermo Electron Co.) with an ultraviolet (210 nm) detector (UV1000, Thermo Electron) and an 100 × 7.8 mm Fast Acid Analysis column (Bio-rad Lab.) using 0.005 M H_2SO_4 as the mobile phase. Aromatic compounds (phenol and benzoate) were determined by HPLC (model FCV-10AL, Shimadzu) with an Eclipse XDB-C18 column (Agilent) and an SPD-M10A UV-detector using a 50% (v/v) acetonitrile as the mobile phase. The liquid samples were pretreated with a 0.45 μm membrane filter before injection to both HPLCs. The concentration of COD, SS, and alkalinity were measured according to the Standard Methods [19].

3. Results and discussion

3.1. Adaptation of anaerobic granules to phenol

Fig. 2 shows the cumulative CH_4 production and residual phenol concentration during repeated-batch operation. The cumulative CH_4 production was fitted by modified Gompertz eq. ($R^2 > 0.99$) [14].

Of note, the lag periods for both phenol degradation and CH_4 production decreased as the batch fermentation was repeated, which indicates a progressive adaptation of the granules to phenol. Drastic phenol degradation started after 10 d, 5 d, and 3 d at the first, second, and third batch operations, respectively. Likewise, the lag period for CH_4 production determined by modified Gompertz eq. decreased as follows: 16.8 d, 8.5 d, and 4.2 d at the first, second, and third phenol additions, respectively. Over 95% of the phenol was degraded in all batch operations, while there was a difference in the total amount of CH_4 produced. The CH_4 production yield (the amount of CH_4 produced/theoretical amount of CH_4 produced from the added phenol) at the first batch operation was 75%, which increased to 95% at the second and third batch operations. This meant that, initially, a large amount of energy expenditure occurred to adapt to toxic environment, for the proliferation of phenol-degrading community, and the synthesis of related enzymes.

As clearly observed in the first batch, a huge lag existed between the actual CH_4 production curve and theoretical one (calculated by assuming that the removed phenol was totally converted to CH_4), indicating the accumulation of intermediates such as benzoate and acetate (Fig. 2). Two mechanisms have been reported in anaerobic biological degradation of phenol: via 4-hydroxybenzoate into the benzoyl-CoA pathway or via caproate to acetate, but the for-

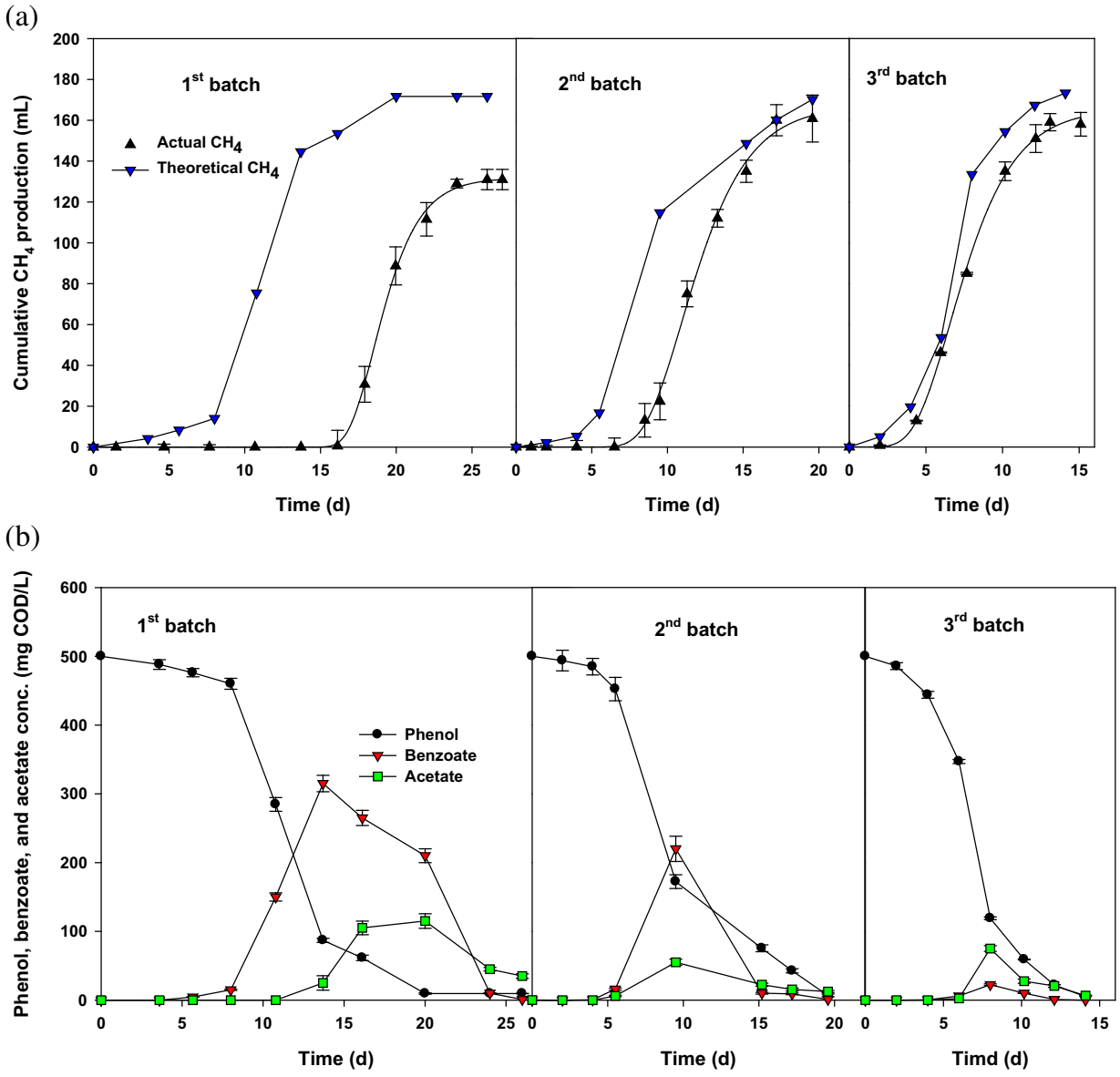

Fig. 2. (a) Cumulative CH4 production, and (b) residual phenol and intermediates concentration during repeated-batch operation.

mer is generally known to be favored in mesophilic condition [2]. According to that pathway, phenol is first reduced to benzoate, and then degraded to acetate, and finally to CH_4 with the participation of various microbial species [4,20]. As in our study, benzoate and acetate have often been detected in mesophilic anaerobic condition, in particular, during the adaptation period and under shock loading [4,5,7].

In the first batch, benzoate started to appear as soon as phenol degradation began, while acetate was detected around 3 d later. This time gap clearly indicates the sequential degradation pathway in the anaerobic conversion of phenol. The maximum concentration of benzoate (315 mg COD/L) was higher than that of acetate (115 mg COD/L), which is ascribed to the fact that the used granules already have a capability to convert acetate to CH_4. As the batch operation was repeated, the total concentration of intermediates in the broth decreased. In the 3rd batch, the sum of intermediates was less than 100 mg COD/L, resulting in a small time gap between the actual and theoretical CH_4 production. It also implies that once the anaerobic consortium is adapted to phenol, the rate-limiting step for methanogenic culture will be the first step: phenol reduction to benzoate. Generally, granules degrading phenolic wastewater exhibit the following order of specific methanogenic activity toward different substrates, acetate > benzoate > phenol [3,21].

3.2. Continuous performance in the UASB

The daily biogas production rate and phenol removal (%) at various operating conditions are shown in Fig. 3. It seemed that an applied starting OLR of 0.5 g COD/L/d (HRT 12 h, phenol concentration 0.25 g COD/L) was the proper condition for the adapted anaerobic granules to utilize phenol. Phenol degradation and the biogas production rate soon reached over 90% and 0.27 $m^3/m^3/d$, respectively, which were kept constant under the given OLR.

As the phenol concentration increased, biogas production proportionally increased, up to 2.0 g COD/L at a fixed HRT of 12 h. The biogas production rate at an OLR of 4.0 kg COD/m^3/d (HRT 12 h, phenol concentration 2 g COD/L) was 1.50 ± 0.07 $m^3/m^3/d$ with showing an average phenol degradation of 88% ((input-residual)/input × 100). The CH_4 content in the biogas was 75.7 ± 1.3%, resulting in a CH_4 yield of 283 mL

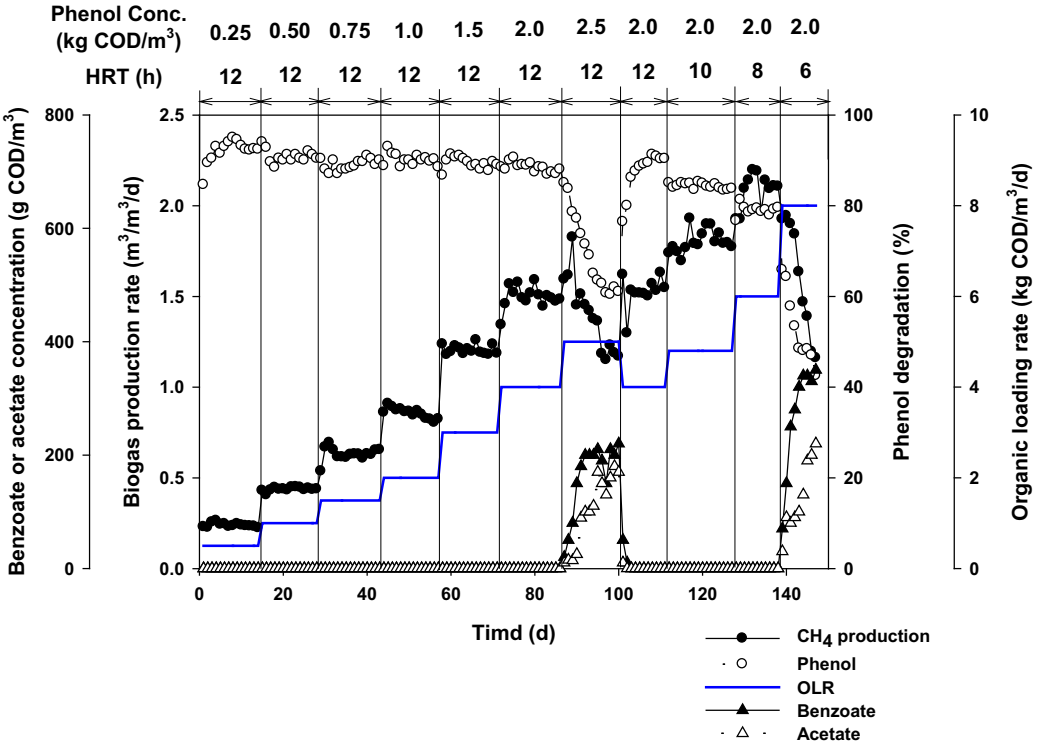

Fig. 3. Daily production of biogas and phenol degradation at various substrate concentrations and HRTs in UASB operation.

CH_4/g COD_{added}. The rest of biogas was mainly composed of CO_2 while H_2 content was not noticeable (<0.1%). The obtained yield indicates an 82% conversion of input phenol to CH_4 (1 g COD = 350 mL CH_4). Compared to the general CH_4 content in organic waste treatment, a higher value was attained in this study, which might be attributed to the fact that phenol is a more reduced form than carbohydrate matter. The theoretical CH_4 content is 58% ($C_6H_6O + 4H_2O \rightarrow 3.5CH_4 3{\cdot}5CH_4 + 2.5CO_2{,}2{\cdot}5CO_2$, $\Delta G = -399$ kJ/mol), in methanogenic phenol degradation, while it is 50% in the case of glucose ($C_6H_{12}O_6 \rightarrow 3CH_4 + 3CO_2$, $\Delta G = -149$ kJ/mol).

When the phenol concentration was increased to 2.5 g COD/L at a HRT 12 h, a drop in biogas production was observed with a gradual decrease in phenol degradation to 60%. In addition, during this period, benzoate and acetate started to appear, which agreed with previous works reporting that the conversion of benzoate to CH_4 could be suppressed by the presence of residual phenol [2,4]. Upon further UASB operation, the phenol concentration decreased to 2.0 g COD/L again, to recover the performance.

From the 112th d, the OLR was increased by reducing the HRT at a fixed phenol concentration of 2.0 g COD/L. Although a slight drop in phenol degradation was observed at a HRT of 10 h and 8 h, a steady-state performance was attained. At an OLR of 6 kg COD/m^3/d, the average biogas production rate, phenol degradation, and CH_4 content were 2.1 ± 0.1 m^3/m^3/d, 79.0 ± 1.0%, and 75.2 ± 1.6%, respectively, which were comparable with previous works [3,9–11]. The calculated CH_4 yield was 261 mL CH_4/g COD_{added}, corresponding to a 75% CH_4 conversion efficiency. Considering of 79% phenol degradation, most of the degraded phenol was converted to CH_4, while only 4% (=79% − 75%) of the input phenol was converted to biomass or intermediates. Benzoate and acetate were not detected in the effluent during this period. The general cell yield of the anaerobic consortia in using phenol as a carbon source was reported to be in the range of 2–3% (COD/COD) [22]. When the OLR was further increased to 8 kg COD/m_3/d by reducing the HRT to 6 h, a drastic drop in performance was observed. Phenol degradation almost dropped down to 40%, and a considerable amount of benzoate and acetate were also detected in the effluent. The concentration of benzoate and acetate increased up to 350 mg COD/L and 220 mg COD/L, respectively.

3.3. Microbial community

To reveal the shift in the microbial community structure in response to phenol, the inoculum, and granules collected on the 80th day and at the end of operation were used to pyro-sequence the former region of the 16S rRNA gene using a 454 GS-FLX sequencer. The sampling was prepared as a mixture of 10 mL mixed liquor at two ports (10 cm, and 36 cm from the bottom). A total of 35,983 (bacteria) and 37,961 (archaea) high quality sequence reads and 74 (bacteria) and 115 (archaea) operational taxonomic units (OTUs) with similarity cutoffs of 3% were obtained from a single lane of an eight-lane pico-titer plate on the Genome Sequencer FLX Titanium system.

Fig. 4 shows the changes in diversity of key bacteria at start-up (inoculum), at the 80th operation (U1), and at the end of operation (U2). Clear differences in the relative abundances were observed as UASB operation continued. At the bacterial class level, there was an apparent increase in relative sequences of *Deltaproteobacteria* (33.6% in U1 and 48.6% in U2), which was only 8.5% in the inoculum. This increase was in accordance with the previous work of Chen et al. [13]. Meanwhile, the vast majority of the total sequence detected in inoculum such as *Anaerolineae* (13.5%), *Synergistia* (17.5%), *Clostridia* (48.5%), *Thermotogae* (5.6%), and *Bacteroidia* (3.1%) decreased in U1 and U2. At the genus level, *Syntrophorhabdus* and *Clostridium* were found to be the main members, whose sum occupied 55.7% and 60.8% of the total sequences in U1 and U2, respectively. In particular, there was a significant increase in *Syntrophorhabdus* (24.5% in U1 and 39.2% in U2), which was negligible in the inoculum. Meanwhile, the relative abundances of other genera such as *Levilinea*, *Desulfotomaculum*, *Aminiphilus*, *Thermoanaerobacterium*, *Syntrophobacter*,

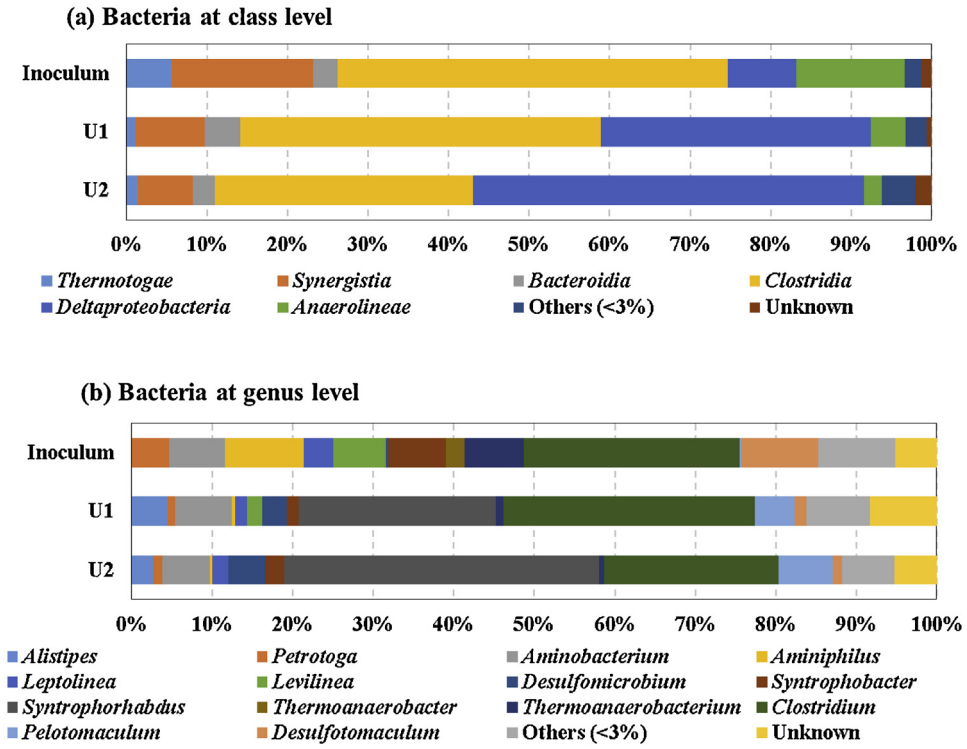

Fig. 4. Changes in diversity of key bacteria acclimated to phenol by next generation sequencing (U1 = samples were taken after 80 days of UASB operation, and U2 = samples were taken at the end of UASB operation).

Thermoanaerobacter, *Petrotoga*, and *Leptolinea* decreased in U1 and U2.

The sequences of bacteria at the species level were further assigned by the EzTaxon server, and ten representative OTUs were chosen shown in Table 1. It was distinctive to see that *Syntrophorhabdus aromaticivorans* emerged and became dominant (24.3% and 32.3% of the total OTU at U1 and U2, respectively) as the UASB operation continued. The role of *S. aromaticivorans* is known to degrade phenol to benzoate under mesophilic condition and subsequently to acetate and hydrogen in syntrophic association with a hydrogenotrophic methanogen [23,24]. Syntrophic benzoate-degrading bacteria such as *Syntrophus* sp., *Sporotomaculum* sp., and *Pelotomaculum* sp. were isolated and described in earlier research [25,26]. Among those, *Pelotomaculum terephthalicum*, growing at mesophilic condition with an optimum of pH 6.8–7.2, was observed to be 0.1% in inoculum and increased to 0.2% and 6.2% in U1 and U2, respectively. Given the co-occurrence of these two types of bacteria at considerable abundance, it can be clearly stated that phenol was degraded via benzoate to acetate pathway in mesophilic UASB operation. Meanwhile, *Syntrophus* sp. and *Sporotomaculum* sp. were not found in all samples.

It was found that the occupation of *Clostridium chauvoei* decreased from 13.6% in inoculum to 10.2% and 3.8% in U1 and U2, respectively, while there was no significant change in the occupation of *Clostridium* sp. *Desulfotomaculum carboxydivorans,* accounting for 8.8% of the total OTU, dropped down to 1.2% and 1.0% in U1 and U2, respectively. *Clostridium* sp. and *Desulfotomaculum* sp. have previously been reported as phenol-degrading microorganism, but their role still remains unclear [27].

Fig. 5 shows the relative abundances of archaeal populations. All classified archaea from the three samples were affiliated with four methanogenic orders, i.e., *Methanobacteriales*, *Methanosarcinales*, *Methanomicrobiales*, and *Methanococcales*. At the genus level, two acetoclastic (*Methanosaeta* and *Methanosarcina*) and five hydrogenotrophic (*Methanobacterium*, *Methanolinea*, *Methanoculleus*, *Methanococcus*, and *Methanotorris*) methanogens were observed. The methanogens in the inoculum mostly belonged to the hydrogenotrophic one (75.3% of the total archaeal sequences), in particular *Methanoculleus* (50.2% of the total archaeal sequences). However, as the UASB operation went on fed with phenol, there was a significant increase in the occupation of acetoclastic methanogens. *Methanosaeta* became dominant, accounting for 36.5% and 42.1% of the total sequences in U1 and U2, respectively. This result can be easily linked to the acetate conversion derived from benzoate degradation. Meanwhile, the low abundance (0.3%) belonging to the *Methanosarcina* in both U1 and U2 corresponds to the previous result of earlier study reporting *Methanosaeta* as a key microorganism in granulation is more dominant than *Methanosarcina* [28].

As a hydrogenotrophic methanogen, *Methanobacterium* became dominant as operation continued, accounting for 14.5% and 24.5% of the total archaeal sequences in U1 and U2, respectively. On the other hand, the occupation of *Methanoculleus* siginificantly decreased. The role of hydrogenotrophic methanogen in phenol degradation is known to be critical, which can make the benzoate conversion reaction (benzoate → acetate + hydrogen) thermodynamically favorable [19,23,29]. *Methanobacterium* is one of the often observed hydrogenotrophic methanogens in the typical anaerobic digestion process treating organic wastes, and also been detected in phenol-degrading enrichment culture [13,30].

This is the first study providing data on the population change of acclimated anaerobic consortia when phenol is used as a sole carbon source. Both bacterial and archaeal communities were thoroughly investigated using NGS tool, which was helpful in understanding the phenol-degrading mechanism. The dominant genera involved in phenol degradation were *Syntrophorhabdus*, *Methanosaeta*, and *Methanobacterium*, which have the following conversion pathways: (phenol → benzoate → acetate + hydrogen), (acetate → methane), and (hydrogen → methane), respectively.

Table 1
Species level identification of the dominant sequences from each sample (>3% of total sequences) (U1 = samples were taken after 80 days of UASB operation, and U2 = samples were taken at the end of UASB operation).

OTU (#)	Similarity (%)	Microorganism	Nearest neighbor accession number	Reads of OTU (%)		
				Inoculum	U1	U2
1	100	*Syntrophorhabdus aromaticivorans*	NR_041306.1	0.0	24.3	32.3
2	100	*Clostridium* sp.	M98449.1	12.1	13.6	12.0
3	98	*Clostridium chauvoei*	NR_026013.1	13.6	10.2	3.8
4	100	*Aminobacterium mobile*	NR_024925.1	6.9	6.8	5.2
5	100	*Desulfotomaculum carboxydivorans*	NR_043297.1	8.8	1.2	1.0
6	98	*Aminiphilus circumscriptus*	NR_043061.1	9.5	0.5	0.0
7	100	*Syntrophobacter sulfatireducens*	NR_043073.1	7.0	1.3	1.5
8	98	*Pelotomaculum terephthalicicum*	NR_040948.1	0.2	4.8	6.2
9	99	*Levilinea saccharolytica*	NR_040972.1	6.4	1.9	0.0
10	100	*Petrotoga halophila*	NR_043201.1	4.6	0.9	1.1

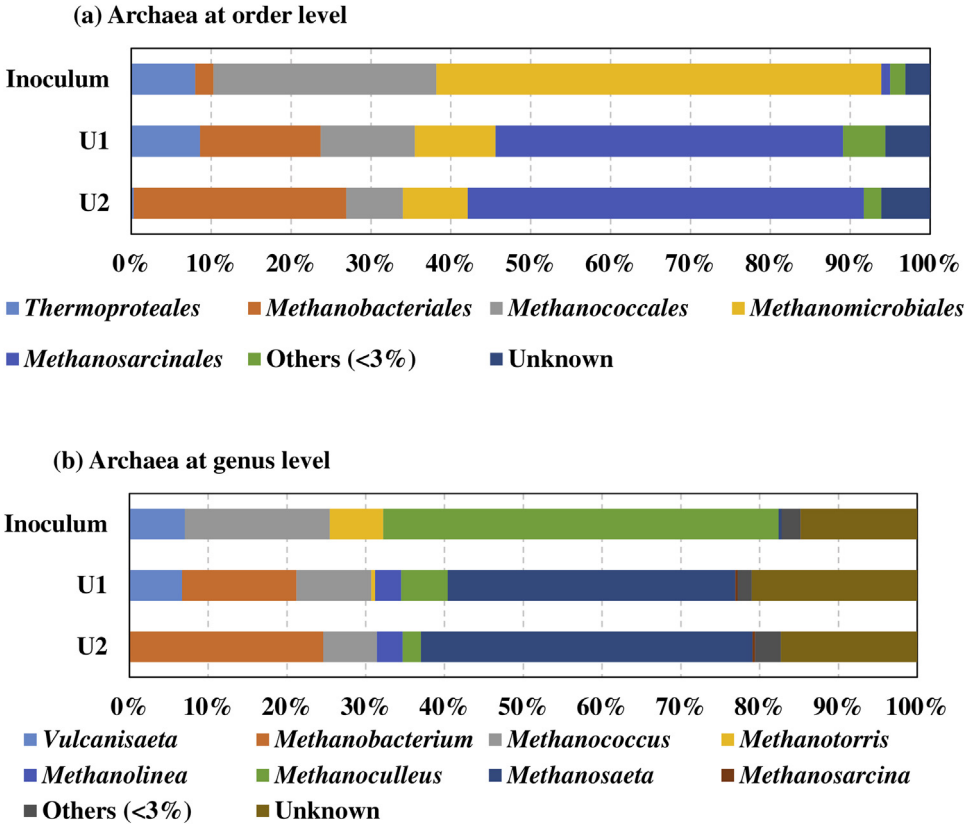

Fig. 5. Changes in diversity of key archaea acclimated to phenol by next generation sequencing (U1 = samples were taken after 80 days of UASB operation, and U2 = samples were taken at the end of UASB operation).

4. Conclusions

The progressive adaptation of granules was observed as phenol addition was repeated. At an OLR of 6 kg COD/m^3/d, the performance of UASB was 2.1 m^3 biogas/m^3/d with 79% phenol degradation. At higher OLR, a phenol degradation efficiency dropped down to 40%, and a considerable amount of benzoate and acetate were detected in the effluent. The NGS results showed that *Syntrophorhabdus*, which is known to degrade phenol to benzoate and subsequently to acetate and hydrogen, was the main genus, accounting for 39.2% of the total bacterial sequences. The existence of acetoclastic methanogens was negligible in the inoculum, but it significantly increased to 42.1% (mainly *Methanosaeta*) of the total archaeal sequences. The main hydrogenotrophic methanogen was found to be *Methanobacterium*, occupying 24.5% of the total archaeal sequences.

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