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Anaerobic treatment of phenol in wastewater under thermophilic condition

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ABSTRACT

Over 99% of phenol was effectively degraded in an upflow anaerobic sludge blanket (UASB) reactor at 55 °C with 40 h of hydraulic retention time (HRT) for a wastewater containing 630 mg/L of phenol, corresponding to 1500 mg/L of chemical oxygen demand (COD) and a loading rate of 0.9 g-COD/L/d. The maximum specific methanogenic activity (SMA) of the phenol-degrading sludge was 0.09 g-CH_4-COD/g-volatile suspended solids (VSS)/d. Based on 16S rDNA analysis, a total of 21 operational taxonomy units (OTUs) were found in the sludge, of which eight (42.6% of the total population) were related to the sequences in the GenBank with similarity of over 97%, and 13 (79.6%) were affiliated with the known thermophilic species. Additional SMA data and phylogenetic analysis suggest that the degradation pathway of phenol for thermophilic sludge was likely via caproate, instead of benzoate as for the mesophilic sludge.

1. Introduction

Phenol is an aromatic compound that is used as raw material for the production of a variety of resins, including phenolic, epoxy, polycarbonate, and polyamide, for various applications (Kirk–Othmer, 1978). In addition, phenol and its derivatives are commonly found in the industrial wastewater from the manufacturing of synthetic chemicals, pesticides, coal conversion, pulp-paper, oil-refining, etc. As a toxic and potentially carcinogenic chemical, the release of phenol into the environment is of great concern (IARC, 1999).

Biodegradation of phenol in wastewater is generally more cost-effective than the physicochemical treatment processes (Loh et al., 2000). The process has been carried out for many years either aerobically or anaerobically. Between the two, the latter is preferred because it saves the energy needed for aeration and produces substantially lower amount of sludge. Anaerobic treatment of phenol-containing wastewater was mostly carried out using the upflow anaerobic sludge blanket (UASB) reactors, and its application has been limited to under mesophilic and ambient temperature (Veeresh et al., 2005). Little information on the anaerobic degradation of phenol under thermophilic condition is available so far. On the other hand, thermophilic UASB treatment of industrial wastewater has attracted much interest in the past two decades (Wiegant, 1985; van Lier et al., 1992). Thermophilic process offers several merits, such as removing pathogenic microorganisms and eliminating the need of cooling for effluent of high temperature. Thus, it is of practical interest to evaluate the performance of anaerobic treatment of phenol in wastewater under thermophilic conditions.

Two possible anaerobic degradation pathways of phenol under mesophilic condition have been reported. In one suggested pathway (Kobayashi et al., 1989), phenol is first converted through carboxylation to produce benzoate. The latter is then de-aromatized to form cyclohexanecarboxylate,

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which is further cleaved to form heptanoate. Heptanoate is then degraded either through β-oxidization to form valerate, propionate and acetate (Keith et al., 1978), or directly to form propionate and butyrate, both of which can be further degraded into acetate (Fina et al., 1978). This pathway was supported by the presence of enzymes performing carboxylation, decarboxylation and dehydroxylation reactions during phenol anaerobic degradation (Gallert and Winter, 1992). In the other degradation pathway (Bakker, 1977), phenol is reduced in the presence of nitrate to cyclohexanone and then *n*-caproate, which is subsequently undergone β-oxidation to form lower volatile fatty acids (VFAs). Whether thermophilic phenol anaerobic degradation follows either of these two pathways is not clear.

Several anoxic phenol-degrading bacteria have been isolated so far, including *Desulfotomaculum* strain (Kuever et al., 1993) and two denitrifying species, *Azoarcus* sp. (van Schie and Young, 1998) and *Magnetospirillum* sp. (Shinoda et al., 2000). But no phenol-degrading bacterium has been isolated under methanogenic condition.

This study was conducted to investigate the degradation efficiency of phenol in wastewater under thermophilic condition (55 °C) using a UASB reactor. The specific methanogenic activities (SMA) of the sludge using various substrates were evaluated, and the microbial community was analyzed using 16S rDNA-based molecular techniques. Based on these results, the possible thermophilic degradation pathway of phenol was proposed.

2. Materials and methods

2.1. Phenol degradation in the UASB reactor

A 2.8 L UASB reactor was used for treating a synthetic phenol-containing wastewater at 55 °C for 224 days. It was operated with an effluent recycle ratio of 1:1 for lowering the toxic effect of phenol. The reactor was inoculated with 1 L of mesophilic phenol-degrading sludge from another UASB reactor operated at 37 °C. Throughout the experiment the wastewater maintained a chemical oxygen demand (COD) of 1500 mg/L with balanced nutrient (Fang et al., 2004). During startup the reactor was fed with phenol plus sucrose as co-substrate with 60 h of hydraulic retention time (HRT), corresponding to 0.6 g-COD/L/d of organic loading rate (OLR). The COD ratio between phenol and sucrose was first fixed at $\frac{1}{4}$, and then increased stepwise to $\frac{2}{3}$, $\frac{3}{2}$, $\frac{4}{1}$, and lastly $\frac{5}{0}$ once the COD removal efficiency reached 96%. At the end of the startup, the reactor continued to treat the wastewater contained 630 mg/L of phenol as sole substrate without sucrose.

The HRT was then lowered stepwise from 60 to 48, 40 and 28 h, once the COD removal efficiency reached 96%. During Days 98–126 operating at 28 h of HRT, the phenol removal efficiency dropped below 77%. The HRT was then returned to 40 h, and the removal efficiency was back to over 96% after 50 days. A second attempt was made to lower the HRT back to 28 h during Days 178–224, but it failed again to achieve satisfactory removal efficiency. The experiment ended on Day 224. Experimental parameters, including biogas production and composition, phenol, COD, and VFA concentrations, were analyzed twice a week following the procedures established from the previous study (Fang et al., 1996). Volatile suspended solids (VSS) in the reactor were analyzed once a month following the Standard Methods (APHA, 1998).

2.2. Specific methanogenic activity tests

The SMA of the phenol-degrading sludge was conducted in 282 mL batch reactors (Fang et al., 2004) at 55 °C. The sludge for the batch tests was sampled from the UASB reactor on Day 177 when the reactor was operated with 40 h of HRT, corresponding to 0.9 g-COD/L/d, removing nearly 100% of phenol from the influent. The sludge concentration in each batch was 2000 mg-VSS/L. Individual substrates used in the batch tests included phenol and eight possible degradation intermediates. Initial phenol concentration in batch tests varied from 50 to 2000 mg/L, corresponding to 119–4760 mg-COD/L. Initial concentrations of other substrates were 1500 mg-COD/L for possible intermediates formate, acetate, propionate, butyrate and benzoate, but 500 mg-COD/L for caproate, cyclohexanone and adipate. Biogas production and composition, VFA, concentrations of residual substrates were analyzed every 1–3 days.

2.3. Chemical analysis

Gas production from each reactor was measured using a glass syringe and the composition was analyzed by a GC-TCD. VFA (from acetate to caproate), benzoate, cyclohexanone and phenol were analyzed by a GC-FID. Sample pretreatments, GC conditions and columns selected were same as the previous study (Fang et al., 1996). Formate was indirectly measured by a total organic carbon analyzer (Shimadzu TOC-5000A). Adipate was measured by HPLC (Shimadzu 10-AD) equipped with a SPD-10A UV-visible detector at 210 nm using a 7.8 × 300 mm Aminex® HPX-87 H LC column (BioRad, CA). The mobile phase was 8 mM H_2SO_4 at 0.6 mL/min.

2.4. Cloning, sequencing and phylogenetic analysis

The DNA of the sludge sampled on Day 177 removing near 100% of phenol in wastewater was selected for DNA analysis. Firstly, the DNA of sludge sample was extracted and amplified by polymerase chain reaction (PCR) using the primer set of EUB8F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and UNIV1392R (5′-ACG GGC GGT GTG TRC-3′) at the annealing temperature of 54 °C. The amplified product was then used to build a clone library with the TA Cloning Kit (Invitrogen Corporation, Carlsbad, CA) (Zhang et al., 2005).

A total of 54 clones were selected for the plasmids recovery. The insert in the plasmids were PCR-amplified using the primer set of 341FGC (5′-CCT ACG GGA GGC AGC AG-3′) with GC-clamp (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G-3′) and 518R (5′-ATT ACC GCG GCT GCT GG-3′) for denaturing gradient gel electrophoresis (DGGE) screening. A total of 21 unique bands on DGGE gel, each indicating a unique sequence, i.e. operational taxonomy units (OTUs), were identified. The 16S rDNA of the 21 OTUs were sequenced using primers UNIV1392R, EUB8F and 907R

(5′-CCGTCAATTCCTTTRAGTTT-3′) with an auto sequencer (ABI model 377A, Perkin-Elmer Ltd., Foster City, CA) and dRhodamine Terminator Cycle Sequencing FS Ready Reaction Kit (Perkin-Elmer Ltd., Foster City, CA).

After the BLAST analysis, the sequences editing and alignment were manually conducted and the results were checked to remove chimeric artifacts (Zhang et al., 2005). Phylogenetic trees were then constructed using the neighbor-joining method with MEGA 2.1. Bootstrap re-sampling analysis for 500 replicates was performed to estimate the confidence of tree topologies.

2.5. *Accession numbers*

The nucleotide sequence data reported in this paper were submitted to the Genbank, EMBL and DDBJ databases, and have been assigned the following accession numbers: AY862518-862538.

3. Results and discussion

3.1. *Phenol degradation in UASB reactor*

After startup using sucrose as co-substrate, the UASB reactor treated the wastewater containing phenol (630 mg/L, equivalent to 1500 mg-COD/L) as sole substrate for over 224 days. During this period, the HRT was lowered stepwise, once the phenol removal efficiency exceeded 96%, from the initial 60 to 48, 40 and 28 h. The phenol concentrations in wastewater and effluent were illustrated in Fig. 1a, and the corresponding phenol removal efficiency in Fig. 1b. Throughout the whole operation there was no detectable VFA in the effluent. The thermophilic phenol-degrading sludge developed into granules of 1–2 mm in the UASB reactor. Based on electron-microscopic examinations, the thermophilic granules in this study, similar to the mesophilic phenol-degrading granules (Fang et al., 1996), lacked a distinct layered microstructure and the distribution of bacterial species was rather uniform.

Results show that as long as the HRT was kept at 40 h, i.e. OLR at 0.9 g-COD/L/d or lower, over 99% of phenol could be removed at 55 °C for wastewater containing 630 mg/L of phenol. When HRT was reduced from 48 to 40 h, the efficiency dropped sharply to 79% but was gradually recovered to 96% after 36 days (Days 61–97). When the HRT was further lowered to 28 h, the removal efficiency dropped to 59% and only recovered to 77% after 28 days (Days 98–126). This indicated that the bioactivity was partially inhibited by the increased phenol-loading rate at low HRT. By increasing HRT back to 40 h, the treatment efficiency had gradually recovered to near 100% by Day 177. During Days 178–224, the HRT was lower again to 28 h, and consequently only 70% of phenol removal could be achieved.

The threshold OLR of 0.9 g-COD/L/d for effective phenol removal at 55 °C is substantially lower than the OLR of 6 g-COD/L/d observed at 37 °C and 26 °C (Fang et al., 1996, 2004).

3.2. *SMA test*

Batch test results showed that sludge in the UASB reactor was able to completely degrade phenol at concentrations up to 1000 mg/L. At 2000 mg/L, no phenol was degraded for 60 days due to phenol's toxic effect. Figure 2 illustrates the degradation of phenol in batches at initial phenol concentrations from 50 to 1000 mg/L. It shows that after an acclimation period (up to 20 days for the batch treating 1000 mg/L of phenol), phenol was degraded completely at a near linear rate, which is defined as SMA. In all batches, there was no detectable benzoate and other intermediates in the mixed liquor throughout the test.

Figure 3 illustrates the SMA of sludge increased with the initial phenol concentration reaching the maximum value of 0.09 g-CH_4-COD/g-VSS/d for initial phenol concentrations of 600–1000 mg/L. This SMA value at 55 °C is substantially lower than the 0.19 g-CH_4-COD/g-VSS/d reported for the sludge

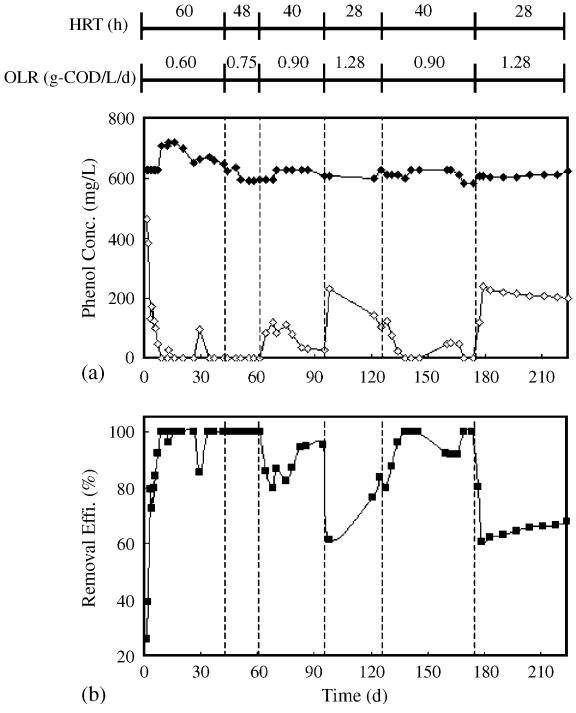

Fig. 1 – Phenol degradation in UASB reactor at 55 °C: (a) phenol concentrations in influent (◆) and effluent (◇); (b) phenol removal efficiency (■).

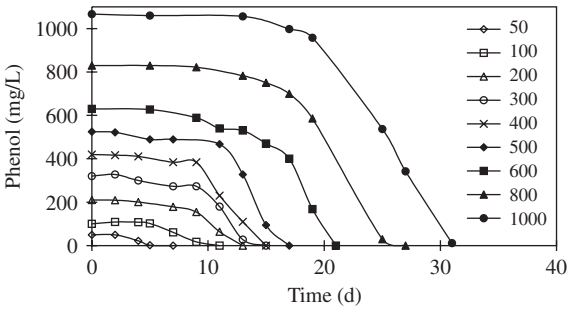

Fig. 2 – Phenol degradation at different initial concentrations in batch reactors.

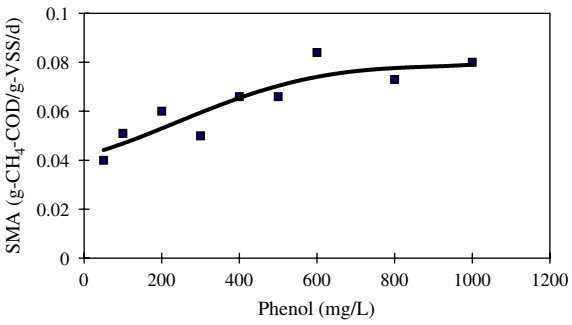

Fig. 3 – SMA of phenol degradation at 55 °C.

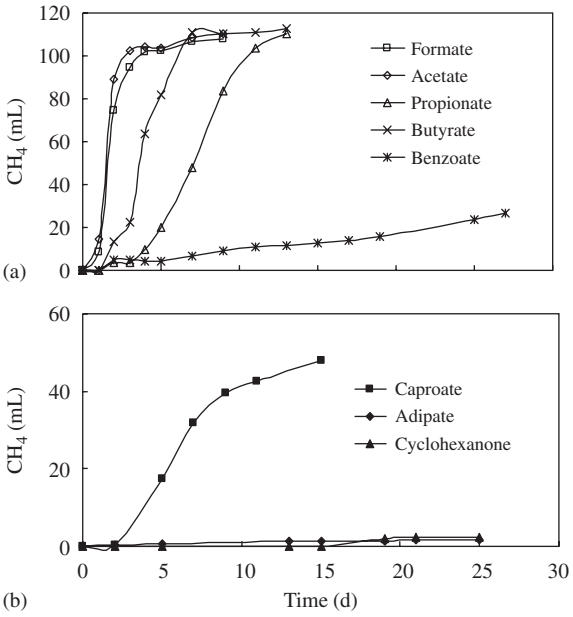

Fig. 4 – Cumulative methane production of different substrates.

degrading phenol at 26 °C, (Fang et al., 2004), and the 0.24 g-CH_4-COD/g-VSS/d for sludge at 37 °C (Fang et al., 1996).

Figure 4 illustrates the cumulative methane productions of eight selected individual possible substrates: (a) formate, acetate, propionate, butyrate and benzoate at the initial concentration of 1500 mg-COD/L; and (b) caproate, adipate and cyclohexanone at 500 mg-COD/L, the concentration of which was to avoid the toxic effect, if any. Table 1 summarizes the SMA of the phenol-degrading sludge at 55 °C along with the corresponding SMA at 37 °C for comparison. It shows that the sludge at 55 °C degraded phenol at 0.09 g-CH_4-COD/g-VSS/d, but degraded propionate and butyrate at 0.22–0.25 g-CH_4-COD/g-VSS/d, and degraded formate and acetate at 0.40–0.45 g-CH_4-COD/g-VSS/d. Under anaerobic condition, phenol is converted to methane through a series of chain reactions, including de-aromatization, acetogenesis, and methanogenesis. The SMA values in Table 1 show that the initial de-aromatization was the rate-limiting step, due to the stable ring structure, for the degradation of phenol. Methanogenesis had the highest rate of the three steps. Results in Table 1 further show that sludge degrading phenol at 55 °C had lower SMA than the sludge at 37 °C.

Figure 4a shows that formate and acetate were rapidly converted to methane. The amounts produced from formate and acetate accounted for 85.6% and 87.5% of respective theoretical values. Figure 4a and Table 1 also show that thermophilic phenol-degrading sludge could degrade propionate and butyrate, but could hardly degrade benzoate with a SMA of only 0.01 g-CH_4-COD/g-VSS/d. These results suggest that propionate and butyrate were likely the degradation intermediates, whereas benzoate was not. This is conflicting with the observations from a previous mesophilic study (Fang et al., 1996), in which it showed that at 37 °C phenol-degrading sludge was able to degrade benzoate at the rate similar to degrading phenol, but could not degrade either propionate or butyrate. These conflicting observations suggest that phenol at 55 °C was not degraded via benzoate, unlike under mesophilic condition.

Furthermore, results of this study also suggest that phenol was not degraded by another pathway, i.e. via cyclohexanone to caproate and adipate, as illustrated in Fig. 5 (Bakker, 1977; Evans, 1988). However, Fig. 4b shows that the phenol-degrading sludge in this study was even though able to convert caproate into methane, but could not degrade cyclohexanone and adipate, suggesting that the degradation pathway was not through cyclohexanone and adipate. The possible degradation pathway of phenol via caproate remains to be further investigated.

4. Microbial analysis

4.1. *Diversity of the microbial community*

Based on DGGE screening results, the 54 clones in the library were classified into 21 OTUs. This shows a high degree of bacteria diversity even under the stress of phenol toxicity and high temperature. Of the 21 OTUs, six had three clones or more, including TPD-2 (15 clones), -1 (6), -3 (4), -45 (4), -85(3), and -60(3). Four OTUs had two clones each including TPD-4, -6, -39, and -89, and the remaining 11 OTUs had only one clone, including TPD-7, -22, -27, -29, -47, -48, -55, -56, -58, -86, -87. Table 2 summarizes the abundance of each OTU in the sludge degrading phenol at 55 °C, its closest species, degree of similarity, and the closest species' characteristics related to thermophilic and anaerobic growth.

Among the 21 OTUs, only eight (23 clones, 42.6% of the population) are related to the known 16S rDNA sequences in the GenBank with similarity of 97% or above, that means over 50% of population in the sludge are unrelated to any known species. For comparison, most of the population (94.4%) of sludge degrading phenol at 26 °C were closely related to known species (Zhang et al., 2005). Five of these eight OTUs are closely related (97% and above) to several uncultured bacteria from various environments (Sekiguchi et al., 1998; Zhang et al., 2005). Results in Table 2 also show that 13 OTUs (79.6% of the population) are related to known thermophilic species.

The phylogenetic tree in Fig. 6 illustrates that all the 21 OTUs belong to eight divisions in the *Eubacteria* domain, i.e.

Table 1 – Comparison of SMA of phenol-degrading sludge at 55 °C and 37 °C

Substrate	55 °C (This study)		37 °C (Fang et al., 1996)	
	COD (mg/L)	SMA (g-CH_4-COD/g-VSS/d)	COD (mg/L)	SMA (g-CH_4-COD/g-VSS/d)
Formate	1500	0.40	1500	0.98
Acetate	1500	0.45	2500	0.64
Propionate	1500	0.22	2500	Nil
Butyrate	1500	0.25	2500	Nil
Benzoate	1500	0.01	2400	0.24
Phenol	1400	0.09	1000	0.23
Caproate	500	0.04	—	—

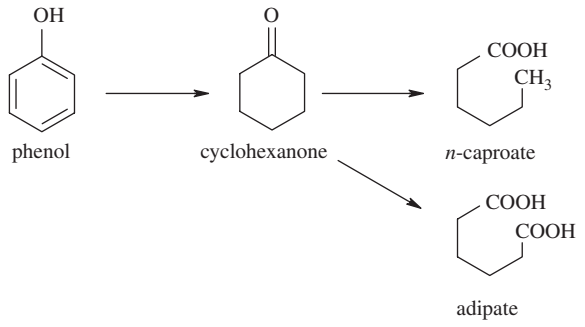

Fig. 5 – Phenol degradation pathway via cyclohexanone.

Thermotogae (four OTUs, 38.9% of the total clones), *Firmicutes* (six OTUs, 27.8%), *Chloroflexi* (four OTUs, 11.1%), candidate division OP8 (two OTUs, 9.3%), candidate division OP5 (one OTU, 5.5%), *Proteobacteria* (two OTUs, 3.7%), *Bacteroidetes* (one OTU, 1.9%), and *Nitrospirae* (one OTU, 1.9%). For comparison, the bacteria in the sludge degrading phenol at 26 °C were only found in two *Eubacteria* divisions, i.e. *Proteobacteria* and *Firmicutes*.

4.2. *The classification of the six major populations*

Six OTUs in this sludge had three or more clones, accounting for 64.8% of the total population. The most abundant species was OTU TPD-2 (15 clones, 27.8% of population), which is closely related to the two uncultured bacteria: TUG22 (99% similarity) from a thermophilic reactor and MUG18 (98%) from a mesophilic reactor, treating synthetic wastewaters of comprising mainly sucrose, acetate, propionate and peptone or yeast extract (Sekiguchi et al., 1998).

The second most abundant population was OTU TPD-1 (six clones, 11.1%), which is remotely related to thermophilic bacterium JA2 (94% similarity) from a thermophilic oleate-degrading culture (Menes et al., 2001), belongs to the *Clostridia* class of *Firmicutes*. Some *Clostridia* species are capable of degrading phenol and chlorophenol (Tartakovsky et al., 2001). Two of them, resembling *Desulfotomaculum* and *Clostridium*, respectively, are responsible for the conversion of phenol to benzoate under ambient temperature (Tartakovsky et al., 2001). The role of TPD-1 in this thermophilic sludge is unclear.

OTU TPD-3 (four clones, 7.4%) is closely related to *Fervidobacterium gondwanense*, a new thermophilic anaerobic bacterium isolated from non-volcanically heated geothermal waters (Andrews and Patel, 1996). TPD-3 and *F. gondwanense* form a group with the *Thermotogales* sp. SRI-251 from a hot spring (Skirnisdottir et al., 2000) in the division of *Thermotogae*. TPD-45 (four clones, 7.4%) is remotely related to *Moorella glycerini* (Slobodkin et al., 1997) and *Moorella thermoacetica* ET-5a (Gossner et al., 1999) with the similarity of 92% in *Clostridia* group.

TPD-60 (three clones, 5.6%) is remotely related to candidate division OP8 clone OPB95 from a hot spring at Yellowstone (Hugenholtz et al., 1998) and an uncultured bacterium TTA B3 from a thermophilic terephthalate-degrading anaerobic sludge (AY297963) with the similarity of 94%. TPD-85 (three clones, 5.6%) is related to uncultured bacteria TTA H122 from a thermophilic anaerobic reactor degrading terephthalate (AY661421) and uncultured bacteria SRI-280 from hot spring (Skirnisdottir et al., 2000) with the similarity of 96%.

4.3. *Microbial community and degradation pathway*

At ambient and mesophilic temperatures, phenol was presumably degraded through benzoate pathway (Kobayashi et al., 1989; Fang et al., 2004) as the sludge has high SMA value using benzoate as the substrate and the benzoate-degrading *Syntrophus*-like bacteria accounted for about 49% of the population (Zhang et al., 2005). However, judging from the SMA data of this study, the thermophilic phenol-degrading sludge could hardly utilize benzoate to produce methane and, instead, its caproate-degrading SMA was higher than the benzoate-degrading SMA. In addition, only one *Syntrophus*-like OTU (TPD-86, 1.9% of the population) was detected in this study using the 16S rDNA method. The thermophilic degradation pathway of phenol was thus likely via caproate, instead of benzoate, judging from the sludge incapability of degrading benzoate and the absence of benzoate-degrading *Syntrophus* bacteria. This could be due to the thermal sensitivity of benzoyl-CoA, a key intermediate in the benzoate pathway. In addition, the bacteria responsible for the conversion of phenol to caproate cannot be identified in this study. Further investigation on the thermophilic degradation pathway of phenol is warranted.

Table 2 – The OTUs abundance, affiliation, closest species, similarity, and their characteristics related to thermophilic and anaerobic growth

OTUs	Abund.[a] (%)	Affiliation	Closest species	Sim.[b] (%)	Thermo.[c]	Anaer.[d]	References
TPD-1	11.1	*Firmicutes; Clostridia*	*Thermophilic bacterium JA2*	94	+	+	Menes et al. (2001)
TPD-2	27.8	*Thermotogae*	TUG22[e]	98[f]	+	–	Sekiguchi et al. (1998)
TPD-3	7.4	*Thermotogae; Thermotogales*	*Fervidobacterium gondwanense*	92	+	–	Andrews and Patel (1996)
TPD-4	3.7	*Chloroflexi*	Sh765B-AG-22[e]	91	–	+	AJ519644 [g]
TPD-6	3.7	*Firmicutes; Clostridia*	*Clostridium roseum*	94	–	+	Stackebrandt et al. (1999)
TPD-7	1.9	*Chloroflexi*	*Anaerobic filamentous bacterium IMO-1*	99	+	+	AY555808 [g]
TPD-22	1.9	*Thermotogae*	TUG22[e]	98[f]	+	+	Sekiguchi et al. (1998)
TPD-27	1.9	*Nitrospirae; Nitrospirales*	*Thermodesulfovibrio islandicus*	98	+	+	X96726 [g]
TPD-29	1.9	*Thermotogae*	TUG22[e]	99[f]	+	+	Sekiguchi et al. (1998)
TPD-39	3.7	Candidate division OP8	TTA B3[e]	94	+	–	AY297963 [g]
TPD-45	7.4	*Firmicutes; Clostridia*	*Moorella glycerini*	92	+	+	AY695836 [g]
TPD-47	1.9	*Bacteroidetes*	PK91[e]	99	+		AY555796 [g]
TPD-48	1.9	*Proteobacteria*	SHA-22[e]	96	–	+	Schlotelburg et al. (2000)
TPD-55	1.9	*Firmicutes*	MBNA03[e]	91	+	+	Tang et al. (2004)
TPD-56	1.9	*Chloroflexi*	SHA-31[e]	93	–	+	Schlotelburg et al. (2000)
TPD-58	1.9	*Firmicutes*	IRR-DS6-19[e]	92	–	+	AJ622007 [g]
TPD-60	5.6	Candidate division OP8	OPB95[e]	94	+	–	Hugenholtz et al. (1998)
TPD-85	5.6	*Candidate division OP5*	TTA H122[e]	96	+	+	AY661421 [g]
TPD-86	1.9	*Proteobacteria; Syntrophus*	PD-UASB-5[e]	98	–	+	Zhang et al. (2005)
TPD-87	1.9	*Firmicutes*	PPf35E8[e]	93	–	+	AY548784 [g]
TPD-89	3.7	*Chloroflexi*	Anaerobic filamentous bacterium IMO-1	98	–	+	AY555808 [g]

[a] Abundance.
[b] Similarity.
[c] Thermophilic.
[d] Anaerobic.
[e] Environmental clone.
[f] Only 500 bps sequence available for TUG22.
[g] Only the accession number available.

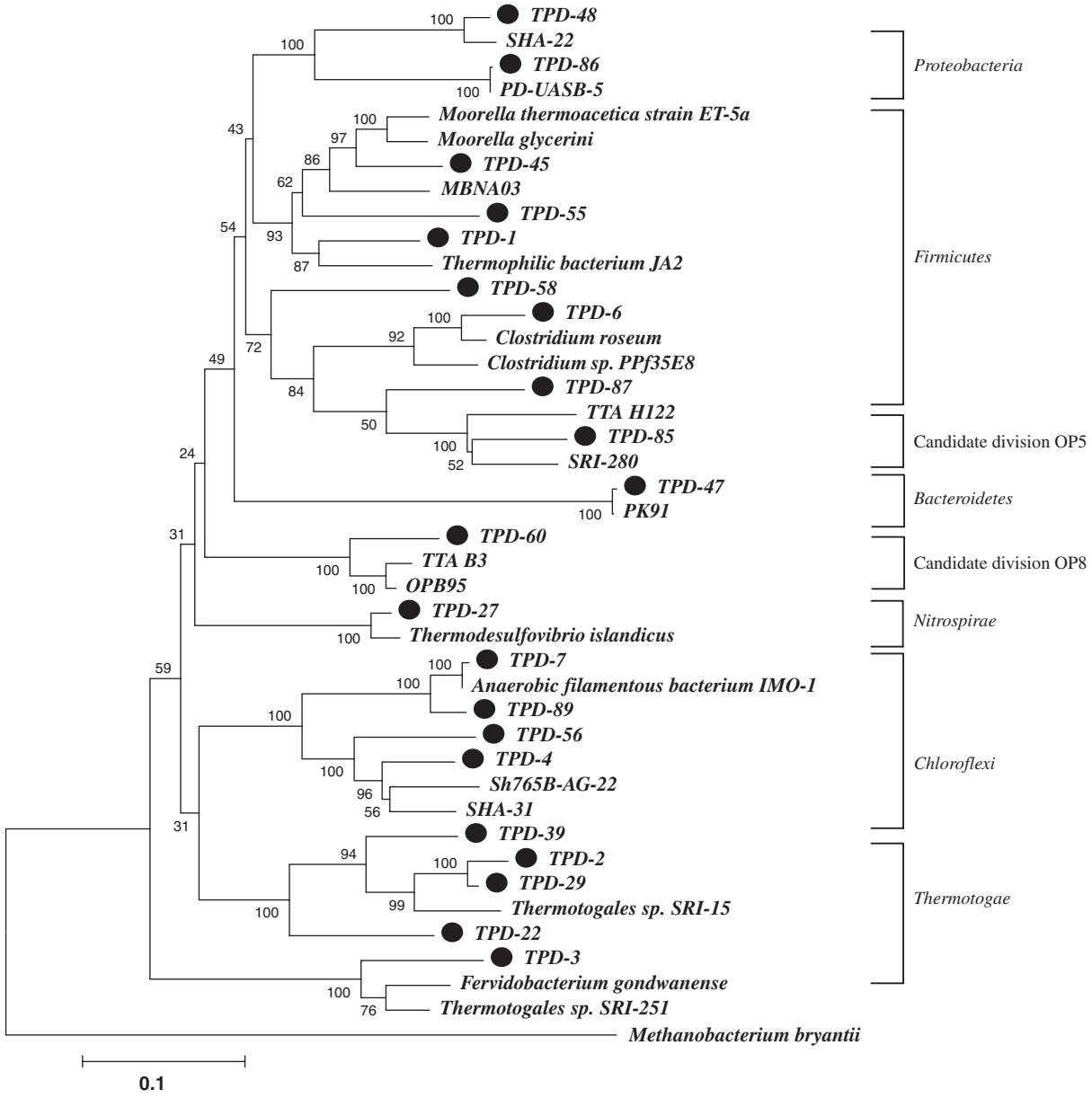

Fig. 6 – Phylogenetic tree of the OTUs in the phenol-degrading sludge and their close relatives based on almost full 16S rDNA sequences. The tree was based on Jukes–Cantor distance and constructed using the neighbor-joining algorithm with 500 bootstrapping. *Methanobacterium bryantii* was selected as the out-group species. The scale bar represents 0.1 substitution per nucleotide position. Numbers at the nodes are the bootstrap values.

5. Conclusion

Over 99% of phenol could be effectively degraded in a UASB reactor under thermophilic condition (55 °C), with 40 h of hydraulic retention time for phenol concentration 630 mg/L, corresponding to 1500 mg/L of COD and a loading rate of 0.9 g-COD/L/d. The SMA of the phenol-degrading sludge was 0.09 g-CH_4-COD/g-VSS/d. The degradation pathway of phenol was likely via caproate, instead of benzoate, judging from the sludge incapability of degrading benzoate and the absence of benzoate-degrading *Syntrophus* bacteria. Based on 16S rDNA molecular analysis, a total of 21 OTUs were found in the sludge, eight (42.6% of the total population) of which were related to the known sequences in the GenBank (over 97% similarity) and 13 (79.6%) were affiliated with the known thermophilic species.

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