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Anaerobic degradation of dimethyl phthalate in wastewater in a UASB reactor

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ABSTRACT

Over 99% of dimethyl phthalate (DMP) and 93% of chemical oxygen demand (COD) were effectively removed in a continuous upflow anaerobic sludge blanket (UASB) reactor from a wastewater containing 600 mg/L DMP at 8 h of hydraulic retention time (HRT), corresponding to a loading rate of 3 g-COD/(L d). Each gram of sludge, expressed as volatile suspended solids (VSS), had a maximum specific methanogenic activity (SMA) of 24 mg-CH₄/(g-VSS d) using DMP as the sole carbon source. The sludge yield was estimated as 0.08 g-VSS/g-COD. During anaerobic degradation, DMP was de-esterified, first to mono-methyl phthalate (MMP) and then to phthalate, before being de-aromatized and subsequently converted to CH₄ and CO₂. The maximum specific degradation rates of DMP, MMP and phthalate were 415, 88 and 36 mg/(g-VSS d), respectively. Analysis based on polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) showed a gradual shift of microbial population with the increase of DMP loading.

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1. Introduction

Phthalic esters (PEs) are a group of chemicals widely used as additive in the manufacturing of plastics. They are listed as priority pollutants in many countries due to their suspected mutagenicity and endocrine disrupting effects. Since PEs are not chemically bound to the host plastics, they are inevitably leached from the products and released to the environment ultimately. They have been detected in surface water, wastewater, sewage sludge and sediment (Fromme et al., 2002), as well as landfill leachate (Marttinen et al., 2003).

PEs can be biodegraded in surface waters, soils and sediments under various conditions, either aerobic, anoxic (Liang et al., 2007) or anaerobic (Staples et al., 1997). Of all PEs, dimethyl phthalate (DMP) is the simplest and the most commonly used. Although DMP has only a moderate toxicity with a LD₅₀ of over 5 mL/kg for rats (Autian, 1973), its metabolite mono-methyl phthalate (MMP) is not only toxic but also an endocrine disruptor, i.e. MMP may interfere with

the development and reproductive system of animals, or even human, by reducing testosterone production and decreased sperm counts (Lottrup et al., 2006). Under aerobic conditions, DMP in wastewater could be effectively removed in batch reactors in less than 5 days at the initial concentration of 400 mg/L (Wang et al., 2003, 2004) as well as in continuous packed-bed reactors at a volumetric rate of 560 mg/(L h) (Juneson et al., 2002). Under anoxic conditions, DMP could be degraded at the rate of 62 mg/(g-VSS h) (Liang et al., 2007). Its degradation under anaerobic conditions is, however, much slower. Batch data show that it required 7 days to degrade DMP using a digester sludge (Shelton et al., 1984), 17 days using a sludge obtained from an upflow anaerobic sludge blanket (UASB) reactor (Kleerebezem et al., 1999), and up to 70 days in a laboratory-scale municipal solid waste landfill (Ejlertsson et al., 1996). Most anaerobic studies of PEs degradation so far were conducted in batches. Little information is available on the degradation of DMP in wastewater in continuous flow reactors.

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This study was conducted to investigate the anaerobic degradation of DMP in wastewater using a UASB reactor under mesophilic condition. The effect of using phenol as a co-substrate during startup for enhancing the microbial biodegradability was evaluated. The maximum loading rate, degradation intermediates, and the specific methanogenic activity (SMA), as well as the microbial population of the DMP-degrading sludge, based on denaturing gradient gel electrophoresis (DGGE) (Muyzer et al., 1993) analysis of extracted DNA, were also investigated.

2. Material and methods

2.1. DMP degradation in a UASB reactor

A 2.8-L UASB reactor (Fang et al., 1996) was used for treating a synthetic DMP-containing wastewater at 37 °C for over 530 days. It was operated with an effluent-recycling ratio of 1:1 in order to reduce the toxic effect of DMP. The reactor was seeded with the phenol-degrading sludge from a previous study (Fang et al., 1996), in which phenol was effectively degraded at concentrations up to 1260 mg/L. For each gram of theoretical oxygen demand, the feed solution consisted of following nutrients: 1 g NaHCO₃, 200 mg NH₄Cl, 42.5 mg MgSO₄·7H₂O, 24.8 mg K₂HPO₄, 9.9 mg KH₂PO₄, 13.0 mg CaCl₂, 5.3 mg NiSO₄·7H₂O, 4.1 mg FeCl₃·6H₂O, 1.1 mg MnCl₂·4H₂O, 0.6 mg ZnCl₂, 0.6 mg CoCl₂·2H₂O, 0.4 mg (NH₄)₂MoO₄·4H₂O, 0.3 mg CuCl₂·2H₂O and 0.2 mg NaBO₂·10H₂O. The 530-day operation was divided into two stages, depending on whether phenol was used as co-substrate. The operational conditions are summarized in Table 1.

In Stage I (Days 1–232), phenol with decreasing concentrations was used as the co-substrate allowing the sludge a gradual acclimation to DMP. The reactor was started by feeding an influent containing DMP/phenol at respective concentrations of 300/1050 (both in mg/L), corresponding to a chemical oxygen demand (COD) loading of 3000 mg/L. The hydraulic retention time (HRT) was kept at 20 h, after a brief period operating at 12 h at which the effluent COD exceeded 400 mg/L. Afterwards, the DMP/phenol concentrations (in mg/L) were changed stepwise from 300/1050 to 600/840, and then

to 800/630, once the COD removal efficiency reached 90% at the given concentration levels. However, when DMP/phenol concentrations at 800/630 (mg/L), the COD removal dropped below 75% and could not recover after several weeks. By changing the concentrations back to 600/840 mg/L, COD removal efficiency was gradually recovered and was kept steady over 90% for the next 50 days.

In Stage II (Days 233–530), phenol was removed from the influent. DMP was kept at 600 mg/L, equivalent to 1000 mg-COD/L, as the sole carbon source. Similar to Stage I, once the COD removal efficiency reached 90%, the organic loading was increased by lowering the HRT stepwise from 20 h to 16, 12, 9.6 and lastly 8 h.

2.2. Specific methanogenic activity (SMA)

The SMA of DMP-degrading sludge at 37 °C was conducted in 282 mL serum bottles in batch mode. The SMA is an indicator of the methanogenic activity of the biomass under a condition in which the supply of substrate is not a limiting factor. Seed sludge was sampled from the DMP-degrading UASB reactor at the end of Stage II on Day 530, when the reactor was operated at 3 g-COD/(L d) removing 100% of DMP and 93% of COD from the influent. In each batch test, the initial sludge concentration, measured as volatile suspended solids (VSS), was 900 mg/L. Substrates in the SMA tests were DMP and eight possible degradation intermediates, including formate, acetate, propionate, butyrate, caproate, benzoate, phenol and phthalate. The initial concentration of each substrate was chosen to ensure that substrate supply was not the limiting factor and yet without causing an inhibitory effect. Initial concentrations of DMP varied from 100 to 1500 mg/L at six levels. Initial concentrations for other substrates were (in mg/L): formate 4320, acetate 1400, propionate 990, butyrate 825, caproate 490, benzoate 505, phenol 420, and phthalate 690. These concentrations were equivalent to 1500 mg-COD/L for the first four substrates, and 1000 mg-COD/L for the others.

2.3. Chemical analysis

In each SMA test, biogas production and composition, DMP and its metabolites, including volatile fatty acids (VFAs), were

Table 1 – Operation conditions of anaerobic DMP degradation in the UASB reactor

Stage	Time (d)	HRT (h)	DMP (mg/L)	Phenol (mg/L)	COD (mg/L)	DMP loading (mg/(L d))
I	1–57	12	300	1050	3000	600
	58–89	20	300	1050	3000	360
	90–110	20	600	840	3000	720
	111–177	20	800	630	3000	960
	178–232	20	600	840	3000	720
II	233–322	20	600	–	1000	720
	323–355	16	600	–	1000	900
	356–372	12	600	–	1000	1200
	373–400	9.6	600	–	1000	1500
	401–530	8	600	–	1000	1800

analyzed every 1–3 days. Gas productions were measured using glass syringes and the gas compositions were analyzed by a gas chromatograph (GC; Hewlett Packard-5890, USA) equipped with a thermal conductivity detector. Benzoate, phenol and VFAs were analyzed by another GC (Agilent-6890N, USA) with a flame ionization detector. Sample pretreatments, GC conditions and column information followed those in a previous study (Fang et al., 1996). DMP and two potential metabolites, i.e. mono-methyl phthalate (MMP) and phthalate, were quantified using a high-performance liquid chromatograph (HPLC) (Shimadzu-10A, Japan) with a UV detector (Shimadzu SPD-10AV, Japan) at 254 nm. The mobile phase was a mixture of acetonitrile and 0.05 M KH_2PO_4 at the ratio of 35/65 (v/v), adjusted to pH 3 using H_3PO_4 , delivered at a flow rate of 1 mL/min through an Allsphere column (Alltech ODS-2 3u, USA; 4.6×150 mm, particle size 3 μm). The detection limit of each phthalate was 0.5 mg/L.

2.4. PCR-DGGE analysis

The sludge was sampled on Days 1, 232, 322, 355, 372, 400 and 530 for microbial analysis. DNA was extracted from 1 mL of sludge sample, followed by PCR amplification using the primer set of 341FGC (5'-CGC CCG CCG CGC GCG GCG GCG GGG CCG GCG GGG GCC TAC GGG AGG CAG CAG-3') and 518R (5'-ATT ACC CCG GCT GCT GG-3') at the annealing

temperature of 54°C in an automated thermal cycler (GeneAmp® PCR 9700, Perkin-Elmer, Foster City, CA). The PCR-amplified products were then separated using DGGE to investigate the microbial population diversity using 8% gel with 40–60% denaturant gradient (Zhang and Fang, 2000).

3. Results and discussion

3.1. DMP degradation in UASB reactor

Fig. 1 illustrates the overall performance of DMP degradation over 530 days in the UASB reactor. Phenol-degrading sludge was very effective for the startup of DMP anaerobic degradation. During Stage I (Days 1–232) over 90% of the 300 mg/L DMP in the influent was removed with 12 h of HRT after only 14 days of acclimation (Stage I-1 in Fig. 1a), whereas phenol was completely degraded. However, over 400 mg/L of COD still remained in the effluent by the end of Stage I-1, indicating that DMP was only partially degraded as confirmed by the finding of MMP and phthalate in the effluent. Complete mineralization of DMP was not accomplished until Day 75. Throughout the whole operation, there was no detectable VFA in the effluent.

After acclimation, the UASB reactor was able to remove 99% of both DMP and phenol for the wastewater containing DMP/

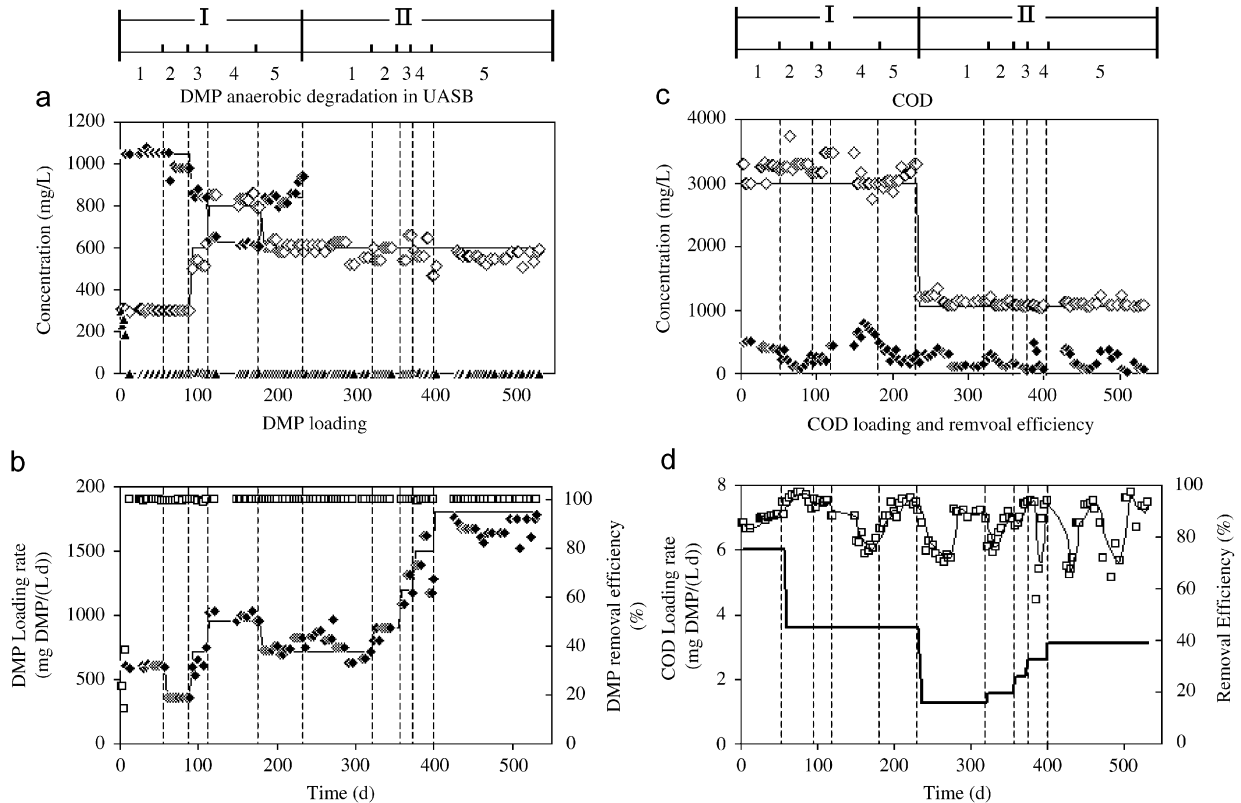


Fig. 1 – Anaerobic DMP degradation in the UASB reactor: (a) influent DMP (\diamond), influent phenol (\blacklozenge), and effluent DMP (\blacktriangle); (b) DMP-loading rate (\blacklozenge) and DMP-removal efficiency (\square); (c) influent COD (\diamond) and effluent COD (\blacklozenge); (d) COD removal efficiency (\square) and COD loading rate (—).

phenol concentrations of 300/1050 (in mg/L) (Stage I-2) and 600/840 (in mg/L) (Stage I-3) with 20 h of HRT. The corresponding COD removal efficiencies were 97% and 93%, respectively. As the DMP/phenol concentrations adjusted to 800/630 (in mg/L) (Stage I-4), both the DMP and phenol removal efficiencies remained at 99%, while the COD removal efficiency was reduced to only 75%, indicating substantial amounts of intermediates remained in the effluent. However, such inhibition effect caused by the concentrated DMP (800 mg/L) was reversible. By changing DMP/phenol concentrations back to 600/840 (in mg/L), the COD removal efficiency was recovered to 94% within 20 days.

During Stage II (Days 233–530), HRT was reduced stepwise, while DMP was the sole carbon source keeping at the constant concentration of 600 mg/L, equivalent to 1000 mg-COD/L. The HRT were 20, 16, 12, 9.6 and 8 h during Stages II-1 (Days 233–322), -2 (Days 323–357), -3 (Days 358–375), -4 (Days 376–425) and -5 (Days 426–530), corresponding to the respective loading rates of 1.3, 1.6, 2.1, 2.6 and 3.0 g-COD/(L d). The HRT was lowered to a new level each time when COD removal reached 90%. During Stage II-1, DMP removal efficiency remained over 99%, while the initial effluent COD increased to 300 mg/L corresponding to a COD removal of 70%. This indicates that the removal of phenol from the influent did not affect the degradation of DMP, but significantly affect the degradation of intermediates. After 40 days, the COD removal efficiency was recovered to 90%.

The DMP-degrading sludge was sensitive to the increase of organic loading, resulting in the sudden drops of COD removal efficiency, each time when the HRT was lowered, as illustrated in Fig. 1d. However, the effect of loading shocks to the DMP-degrading sludge was reversible. COD removal efficiency returned to 90% within 2–4 weeks. The reactor was also tolerant to small temperature shock. During Days 474–477, the temperature of the UASB was dropped from 37 to 25 °C for about 72 h, due to the malfunction of the water heater. The shock resulted in the lowering of COD removal efficiency to 62% by Day 483, but was fully recovered in 3 weeks. Fig. 1d shows that DMP was effectively degraded at 3.0 g-COD/(L d).

3.2. Biomass yield and gas production

The DMP-degrading sludge, which was seeded from a phenol-degrading reactor, remained granular throughout the study. During Days 503–530, when the reactor was operated steadily at 3 g-COD/(L d), the sludge bed occupied 50% of the reactor volume containing an average biomass concentration of 40 g-VSS/L. The solids retention time was 90 days, which is estimated from the average daily discharge of 0.6 g VSS in the effluent. On average, the reactor produced daily 4.0 L of biogas containing 65% of methane. Since each gram of methane is equivalent to 4 g of COD, methane accounted for 89% of the COD removed and the remaining 11% was presumably converted to biomass. Assuming each gram of biomass had an average COD of 1.41 g (Fang et al., 1996), the sludge yield based on COD balance was thus estimated as 0.08 g-VSS/g-COD.

3.3. Maximum specific degradation rates of DMP, MMP and phthalates

Fig. 2 shows the concentrations of DMP, MMP and phthalate in the mixed liquor, as well as the production of methane, in the six SMA tests using DMP as substrate. It shows that the DMP-degrading sludge was able to completely degrade DMP at concentrations up to 1500 mg/L, despite it had only been exposed to a maximum of 600 mg/L of DMP in the UASB reactor. Throughout these SMA tests, only trace amount of benzoate was detected in the mixed liquor, but no VFAs. Fig. 2a shows that the DMP was completely removed in 6 days without a lag phase. Comparing Figs. 2a and b shows that DMP was first converted to MMP, the concentration of which reached maximum after 5–8 days, and then degraded linearly over time. Comparing Figs. 2b and c shows that phthalate concentration in the mixed liquor increased corresponding to the decrease of MMP, and then decreased also linearly over time. Fig. 3 illustrates the maximum specific degradation rates of DMP, MMP and phthalate, based on the degradation slopes in Figs. 2a and c, and the corresponding SMA at various initial DMP concentrations. It shows that the maximum specific degradation rates of DMP, MMP and phthalate were 415, 88 and 36 mg/(g-VSS d), respectively, which were substantially lower than the corresponding rates of 1490, 821 and 558 mg/(g-VSS d) for a denitrifying DMP-degrading sludge (Liang et al., 2007).

3.4. SMA and probable degradation pathway

Fig. 3 also illustrates that the SMA increased with the initial DMP concentration, ranging 12–24 mg-CH₄/(g-VSS d). Table 2 shows the SMAs of the sludge using eight other individual substrates, plus the corresponding SMAs of the phenol-degrading sludge (Fang et al., 1996) for comparison. Results show that these two sludges varied substantially in their substrate degrading abilities. Both sludges could degrade benzoate, but nearly no propionate. The phenol-degrading sludge could not degrade butyrate, whereas the DMP-degrading sludge could not degrade phenol.

SMA may also reveal the possible degradation pathway. Since the phenol-degrading sludge was capable of degrading benzoate and acetate, but not propionate and butyrate, phenol was presumably degraded first to benzoate, which was then directly converted to acetate before methanogenesis (Fang et al., 1996). On the other hand, Figs. 2a and c show that during anaerobic degradation DMP was first converted to MMP, and then phthalate, similar to the anaerobic degradation of dimethyl terephthalate (Kleerebezem et al., 1999), and the aerobic (Fang et al., 2007) and anoxic degradation of phthalates (Liang et al., 2007). Judging from DMP-degrading sludge's SMA data, DMP after de-esterification was also likely degraded to benzoate, which was then further degraded, unlike in phenol degradation, via caproate, butyrate and then acetate presumably by β -oxidation.

Results in Table 2 also show that the SMA of the sludge were lower in degrading DMP, phthalate and benzoate, ranging 24–45 mg-CH₄/(g-VSS d), as compared to those in degrading the downstream intermediates, i.e. caproate, butyrate, acetate and formate. This suggests that the initial

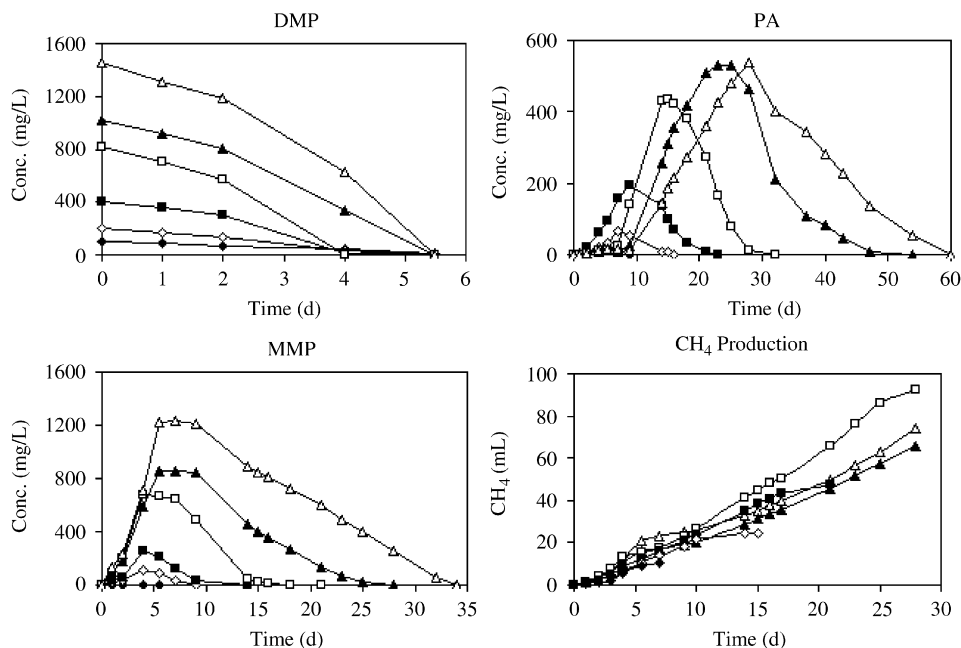


Fig. 2 – Time profiles of (a) DMP, (b) MMP and (c) phthalate concentrations in the mixed liquor, and (d) methane production during DMP degradation. Initial DMP concentration: (Δ) 1500 mg/L, (\blacktriangle) 1000 mg/L, (\square) 800 mg/L, (\blacksquare) 400 mg/L, (\diamond) 200 mg/L, (\blacklozenge) 100 mg/L.

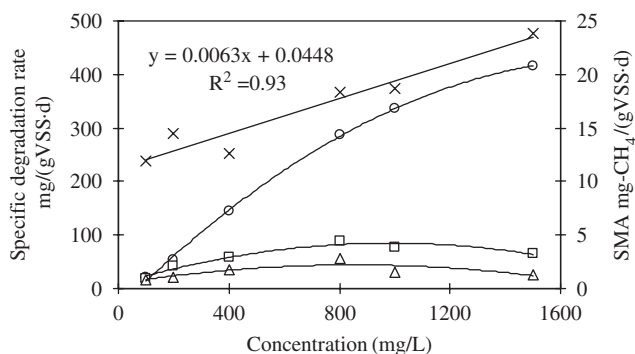


Fig. 3 – Maximum specific degradation rates of DMP (○), MMP (□) and phthalate (△) of the DMP-degrading sludge and its SMA (×) using DMP as a sole carbon source.

de-esterification was likely the rate-limiting step in the anaerobic degradation of DMP.

3.5. Microbial population shift in DMP-degrading sludge

Fig. 4 compiles the DGGE profiles of sludge sampled at various stages of operation, including the seed (Day 1), one using phenol as co-substrate (Day 232), and five using DMP as the sole substrate at various HRTs (Days 322, 355, 372, 400, and 530). In a DGGE image, each band represents a distinct species, which can be identified by its location. A total of at least 10 species were found in the seven samples. However, microbial community changed greatly from the phenol-degrading sludge to the DMP-degrading sludge. After phenol was removed from the influent, six bands in the seed sludge disappeared after Day 232, replacing by six new bands,

Table 2 – SMA of DMP- and phenol-degrading sludge using various substrates

Source of substrates	COD (mg/L)	SMA (mg-CH ₄ /(g-VSS d))	COD (mg/L)	SMA ^a (mg-CH ₄ /(g-VSS d))
Formate	1500	103	1500	245
Acetate	1500	173	2500	160
Propionate	1500	15	2500	Nil
Butyrate	1500	62	2500	Nil
Caproate	1000	53	–	–
Phenol	1000	8	1000	58
Benzoate	1000	45	2400	60
Phthalate	1000	40	–	–
DMP	1500	24	–	–

^a Fang et al., 1996.

marked as Bands-d, -e, -f, -g, -h and -i in Fig. 4. The new bands were possibly responsible for the degradation of DMP and its intermediates. Identifications of these new species warrant a further study.

4. Conclusion

Over 99% of DMP and 93% of COD were effectively removed in a continuous UASB reactor from a wastewater containing 600 mg/L DMP at 8 h of HRT, corresponding to a loading rate of 3 g-COD/(L·d). The sludge yield was estimated as

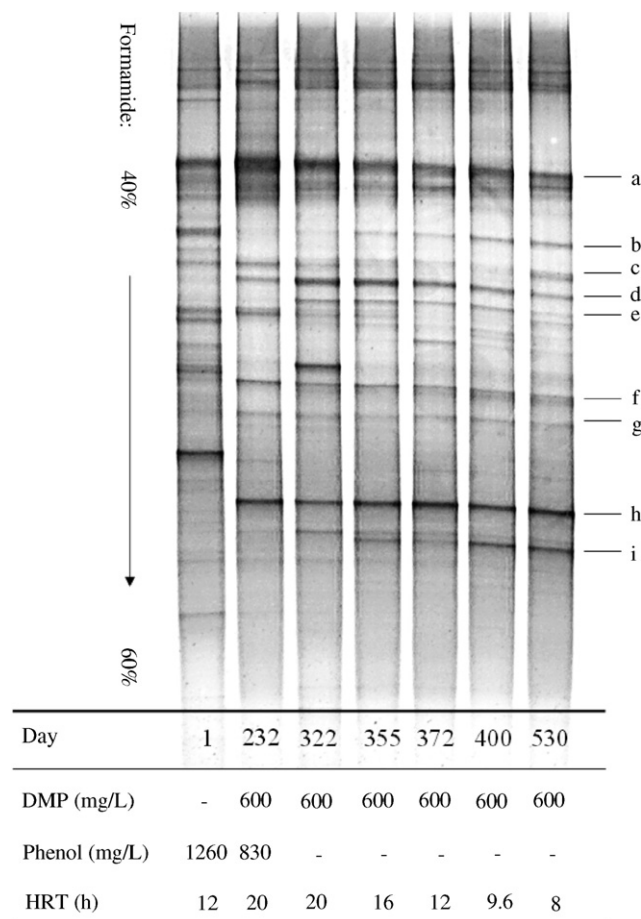


Fig. 4 – Microbial shift in DMP-degrading sludge under co-substrate and various loading rates.

0.08 g-VSS/g-COD. The maximum SMA using DMP as substrate was 24 mg-CH₄/(g-VSS d). During anaerobic degradation, DMP was de-esterified, first to MMP and then phthalate, before being de-aromatized and subsequently converted to CH₄ and CO₂. The maximum specific degradation rates of DMP, MMP and phthalate were 415, 88 and 36 mg/(g-VSS d), respectively. Analysis based on PCR-DGGE showed a gradual shift of microbial population with the increase of DMP loading.

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