Anaerobic Degradation of Terephthalic Acid and Aniline by Methanogenic Consortia

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Abstract

The biodegradability of eight aromatic compounds; benzyl alcohol, phenol, toluene, terephthalic acid, aniline, benzenesulfonic acid, 2,4,6-trichlorophenol and *m*-nitrophenol; was evaluated under methanogenic conditions. Benzyl alcohol and phenol were termed as readily biodegradable aromatic compounds based on the net gas production. Toluene, terephthalic acid, aniline, and benzenesulfonic acid were termed as persistent compounds. 2,4,6-trichlorophenol and *m*-nitrophenol were caused a toxicity response of the methanogenesis. All compounds, which were suggested to be toxicity compounds by the biodegradability test, showed toxicity to acetoclastic methanogens. The anaerobic continuous treatments of synthetic wastewaters with aniline or terephthalic acid as the carbon sources were carried out. Glucose or acetic acid, used as co-substrates, showed stimulatory effect on the degradation of aniline or terephthalic acid. The degradation efficiency of aniline and terephthalic acid were more than 80% and 90 %, respectively, in the treatments of synthetic wastewaters with acetic acid and aniline or terephthalic acid at the mole ratio (acetic acid / aniline or terephthalic acid) over 10.0 at a TOC loading rate of 0.5 g/l·d.

Key words: terephthalic acid, aniline, anaerobic degradation, methane fermentation

INTRODUCTION

Significant amounts of aromatic compounds are present in the wastewaters discharged from the petrochemical factories. The biodegradation of aromatic compounds is of interest based on their toxicity to human health. Aniline (aminobenzene) is one of the harmful compounds and its concentration in wastewater is restricted in Japan¹⁾. This compound is used as the standard compound for MITI Biodegradation Test (OECD 301C)²⁾. Terephthalic acid (1,4-benzendicarboxic acid) is aromatic compound that used in the

production of poly (ethylene terephthalate, PET) bottle, and is one of the top 50 chemicals manufactured in the world.³⁾ A significant amount of terephthalic acid is discharged from PET bottle producing process or purification of terephthalic acid.

Methane fermentation process, or microbial degradation of organic matters under anaerobic conditions, has been regarded as an energy-saving wastewater treatment process. However, the introduction of the anaerobic processes for wastewater containing aromatic compounds has been recognized to be difficult, because of their

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presumed toxicity and poor biodegradability. Recently, it has been clear that most aromatic compounds can be degraded under anaerobic conditions, such as methanogenic and /or sulfate-reducing conditions.⁴⁾ Based on these findings, application of anaerobic treatment on wastewaters including aromatic compounds is expected. But there are only a few reports on the anaerobic biodegradation of terephthalic acid and aniline or on the construction of treatment processes.⁵⁻⁷⁾

Kawahara *et al.* carried out the anaerobic biodegradability tests of 13 compounds, and demonstrated that 5 aromatic compounds showed persistent under methanogenic conditions⁸⁾. We estimated here, therefore, the biodegradability of eight aromatic compounds with different functional groups including aniline ($-\text{NH}_2$) and terephthalic acid ($-\text{COOH}$) under methanogenic conditions. We also estimated and their toxicity to aceticlastic methanogenesis. Then, we constructed two continuous anaerobic treatments decomposing aniline or terephthalate, which both showed no toxicity to aceticlastic methanogenesis but showed low degradability under methanogenic conditions. Moreover, we estimated the stimulative effect of glucose or acetate on the anaerobic biodegradations of the two aromatic compounds.

MATERIALS AND METHODS

Synthetic wastewater A modified methanotrix medium (DSMZ medium 334 provided by Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH [DSMZ], Braunschweig, Germany) was prepared for synthetic acetate wastewater without Ni^{2+} and Co^{2+} as follows (g/l): sodium acetate, 5.46; acetic acid, 16.0; KH_2PO_4 , 0.3; KHCO_3 , 4.0; NH_4Cl , 1.0; NaCl , 0.6; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.82; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.08; cystein-HCl $\cdot$ H_2O , 0.1; 10 ml of trace element solution without Ni^{2+} and Co^{2+} ; and 10 ml of vitamin solution without B_{12} . $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ were added to give Ni^{2+} and Co^{2+} concentrations of 0.5 and 0.2 mg/l, respectively. The TOC concentration of the synthetic wastewater was 4,000 mg/l.

The acclimatized digested sludge Mesophilic digested sludge from Kumamoto-

Tobu sewage works (Kumamoto-city) was introduced into a completely stirred tank reactor MD-500 (Marubishi, Tokyo) with a working volume of 10 l. This sludge was then acclimatized with a mixed sludge, 2:1 mixture of primary sludge and thickened surplus sludge, in an organic matter loading rate of $1.0 \text{ g/l} \cdot \text{d}$ by draw and fill method⁹⁾ at 37°C for 6 months.

Aceticlastic methanogenic sludge The acclimatized digested sludge was washed and diluted with the synthetic acetate wastewater to give a final OD_{660} of 3.0. A 0.4 l of the resulting sludge was introduced into a completely stirred tank reactor MD-300 (Marubishi, Tokyo) with a working volume of 3 l. The continuous cultivation of the sludge was carried out at 37°C supplied with the synthetic acetate wastewater at a dilution rate of 0.6 d^{-1} for one year. Phase contrast microscopic observation of the culture broth was carried out using type BX60 (Olympus, Tokyo).

Biodegradability test of aromatic compounds under methanogenic conditions A 90 ml of non organic salts medium (in 1 l; KH_2PO_4 , 0.27 g; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 1.12 g; NH_4Cl , 0.53 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.075 g; $\text{MgCl}_2 \cdot 2\text{H}_2\text{O}$, 0.10 g; $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 0.20 g; resazurin, 0.001 g) which was purged with nitrogen gas, mixed with a 10 ml of the acclimatized digested sludge, was introduced into a 125 ml serum bottle capped with a butyl rubber stopper. Aromatic compound of interest was, then, added to give a total organic carbon (TOC) concentration of 100 mg/l and was incubated at 37°C for 8 weeks according to the method by Kawahara *et al.*⁸⁾. Biogas production, due to the complete degradation of the aromatic compound, is theoretically calculated to be approximately 21 ml at 37°C . Produced biogas was measured on every week.

Toxicity test of the aromatic compounds to aceticlastic methanogens The toxicity of persistent compounds to aceticlastic methanogens were estimated as follows. A 0.2 ml of 298.6 mg/l sodium acetate solution was introduced into a 15 ml serum bottle. Next, a 0.2 ml of the test compound solution were added to final concentrations of 50, 100 or 200 mg/l (20 mg/l was adapted for *m*-

nitrophenol). A 15 mm stirrer bar was put to each serum bottle, which was then tightly capped with a rubber stopper and screw cap. Nitrogen gas was purged for one minute into the headspace with a syringe needle. After connecting a 3 ml volumetric pipette using vinyl tubes, the bottles were placed in a water bath at 37°C and left for 1-2 hrs. A 10 ml of aceticlastic methanogenic sludge was added into the bottle and incubated with mixing at 37°C. After one hour of incubation, the gas production volume was measured at 0.5 h intervals manually. The relative gas production volume in the presence and absence of the test substance was compared and the toxicity effect of the test compounds to the aceticlastic methanogenic sludge was determined.

Continuous treatment test using UAFP (Upflow anaerobic filter process) bioreactor system UAFP bioreactor system used in this report is glass with a working volume of 0.78 l (Fig. 1). The apparatus for the UAFP was described previously¹⁰⁾. The acclimatized digested sludge was circulated in the reactor for one over night to allow the microorganisms attach the carrier. Then, the synthetic wastewater, shown in Table 1, 2, was added continuously at a TOC loading rate of 0.5 g/l·d for the synthetic wastewater with multiple carbon sources, 0.38 g/l·d for the synthetic wastewater with aniline, or 0.29 or 0.58 g/l·d for the synthetic wastewater with terephthalic acid. Glucose or acetic acid as easily biodegradable substrate was added to the synthetic wastewater at mole ratios of 0-35 to the test compound. The treatments were carried out over 200 days for each carbon sources; aniline and glucose, aniline and acetic acid, terephthalic acid and glucose, or terephthalic acid and acetic acid.

In order to estimate the amount of aniline and terephthalic acid absorbed in the microorganisms, the solid fraction in the effluent was treated by ultrasonic and the concentration of aniline in the solid fraction was measured. A 5 ml of the effluent was centrifuged at 6,500 rpm for 10 min. The pellet was washed twice by ice chilled sterilized water and resuspended in sterilized water to give an OD₆₆₀ of 3.5. A 1 ml of resulting solution was then introduced into a

2 ml-volume polypropylene tube with 1 g of glass beads (5 mm ϕ) and then beads-beating was carried out for 15 min using Mini-Bead-Beater-8 (Central Kagaku, Tokyo). The cell lysate was centrifuged at 10,000 rpm for 30

Fig. 1 Schematic diagram of equipment used for upflow anaerobic filter process (UAFP) used for the continuous treatment tests

Table 1 Compositions of the synthetic wastewater for continuous treatment test

Carbon sources		
glucose (4.7 g/l), aniline (0.5 g/l), terephthalic acid (0.5, 1.0 g/l), or multiple carbon sources ^{a)} (aniline + glucose, aniline + acetic acid, terephthalic acid + glucose, or terephthalic acid + acetic acid)		
Mineral nutrients		
K ₂ HPO ₄	3.48	g/l
KH ₂ PO ₄	0.435	g/l
NH ₄ Cl	0.056	g/l
MgCl ₂ ·6H ₂ O	0.045	g/l
NaCl	0.125	g/l
CaCl ₂ ·2H ₂ O	0.1	g/l
FeSO ₄ ·6H ₂ O	0.0001	g/l
Na ₂ S·9H ₂ O	0.02	g/l
Vitamin solution ^{b)}	1.0	ml/l
Trace element solution ^{c)}	0.3	ml/l
pH	7.6	

^{a)} The concentrations of each carbon sources for the wastewater containing multiple carbon sources are shown in Table 2.

^{b)} Vitamin solution (in 1 l of distilled water): biotin, 2.0 mg; folic acid, 2.0 mg; pyridoxine·HCl, 10.0 mg; thiamine·HCl, 5.0 mg; riboflavin, 5.0 mg; nicotinic acid, 5.0 mg; DL-calcium pantothenate, 5.0 mg; vitamin B₁₂, 0.1 mg; *p*-aminobenzoic acid, 5.0 mg; lipoic acid, 5.0 mg.

^{c)} Trace element solution (in 1 l of distilled water): FeCl₃, 1.35 g; MnCl₂·4H₂O, 0.10 g; CoCl₂·6H₂O, 0.024 g; CaCl₂·2H₂O, 0.10 g; ZnCl₂, 0.10 g; CuCl₂·2H₂O, 0.025 g; H₃BO₃, 0.01 g; Na₂MoO₄·2H₂O, 0.024 g; NaCl, 1.0 g; NiCl₂·6H₂O, 0.12 g; Na₂SeO₃·5H₂O, 0.026 g; nitrilotriacetic acid (NTA), 12.8 g.

min. The supernatant was used for HPLC analysis to detect aniline or terephthalic acid. The beads-beating condition was decided judging from the protein concentration of the supernatant after beads-beating, which was 5,500 mg/l in the plateau value.

Other analytical methods The TOC concentration and the concentrations of the test compounds in the effluent were measured using the supernatant after centrifugation at $6,000 \times g$ for 10 min of the effluent as follows. The concentrations of TOC and inorganic carbon (IC) in the effluent were measured using a TOC analyzer TOC-500 (Shimadzu, Kyoto). Volatile fatty acids (VFAs) were analyzed in supernatants obtained after centrifugation at $6,000 \times g$ for 10 min of the effluent as described previously⁹⁾. The methane concentration in the produced biogas was analyzed using a gas chromatography type GC-323 (GL Sciences, Tokyo) equipped with a $2m \times 1/8"$ SUS Porapak N 50/80 column (GL Sciences, Tokyo).

The concentrations of aniline, terephthalic acid and phenol in the effluent were determined by reversed-phase high-performance liquid chromatography (Shimadzu model LC-10AT system). Separation was achieved at 35°C by using a column of L-column ODS (Chemicals Evaluation Research Institute, Tokyo) at a flow rate of 1.0 ml/min in which 0.8 ml/min of phosphate buffer (20 mM Na_2HPO_4 solution mixed with 50 mM KH_2PO_4 solution to give a pH of 7.4) and 0.2 ml/min of acetonitrile as the mobile phase. For the separation of terephthalic acid, 0.9 ml/min of phosphate buffer and 0.1 ml/min of acetonitrile were used as the mobile phase.

RESULTS AND DISCUSSION

Biodegradability of eight aromatic compounds under methanogenic conditions

Biodegradability of eight aromatic compounds, benzyl alcohol, phenol, toluene, terephthalic acid, aniline, benzenesulfonic acid, 2,4,6-trichlorophenol and *m*-nitrophenol (100 mg-C/l) using the acclimatized sludge under methanogenic conditions was estimated based on measuring of the integrated gas production (Fig. 2). The gas production curves of benzyl alcohol and

phenol reached a plateau in two to three weeks, being approximately 20 ml. The methane concentration of the produced biogas was 60-70%. The gas production curves of toluene, terephthalic acid, aniline and benzenesulfonic acid were similar to the blank curve within eight weeks. These results suggest that benzyl alcohol and phenol are relatively easy to be degraded. On the other hand, toluene, terephthalic acid, aniline and benzenesulfonic acid were suggested to be persistent. The gas production curves of 2,4,6-trichlorophenol and *m*-nitrophenol were shown lower than the blank, suggesting that these compounds caused a toxicity response of the microorganisms in the acclimatized sludge.

Toxicity of on the aromatic compounds to acetitlastic methanogens

The toxicity effects of six aromatic compounds, which showed persistent or toxicity by the biodegradation tests, to acetitlastic methanogens were measured based on the specific gas evolution rates using the acetitlastic methanogenic sludge (Fig. 3). Microscopic observation of the acetitlastic methanogenic sludge revealed that *Methanosaeta*-like organisms and *Methanosarcina*-like organisms were predominant (data not shown). 200 mg/l of 2,4,6-trichlorophenol and 20 mg/l of *m*-nitrophenol completely inhibited the methanogenesis from acetate. Other compounds and phenol, used as a control, did not inhibit the methanogenesis from acetate even at a high concentration (up to 200 mg/l).

Anaerobic digestion of organic matters consists of a series metabolism; (i) hydrolysis and acidogenesis, (ii) acetogenesis and dehydrogenation and (iii) methanogenesis¹¹⁾. The inhibitory effect of a chemical on anaerobic degradation could depend on its inhibition of single or multiple steps in the series metabolism. In general, methanogenesis from acetate has been recognized the rate-limiting step in anaerobic treatment of soluble organic matters, because there have been several findings that acetitlastic methanogens is the most sensitive to the toxic chemicals¹²⁾. It has been considered that approximately 70% of methane is derived from acetate by

aceticlastic methanogenesis in anaerobic digestion of sewage sludge^{11,13} and cattle waste¹⁰, and in anoxic sediments of a eutrophic lake¹⁵.

In our experiments, *m*-nitrophenol and 2,4,6-trichlorophenol which suggested to be toxicity response in the biodegradability test (Fig. 2), showed a strong toxicity to aceticlastic methanogens (Fig. 3). These results indicate a positive correlation between the toxicity response in the microorganisms in the acclimatized sludge and the toxicity response of aceticlastic methanogens. One possible explanation is that these compound inhibit the anaerobic degradation by their toxicity effect to aceticlastic methanogens.

The degradation of aniline and terephthalic acid in batchwise cultures The biodegradation tests of aniline and terephthalic acid, which showed persistent based on the integrated gas production (Fig. 2) and no toxicity to aceticlastic methanogens (Fig. 3), were carried out. Only 9.8-15.3% of aniline and 5.1-6.4% of terephthalic acid were degraded in 56 days incubation, although 100% of phenol used as a control was degraded (data not shown). However, the degradation efficiencies of aniline and terephthalic acid were improved to 29.2-35.2% and 12.0-14.7%, respectively, by addition of glucose (data not shown).

The continuous treatment tests of aniline and terephthalic acid As the degradation efficiency of aniline and terephthalic acid in batchwise cultures were very low, the degradation tests by continuous treatment using the UAFP were carried out. The concentrations of TOC and aniline in the soluble fraction of the effluent reached steady states, being 240 mg/l and 300 mg/l, respectively, after 172 days treatment of the synthetic wastewater with a 500 mg/l of aniline as the sole carbon source at a TOC loading rate of 0.38 g/l·d. The removal efficiency of TOC and degradation efficiency of aniline were 37 and 40%, respectively. The concentration of suspended solid (SS) in the effluent was 14.9 g/l after 172 days continuous treatment. In order to estimate the amount of aniline absorbed in the microorganisms, the solid fraction in the

Fig. 2 Integrated gas production caused by degradation of eight aromatic compounds. Symbols: ▲, benzyl alcohol; ◇, phenol; ■, toluene; ○, terephthalic acid; ▼, aniline; △, benzensulfonic acid; ◆, 2,4,6-trichlorophenol; □, *m*-nitrophenol; ●, without addition of test compounds (negative control).

Fig. 3 Toxicity test of aromatic compounds to aceticlastic methanogens based on the relative gas production rate. Symbols: ◇, phenol; ■, toluene; ○, terephthalic acid; ▼, aniline; △, benzensulfonic acid; ◆, 2,4,6-trichlorophenol; □, *m*-nitrophenol.

culture broth was treated by ultrasonic and the concentration of aniline in the solid fraction was measured. As the amount of aniline absorbed in SS was 0.064 mg/g-SS, the degradation efficiency of aniline was almost same as that without considering the absorption in SS. The decrease of aniline was suggested to due to degradation by microorganisms in the UAFF.

The continuous treatment test of the synthetic wastewater with terephthalic acid was carried out with 39 days treatment with a 500 mg/l of terephthalic acid as the sole carbon source at a TOC loading rate of 0.29 g/l·d, followed by 186 days treatment with a 1,000 mg/l of terephthalic acid at a TOC loading rate of 0.58 g/l·d. After the total 225 days treatment, the concentrations of TOC and terephthalic acid in the soluble fraction of effluent reached steady states, being 420 mg/l and 680 mg/l, respectively. The removal efficiency of TOC and degradation efficiency of terephthalic acid were 28 and 32%, respectively. The concentration of SS in the

effluent was 24.5 g/l after 255 days treatment. A 0.8135 mg of terephthalic acid was detected in one gram of SS. The decrease of terephthalic acid was also suggested to due to degradation by microorganisms in the continuous treatment.

The continuous treatment tests of aniline and terephthalic acid with glucose As the degradation efficiency of aniline and terephthalic acid were still low even in the continuous treatments, the influence of the addition of glucose as a co-substrate¹⁶⁾ was investigated in the continuous treatment. The mole ratio of glucose and aniline or terephthalic acid was varied as described in Table 2.

Using the steady-state data at each mole ratio, the removal efficiency of TOC and the degradation efficiency of aniline or terephthalic acid were depicted in Fig. 4. The degradation efficiency of aniline increased from 40 to 91% with an increase in mole ratio of glucose to aniline, and the removal efficiency of TOC was also increased at a

Table 2 Carbon sources in the synthetic wastewaters containing multiple carbon sources for the continuous treatment tests

aniline + glucose						
mole ratio (glucose/aniline)	4.9	2.4	1.2	0.6		
aniline (mg/l)	500	500	500	500		
glucose (mg/l)	4700	2350	1175	588		
TOC (mg/l)	2270	1330	860	620		
terephthalic acid + glucose						
mole ratio (glucose/terephthalic acid)	8.7	4.3	2.2	1.1	0.5	
terephthalic acid (mg/l)	500	500	500	500	500	
glucose (mg/l)	4700	2350	1175	588	294	
TOC (mg/l)	2170	1230	760	520	410	
aniline + acetic acid						
mole ratio (acetic acid/aniline)	35.0	20.0	10.0	5.0	3.0	1.0
aniline (mg/l)	500	500	500	500	500	500
sodium acetate (mg/l)	9247	5284	2642	1321	792	264
acetic acid (mg/l)	4515	2580	1290	645	387	129
TOC (mg/l)	4890	2960	1680	1030	770	520
terephthalic acid + acetic acid						
mole ratio (acetic acid/terephthalic acid)	35.0	20.0	10.0	5.0	3.0	1.0
terephthalic acid (mg/l)	500	500	500	500	500	500
sodium acetate (mg/l)	4407	2962	1481	740	444	148
acetic acid (mg/l)	3226	1446	723	360	216	72
TOC (mg/l)	2870	1730	1010	650	510	360

Fig. 4 The degradation efficiency of aniline (A) and terephthalic acid (B) at each mole ratio of glucose to aniline or glucose to terephthalic acid in the continuous treatment tests with addition of glucose.

Symbols: ▼, degradation efficiency (%) of aniline; ○, degradation efficiency (%) of terephthalic acid; □, removal efficiency (%) of TOC.

TOC loading rate of $0.5 \text{ g/l} \cdot \text{d}$ as shown in Fig. 4A. The rate of biogas production at a mole ratio of 4.9 was $150\text{--}180 \text{ ml/l} \cdot \text{d}$. The methane concentration was approximately 50 %. The degradation efficiency of terephthalic acid increased from 48 to 95% with an increase in mole ratio of glucose to terephthalic acid, and the removal efficiency of TOC was also increased at a TOC loading rate of $0.5 \text{ g/l} \cdot \text{d}$ as shown in Fig. 4B. The biogas production rate at a mole ratio of 4.3 was $100\text{--}150 \text{ ml/l} \cdot \text{d}$. The methane concentration in the biogas was approximately 65 %.

These results indicated that addition of glucose improved the biodegradation of aniline or terephthalic acid under continuous treatment drastically compared with the results of batch cultivations.

The continuous treatment tests of aniline and terephthalic acid with acetate In order to investigate the effect of the co-substrate at a simplified condition, acetate was used instead of glucose. The mole ratio of glucose and aniline or terephthalic acid was varied as described in Table 2.

Using the steady-state data at each mole ratio, the removal efficiency of TOC and the degradation efficiency of aniline or terephthalic acid were depicted in Fig. 5. The

degradation efficiency of aniline increased from 45 to 90% with an increase in mole ratio of acetate to aniline, and the removal efficiency of TOC was also increased at a TOC loading rate of $0.5 \text{ g/l} \cdot \text{d}$ as shown in Fig. 5A. The biogas production rate was $80\text{--}100 \text{ ml/l} \cdot \text{d}$ and the concentration of acetic acid in the effluent was approximately $58\text{--}60 \text{ mg/l}$ at a mole ratio of 10.0. The degradation efficiency of terephthalic acid increased from 85 to 95% with an increase in mole ratio of acetate to terephthalic acid, and the removal efficiency of TOC was also increased at a TOC loading rate of $0.5 \text{ g/l} \cdot \text{d}$ as shown in Fig. 5B. The rate of biogas production was $130\text{--}150 \text{ ml/l} \cdot \text{d}$ and the methane concentration in the biogas was approximately 60% under 10.0 mole-ratio conditions. Acetic acid was not detected in the effluents over all mole-ratio conditions.

Glucose and acetic acid showed stimulative effects for anaerobic degradation of aniline and terephthalic acid, which were suggested as persistent chemicals by the biodegradability test. These results suggest that the additional carbon sources made a change of microbial consortia and/or the metabolic activities of individual cells in the methanogenic sludge

Fig. 5 The degradation efficiency of aniline (A) and terephthalic acid (B) at each mole ratio of acetate to aniline or acetate to terephthalic acid in the continuous treatment tests with addition of acetate. Symbols: ▼, degradation efficiency (%) of aniline; ○, degradation efficiency (%) of terephthalic acid; □, removal efficiency (%) of TOC.

No microorganism capable of degradation of terephthalic acid anaerobically has been isolated. The anaerobic degradation pathway of terephthalic acid is considered as three steps of reactions as follows: (i) the decarboxylation of terephthalic acid causing benzoate⁶⁾, (ii) acetate and hydrogen production from benzoate¹⁷⁾, and (iii) methanogenesis. Kleerebezem *et al.* reported the inhibition of terephthalate degradation by acetate using anaerobic batch experiment⁶⁾. Fajardo *et al.* reported the inhibition of terephthalic acid degradation by glucose¹⁸⁾. These results seem to be different from our finding that acetic acid and glucose showed stimulative effect to anaerobic degradation of terephthalic acid. The difference might be due to the low concentration of glucose or acetate, and/or might be caused by the microbial consortia that the cultures by Kleerebezem or Fajardo consisted of relatively pure microorganisms compared with our culture. In our more complex microbial consortium, a higher population of aceticlastic methanogens or other microorganisms related to degradation of glucose might be needed for the efficient continuous degradation of terephthalic acid. Therefore, additional acetic acid or glucose

was suggested to show stimulative effects, although analysis of the microbial flora in the methanogenic consortium is needed for further understanding.

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- (Submitted 2001. 5. 14)
(Accepted 2001. 10. 17)