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Variation of bacterial community immobilized in polyethylene glycol carrier during mineralization of xenobiotics analyzed by TGGE technique

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Abstract−*Acinetobacter* sp. SMIC-1, *Cupriavidus* sp. SMIC-2, *Pseudomonas* sp. SMIC-3, *Paracoccus* sp. SMIC-4, and *Pseudomonas* sp. SMIC-5 capable of mineralizing xenobiotics (manmade organic compounds) that are diethyleneglycol monomethyleher (DGMME), 1-amino-2-propanol (APOL), 1-methyl-2-pyrrolidinone (NMP), diethyleneglycol monoethylether (DGMEE), tetraethyleneglycol (TEG) and tetrahydrothiophene 1,1-dioxide (Sulfolane) were immobilized mixedly in polyethyleneglycol carrier (SMIC-PEG). TGGE technique was employed to analyze variation of the immobilized bacterial community during xenobiotics being mineralized. The SMIC-PEG mineralized more than 95% of the xenobiotics except sulfolane in 6 days. When activated sludge (AS) was co-immobilized with SMIC community in PEG carrier (AS-SMIC-PEG), degradation efficiency of DGMEE, NPM was a little decreased; however, the degradation of other xenobiotics was neither increased nor decreased significantly. The bacterial community diversity in the SMIC-PEG was gradually decreased in proportion to incubation time in a batch cultivation reactor. SMIC strains in AS-SMIC-PEG were substituted by other bacterial community after 6 days of incubation time in batch cultivation reactor. The SMIC-PEG mineralized around 90% of xenobiotics in a continuous pilot reactor when 100 or 200 mg/L of xenobiotics was fed for 8 hr of hydraulic retention time (HRT); however, the mineralization efficiency was decreased significantly to around 75% when 200 mg/L of xenobiotics was fed for 4 hr of HRT. The mineralization effect of AS-SMIC-PEG for xenobiotics was lower than SMIC-PEG. Bacterial community diversity in both SMIC-PEG and AS-SMIC-EG was decreased in proportion to operation time in the continuous pilot reactor; however, some of them were maintained during operation for more than 50 days.

Key words: Xenobiotics, PEG Carrier, Ethyleneglycol, Pyrrolidinone, Thiophene, TGGE Technique

INTRODUCTION

Manmade organic compounds that have been utilized variously in industrial fields cause considerable environmental pollution and human health problems as a result of their persistence, toxicity, and conversion into undesirable byproducts [1]. Some of the manmade organic compounds, which are alien to existing enzymes or metabolic systems of microorganisms, are called xenobiotics [2]. The low biodegradability of xenobiotics may be due to the incapacity of microorganisms to effectively metabolize the organic compounds with uncommon chemical structures or properties; however, the microbial ability for the biodegradation of xenobiotics can be improved biologically by enrichment culture or adaptation to the specific organic compounds [3]. The time required for a bacterial community to mineralize xenobiotics and the concentration of xenobiotics may be a critical factor for determining degradation efficiency in wastewater treatment reactor [4,5]. Especially, the difference of persistence and initial concentration among xenobiotics contained in wastewater may be an important determinant for maintenance of bacterial community and biodegradability of individual xenobiotic [6-8].

Mixture of structurally different xenobiotics may be degraded or mineralized by using the bacterial community composed of metabolically different species [9]. A bacterial species depending on a specific xenobiotic may maintain its population in the condition without depletion of the xenobiotic [10]. Theoretically, minimal concentration of xenobiotic has to be maintained to conserve the bacterial population in both batch and continuous cultivation system [11, 12]. Some of xenobiotics that are broken down or degraded in vitro by microorganisms may be mineralized much more slowly than desired from environmental consideration in the wastewater treatment reactor [13]. In a wastewater treatment reactor containing various xenobiotics, an individual xenobiotic may be consumed differently because the newly produced metabolites or byproducts may influence the bacterial metabolism [14,15]. The slow mineralization may be solved by the immobilization technique of microorganisms, by which a stable environment for microorganisms may be developed, metabolic activity of biocatalyst may be maintained, and the bacterial community may be recycled and reused [16-19].

In previous research, we isolated DGMME-, TEG-, DGMEE-, APOL-, NMP-, and Sulfolane-degrading bacteria from a specially designed enrichment culture, such as *Acinetobacter* sp. SMIC-1,

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Cupriavidus sp. SMIC-2, *Pseudomonas* sp. SMIC-3, *Paracoccus* sp. SMIC-4, and *Pseudomonas* sp. SMIC-5. These microorganisms were immobilized mixedly in polyethyleneglycol (PEG) carrier and employed to mineralize wastewater containing the xenobiotics that are released from an electronic company. The electronic wastewater is difficult to mineralize because the initial concentrations of individual xenobiotic are different from each other and the metabolic activity of the SMIC community is also not the same. Objective of this study is to estimate the mineralization efficiency of the electronic wastewater by xenobiotic-degrading bacterial community or AS community immobilized in PEG carrier in coupling with variation of bacterial diversity.

MATERIALS AND METHODS

1. Chemicals

DGMME, TEG, DGMEE, APOL, NMP, Sulfolane, and other chemicals used in this research were purchased from the Korean branch of Sigma-Aldrich (Yongin-city, Korea).

2. Wastewater

Wastewater obtained from an electronic company located in Yongin City was used for continuous cultivation reactor without modification but was modified to be finally 173 mg of DGMME, 10 mg of TEG, 33.6 mg of DGMEE, 5 mg of APOL, 93.2 mg of NMP and 40.4 mg of Sulfolane per liter by addition of individual xenobiotic for batch cultivation reaction.

3. Activated Sludge (AS)

The AS was obtained from the sedimentation reactor of a wastewater treatment system, in which the mixed wastewater released from an industrial complex is treated synthetically and mineralized finally.

4. Cultivation of SMIC Community

Acinetobacter sp. SMIC-1, *Cupriavidus* sp. SMIC-2, *Pseudomonas* sp. SMIC-3, *Paracoccus* sp. SMIC-4, and *Pseudomonas* sp. SMIC-5 were cultivated separately in a nitrogen phosphate basal medium (NPBM) composed of 2 g/L of NH_4Cl, 2 g/L of KH_2PO_4, 2 g/L $NaNO_3$, and 2 ml/L of trace mineral stock solution, to which each 2 g/L of TEG, DGMME, DGMEE, APOL, NMP and Sulfolane were added. The trace mineral stock solution contains 0.01 g/L of $MnSO_4$, 0.01 g/L of $MgSO_4$, 0.01 g/L of $CaCl_2$, 0.002 g/L of $NiCl_2$, 0.002 g/L of $CoCl_2$, 0.002 g/L of $SeSO_4$, 0.002 g/L of WSO_4, 0.002 g/L of $ZnSO_4$, 0.002 g/L of $Al_2(SO_4)_3$, 0.0001 g/L of $TiCl_3$, 0.002 g/L of $MoSO_4$, and 10 mM EDTA. Each bacterial culture was incubated at 30 °C for 72 hr under agitation condition at 120 rpm and harvested to immobilize in PEG carrier by centrifugation at 5,000 ×g, 4 °C for 30 min.

5. Immobilization of Bacterial Cells in PEG Carrier

Bacterial immobilization technique used in this study was modified from the procedure of Sumino et al. [20]. The harvested SMIC community or AS was suspended in a PEG aqueous solution containing an inorganic additive and D-sorbitol (crosslinker), N,N,N,N′-tetramethylethylendiamine (promoter), to which potassium sulfate (initiator) was added and mixed thoroughly. The composition of immobilizing materials was 180 g/L of PEG prepolymer, 40 g/L of inorganic additive, 90 g/L of crosslinker, 5 g/L of promoter, and 25 g/L of initiator, to which 5 g/L of SMIC community (SMIC-PEG) or 2.5 g/L of SMIC and 2.5 g/L of AS mixture (AS-SMIC-PEG) was added based on dry weight. The elastic polymerized gel containing bacterial mixture was processed to be uniform globular shape (4 mm diameter). The pellets were washed thoroughly with water before being used.

6. Estimation of Xenobiotics Mineralization in Batch and Continuous Reactor

The previously prepared SMIC-PEG or AS-SMIC-PEG was filled in a 20 L-reactor at 10% of bed-volume level for batch cultivation reactor and a 500 L-pilot scaled-reactor at 80% of bed-volume level for continuous cultivation reactor. Both reactors were filled with a modified wastewater containing xenobiotics released from an electronic company. The wastewater was analyzed precisely to determine the concentration of individual xenobiotic in batch cultivation reactor. The xenobiotics concentration of inflow wastewater was controlled to be from 100 mg/L to 250 mg/L by adjustment of HRT or proper dilution in the continuous cultivation reactor. Both reactors were begun to be operated by aeration after temperature was controlled to be 30 °C. Dissolved oxygen and pH were adjusted to be 3 mg/L by aeration and 7±0.5, respectively, which was real time monitored automatically. The individual xenobiotic in the batch cultivation reactor was analyzed separately by GC-MS and LC-MS for 9 days at the intervals of 3 days. Meanwhile, the xenobiotics in the continuous cultivation reactor were analyzed based on the total organic carbon (TOC) at the intervals of 2 days for 68 days. The initial TOC of wastewater and HRT were controlled to determine optimal concentration and HRT for maximal treatment efficiency. DNA was isolated directly from the bacterial community in SMIC-PEG or AS-SMIC-PEG.

7. Temperature Gradient Gel Electrophoresis

The 16S-rDNA amplified from chromosomal DNA was used as a template for TGGE sample (16S-rDNA variable region) preparation. A variable region of 16S-rDNA was amplified with forward primer (eubacteria, V3 region) 341f 5′-CCTACGGGAGGCAGCAG-3′ and reverse primer (universal, V3 region) 518r 5′-ATTACCGCGGCTGCTGG-3′. GC clamp (5-CGCCCGCCGCGCGCGGCGGG CGGG GCGGGGGCACGGGGGGCCTACGGGAGGCA GCAG-3′) was attached to the 5′-end of the GC341f primer [21]. The procedures for PCR and DNA sequence were same as the 16S-rDNA amplification condition except for the annealing temperature of 53 °C. The TGGE system (Bio-Rad, Dcode™, Universal Mutation Detection System, USA) was operated as specified by the manufacturer. Aliquots (45 ml) of PCR products were electrophoresed in gels containing 8% acrylamide, 8 M urea, and 20% formamide with a 1.5×TAE buffer system at a constant voltage of 100 V for 12.5 hr and then 40 V for 0.5 hr, applying a thermal gradient of 39 to 52 °C. Before electrophoresis, the gel was equilibrated to the temperature gradient for 30 to 45 min.

8. Amplification and Identification of TGGE Band

DNA was extracted from TGGE band and purified using a DNA gel purification kit (Accuprep, Bioneer, Korea). The purified DNA was amplified with the same primers and procedures used for TGGE sample preparation, in which the GC clamp was not attached to the forward primer. Species-specific identity of the amplified variable 16S-rDNA was determined based on the sequence homology in GenBank database system.

9. Analysis

DGMME, DGMEE, NMP, and Sulfolane were quantitatively

Table 1. Degradation efficiency of Xenobiotics contained in electronic wastewater by SMIC-PEG and AS-SMIC-PEG in lab-scaled reactor under batch cultivation reactor for 9 days

Chemicals (mg/L)	SMIC-PEG (days)				AS-SMIC-PEG (days)			
	(0)	(3)	(6)	(9)	(0)	(3)	(6)	(9)
Diethyleneglycol monomethylether (DGMME)	173	26	<0.1	<0.1	173	43	<0.1	<0.1
Tetraethyleneglycol (TEG)	10	<0.1	<0.1	<0.1	10	<0.1	<0.1	<0.1
Diethyleneglycol monoethylether (DGMEE)	33.6	<0.1	<0.1	<0.1	33.6	23.3	<0.1	<0.1
1-Amino-2-propanol (APOL)	5.0	3.33	0.28	<0.1	5.0	3.4	0.23	<0.1
1-Methyl-2-pyrrolidinone (NMP)	93.2	41.3	1.26	<0.1	93.2	71.1	17.3	<0.1
Tetrahydrothiophene 1,1-dioxide (Sulfolane)	40.4	39.5	30.2	22.1	40.4	38.0	29.9	21.4

*All data were mean value obtained from triple test and standard deviation was not higher than 3%

*<0.1 Indicates the concentration that was not detected or detected at trace level

and qualitatively analyzed by GC-MS based on the modified procedures, which was adapted from technique developed by Shin and Jung [22]. 100 ml of the diluted bacterial culture was placed in a 250-ml separating funnel. About 30 g of NaCl and 100 µl of Ethylene glycol butyl ether internal standard solution (1,000 mg/L) were added to this solution, and the sample was extracted with 20 ml of methylene chloride by mechanical shaking for 20 min. Then the solvent layer was recovered in a flask. One thousand µl of the solvent phase was transferred to a GC vial. At appropriate times, a 1 µl-sample of the solution was analyzed by GC. GC-MS analyses were performed using a PerkinElmer Clarus 600 gas chromatography (GC) interfaced with a mass spectrometer detector (PerkinElmer Clarus 600T mass spectrometer). The GC separation was carried out with Agilent DB-WAXETR capillary column (30 m by 0.32 mm; film thickness, 1 µm) using helium as the carrier gas (flow rate, 1.0 ml min^{-1}). The following GC temperature program was used: injector temperature, 250 °C; initial oven temperature, 80 °C; rate of temperature increase, 10 °C min^{-1} up to 250 °C; holding time, 2 min. Samples were injected in a splitless injection mode. The injector was switched to split mode in 2 min after sample was injected.

High performance liquid chromatography (HPLC; HP1200, Agilent, USA) coupled with ESI-ion trap MS was adopted for the analysis of TEG and APOL. The HPLC apparatus consisted of an Rx-SIL column (3×50 mm, 1.8 µm; Agilent, USA) connected to an LXQ ion trap mass spectrometer (Thermo Scientific, USA) through its ESI interface. A small aliquot of the filtrated bacterial culture (10 µl) was injected into the chromatographic system. The mobile phase consisted of Methanol/H_2O/Formic acid (80/20/0.05, V/V/V) with flow rate of 0.1 ml/min. The LXQ spectrometer, completely controlled by the Xcalibur software (Thermo Scientific, USA), was operated in the positive ion mode; selected ion monitoring (for an *m/z* of 195.0 and 76.0, respectively, corresponding to the charged ion $(M+H)^+$ of TEG and APOL) modes was recorded for each sample.

RESULTS

1. Biodegradation of Individual Xenobiotic in Batch Cultivation Reactor

When the high-concentrated modified electric wastewater was treated by SMIC-PEG and AS-SMIC-PEG in batch cultivation reactor, the individual xenobiotic was degraded differently. The ethyleneglycol derivatives that are DGMME, TEG and DGMEE were mineralized completely in 6 days of incubation time, and APOL and NMP were mineralized completely in 9 days of incubation time by both SMIC-PEG and AS-SMIC-PEG as shown in Table 1; however, about 50% of Sulfolane remained at even the 9^{th} day of incubation time. The mineralization effect of AS-SMIC-PEG for DGMME and NMP was a little lower than the SMIC-PEG. Totally, about 32 mg/L and 47 mg/L of Xenobiotics remained in the batch cultivation reactor operated by SMIC-PEG and AS-SMIC-PEG, respectively, at the 6^{th} day of incubation time, which corresponded to 9% and 13% of initial concentration (355.2 mg/L), respectively. The relatively lower rates of mineralization may be solved by extension of incubation time; however, ethyleneglycol derivatives-degrading bacteria may lose their biological activity for ecological competition by depletion of substrate during long-term incubation. Metabolic intermediates and byproducts were not detected by GC- and LC-MS, which can precisely detect all organic compounds contained in wastewater samples at the level of 0.2 mg/L.

2. Time-coursed Variation of Bacterial Community in Batch Cultivation Reactor

Bacterial community diversity estimated by the TGGE technique in batch cultivation reactors was not only decreased gradually but also changed in proportion to incubation time as shown in Fig. 1. Most SMIC strains remained at 6^{th} of incubation time (Fig. 1(a)); however, some of them disappeared or were substituted by other bacterial species at the 9^{th} day of incubation time in the reactor with SMIC-PEG. This result is related to the xenobiotics concentration that remained in both reactors with SMIC-PEG and AS-SMIC-PEG at the 6^{th} and 9^{th} day of incubation time (Table 1). Meanwhile, most SMIC strains disappeared and were substituted by other bacterial species in the AS-SMIC-PEG after the 6^{th} day of incubation time (Fig. 1(b)). From these results, the SMIC community is supposed to lose their biological activity by competition with the bacterial community originating from AS for substrate and space within PEG carrier, by which the mineralization efficiency of xenobiotics may be decreased (Table 1). At least 9% of xenobiotics is supposed to be required for SMICs community to survive within SMIC-PEG carrier based on 32 mg/L of xenobiotics remaining in the reactor and TGGE pattern (lane 3 in Fig. 1(a)).

3. Mineralization of Xenobiotic in Continuous Cultivation Reactor

Influx concentration of the electronic wastewater and HRT was maintained to be 100 mg/L and 8 hr from initial time to 40^{th} day,

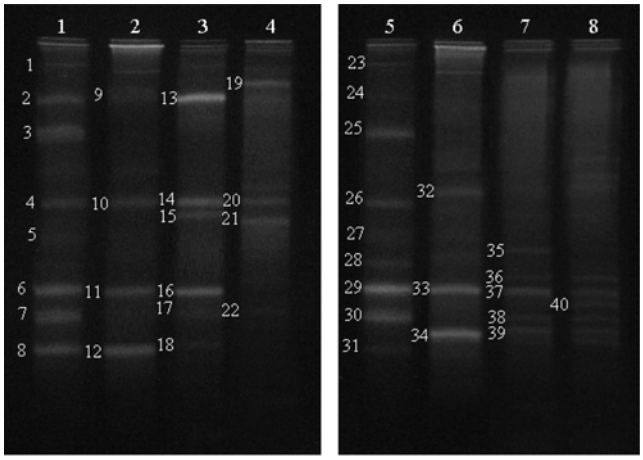

Fig. 1. Time-coursed variation of bacterial community in SMIC-PEG (a) and AS-SMIC-PEG (b) analyzed by TGGE. The SMIC-PEG and AS-SMIC-PEG were cultivated in the electronic wastewater containing DGMME, TEG, DGMEE, APOL, NMP, and Sulfolane. Genomic DNA was directly extracted from bacterial community in SMIC-PEG or AS-SMIC-PEG right after the reactor was operated (lane 1 and 5), at 3rd day (lane 2 and 6), at 6th day (lane 3 and 7) and at 9th day (lane 4 and 8) of incubation time. DNAs extracted from the numbered bands were 90-100% identified with the following: 1, 23. *Acinetobacter* sp. (FJ877153).; 2, 9, 13, 24. *Pseudomonas* sp. (FJ877154).; 3, 25. *Pseudomonas* sp. (FJ877156); 4, 10, 14, 20, 26. *Alkaligenes* sp. (FJ581029); 5, 27. *Pseudomonas* sp. (EF514898); 6, 11, 16, 29, 33, 37. *Paracoccus* sp. (FJ877155); 7, 17, 22, 30, 38. *Paracoccus* sp. (DQ652555); 8, 12, 31. *Cupriavidus* sp. (FJ877152); 15, 21. *Pseudomonas* sp. (EU139849); 19. *Pseudomonas* sp. (AM911667); 28. *Acinetobacter* sp. (EF525671); 34. *Pseudomonas* sp. (EF153193); others: uncultured or unidentified bacteria.

200 mg/L and 8 hr from 41st to 53rd day, and 200 mg/L and 4 hr to optimize the mineralization efficiency in coupling with maintenance of bacterial community. Mineralization efficiency of xenobiotics contained in the electronic wastewater was maintained more than 85% at 200 mg/L of influx concentration for 8 hr of HRT but decreased less than 70% at 200 mg/L of influx concentration for 4 hr of HRT by SMIC-PEG as shown in Fig. 2; however, the mineralization efficiency by AS-SMIC-PEG was less than 80% and fluctuated irregularly at even 100 mg/L of influx concentration for 8hr of HRT. This result may be caused by competition between SMIC strains and AS community for substrates. The initially prepared SMIC-PEG mineralized xenobiotics effectively without addition of new ones in the continuous cultivation reactor for at least more than 65 days. It is possible that some of bacterial species immobilized in the PEG carrier may regenerate and maintain their biological activity using the xenobiotics as substrate.

4. Time-coursed Variation of Bacterial Community in Continuous Cultivation Reactor

Bacterial community diversity was estimated in the continuous cultivation reactor by the TGGE technique as shown in Fig. 3, in which the bacterial diversity was not decreased significantly but their community was changed gradually in proportion to incubation time. More than five bacterial species within the SMIC-PEG were maintained at 50th day of incubation time and four species were identified with SMIC strains. From these results, it can be supposed that the xenobiotics-degrading bacteria may be maintained minimally in the condition without depletion of substrate that was fed continuously to the reactor. The difference of TGGE band pattern between bacterial communities within the SMIC-PEG and the AS-SMIC-PEG may be caused by the bacterial community contained in AS. The TGGE pattern of bacterial community within the AS-SMIC-PEG was maintained more than 90% during incubation for at least 50 days; however, the bacterial species identified based on sequence of DNA extracted from TGGE bands were different mostly from the SMIC strains. The different bacterial species from SMIC strains may be originated from AS, which may actively mineralize specific xenobiotics that are DGMME, TEG, and APOL. The difference of SMIC community diversity between SMIC-PEG and AS-SMIC-PEG corresponds to the mineralization efficiency of xenobiotics by SMIC-PEG and AS-SMIC-PEG (Fig. 2).

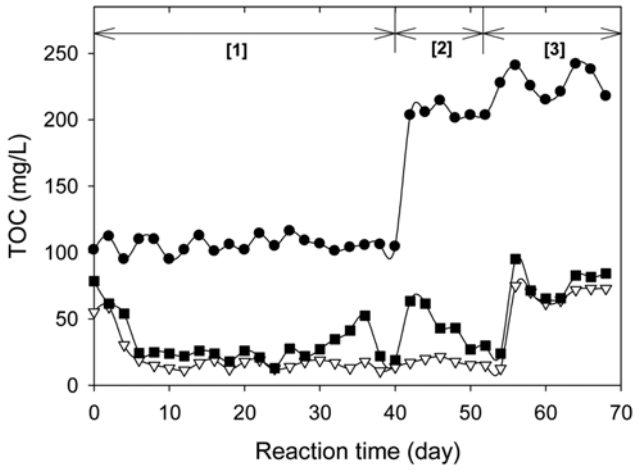

Fig. 2. Time-coursed mineralization efficiencies of electronic wastewater composed of DGMME, TEG, DGMEE, APOL, NMP and Sulfolane in a continuous culture system. The SMIC-PEG (▽) and AS-SMIC-PEG (■) were cultivated in a pilot-scaled reactor (500 L) containing electronic wastewater composed of Xenobiotics mixture. Concentration of organic compounds based on TOC and HRT of influx wastewater (●) was adjusted to 100 mg/L and 8 hr, respectively, from initial time to 40th day [1], 200 mg/L and 8 hr, respectively, from 41st to 52nd [2], and 200 mg/L and 4 hr, respectively, from 53rd to 68th day [3].

DISCUSSION

An environment polluted with various xenobiotics can be purified by microorganisms capable of mineralizing xenobiotics [1,23]. DGMME, TEG, DGMEE, APOL, NMP, and Sulfolane are the typical xenobiotics that are difficult to mineralize by microorganisms without adaptation to the xenobiotics or microorganisms growing in natural habitats [24-27]. The SMIC strains isolated from a specially designed enrichment culture grew on the individual or mixture of xenobiotics without other organic nutrients [2,3]. The treatment time required to biologically mineralize individual xenobiotic

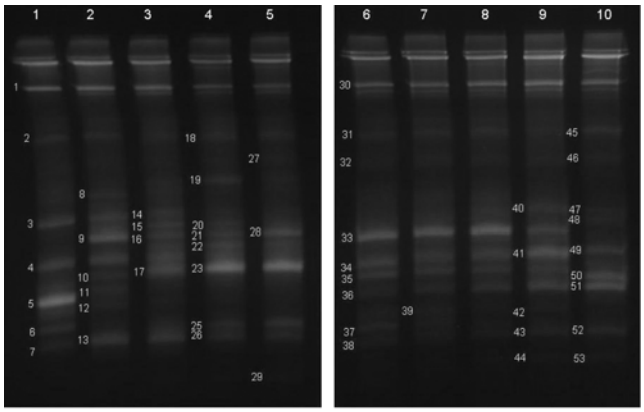

Fig. 3. Time-coursed variation of bacterial community immobilized in the PEG carrier, which was analyzed by TGGE. The electronic wastewater containing DGMME, TEG, DGMEE, APOL, NMP, and Sulfolane was cultivated in a pilot-scaled reactor composed of SMIC-PEG carrier, to which activated sludge was not added (a) or added (b) under continuous-cultured condition. Genomic DNA was extracted directly from both SMIC-PEG carrier and culture fluid at 10^{th} day (lane 1, 6), 20^{th} day (lane 2, 7), 30^{th} day (lane 3, 8), 40^{th} day (lane 4, 9) and 50^{th} day (lane 5, 10) of incubation time. DNAs extracted from the numbered bands were 93-98% identified with the following: 1. *Pseudomonas* sp. (FJ877156); 2, 18. *Pseudomonas* sp. (EU729354); 3. *Pseudomonas* sp. (FJ608757); 4. *Acinetobacter* sp. (FM213385); 5, 11, 36, 51. *Alkaligenes* sp. (AY346141); 6, 25. *Cupriavidus* sp. (EU221391); 7, 37, 43, 52. *Methylopila* sp. (AY466434); 9, 28, 33. *Burkholderia* sp. (AF214132); 17, 23. *Paracoccus* sp. (EF103199); 30. *Pseudomonas* sp. (AB366320); 31, 45. *Klebsiella* sp. (AM922113); 32, 46. *Paracoccus* sp. (FJ581419); 35. *Pseudomonas* sp. (EU194332); 39, 42. *Methylobacterium* sp. (AM940883); 48. *Klebsiella* sp. (GQ339109); 50. *Klebsiella* sp. (GU195170); others; uncultured or unidentified bacteria.

is different according to the structural stability [28]. The structurally various xenobiotics contained in the electronic wastewater may cause SMIC-PEG to catabolize the individual xenobiotic differently [29]. The ring-structured NMP and Sulfolane may be more stable chemically than chain-structured ethyleneglycol-derivatives and APOL, which was proved experimentally (Table 1) [30]. The difference of biodegradability among xenobiotics may cause some bacterial species to lose their biological activity by depletion of substrates (Fig. 1). The difference of mineralization efficiency between the ring- and chain-structured xenobiotics may be a weak point for effective treatment of wastewater but a strong point for maintenance of nutritional condition. SMIC community immobilized in PEG carrier is very possible to generate metabolic energy from the chain-structured xenobiotics during short-term reaction and from the ring-structured xenobiotics during long-term reaction. PEG used as a carrier for bacterial immobilization is strong for high water pressure and friction, does not affect bacterial viability during polymerization and can be easily handled for molding and cutting. Especially, bacteria-immobilized PEG carrier is useful for continuous flow reactor in comparison with free cell reactor because the biocatalysts immobilized in PEG carrier can be maintained in the bioreactor without outflow.

The decrease of bacterial community diversity may cause an individual xenobiotic to be mineralized limitedly. The biodegradability of NMP and Sulfolane is inversely proportional to the TGGE band diversity, which may be an indicator to estimate the treatment efficiency of electronic wastewater. Practically, AS that was co-immobilized with SMIC in PEG carrier decreased SMIC community diversity but did not activate mineralization of NPM and Sulfolane. Some of AS community may catabolize xenobiotics, but some of them may compete for habitat with the SMIC community within the PEG carrier. Accordingly, the application of specific bacterial species capable of mineralizing xenobiotics or xenobiotics is more important than increase of biomass or bacterial diversity in order to improve the mineralization efficiency of xenobiotics.

The depletion of xenobiotics caused the SMIC community diversity to be decreased or some species to be disappeared from the PEG carrier in the batch cultivation reactor. However, NMP and Sulfolane degraded slowly by the SMIC-PEG may rather function as a stable substrate for long-term survival of bacteria than induce the mineralization efficiency to be decreased in the continuous cultivation reactor. The ratio of NMP and Sulfolane to xenobiotics was about 0.089 {(30.2+1.26)/355.2} and 0.133 {(29.9+17.3)/355.3} at 6^{th} day in batch cultivation reactor with SMIC-PEG and AS-SMIC-PEG (Table 1), respectively, which is similar to the mineralization tendency of xenobiotics in the continuous cultivation reactor (Fig. 2). Four or 8 hr of HRT may be not sufficient for mineralization of both ring- and chain-structured xenobiotics based on the TOC variation in the continuous cultivation reactor. However, the low mineralization efficiency of xenobiotics might be a necessary and sufficient condition for maintenance of SMIC community diversity for more than 50 days.

HRT and initial concentration of xenobiotics can be controlled mechanically; however, the biological activity of SMIC community that depends absolutely upon substrate concentration is difficult to control physically. The estimation of SMIC community diversity in coupling with TOC variation may be a practical technique capable of being employed in the biological treatment of xenobiotics. TGGE technique is useful for obtaining the visual data, which can be substituted by the real-time PCR technique for field application.

Conclusively, SMIC community immobilized in PEG carrier mineralized the xenobiotics successively at a level of higher than 85% in the continuous cultivation reactor for more than 55 days. The 85% of efficiency may be not enough for post treatment of residual xenobiotics by chemical reaction. The cost for purification of wastewater by chemical treatment can be saved in proportion to bacterial mineralization efficiency of xenobiotics. At the present time, we are operating a continuous cultivation reactor composed of the SMIC-PEG to improve the mineralization efficiency to be more than 95% by precise control of HRT, SMIC-PEG bed volume, initial concentration of xenobiotics, and aeration rates. New bacterial species capable of mineralizing Sulfolane also are enriched to reinforce the SMIC-PEG activity.

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