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Effect of Electrochemical Oxidation Potential on Biofilter for Bacteriological Oxidation of VOCs to CO_2

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Abstract In this study, an electrical conductive carbon fiber was used as a biofilter matrix to electrochemically improve the biofilter function. A bioreactor system was composed of carbon fiber (anode), titanium ring, porcelain ring, inorganic nutrient reservoir, and VOC reservoir. Electric DC power of 1.5 volt was charged to the carbon fiber anode (CFA) to induce the electrochemical oxidation potential on the biofilter matrix, but not to the carbon fiber (CF). We tested the effects of electrochemical oxidation potential charged to the CFA on the biofilm structure, the bacterial growth, and the activity for metabolic oxidation of VOCs to CO_2. According to the SEM image, the biofilm structure developed in the CFA appeared to be greatly different from that in the CF. The bacterial growth, VOCs degradation, and metabolic oxidation of VOCs to CO_2 in the CFA were more activated than those in the CF. On the basis of these results, we propose that the biofilm structure can be improved, and the bacterial growth and the bacterial oxidation activity of VOCs can be activated by the electrochemical oxidation potential charged to a biofilter matrix.

Key words: Electrochemical reactor, electric conductive biofilter, BTEX degradation, VOC, biofilm, *Burkholderia cepacia*

VOCs such as BTEX (benzene, toluene, ethylbenzene, and xylene isomers) are toxic to humans and their removal from polluted environments is of special interest [23]. Frequently, a considerable amount of gasoline enters the environment as a result of leakage from underground storage reservoirs, accidental spills, or improper waste disposal practice [5]. When gasoline is in contact with water, the benzene, toluene, ethylbenzene, and xylene isomers (BTEX) that account for as much as 90% of the gasoline components are found in the water-soluble fraction [36]. These chemicals are the most common contaminants found in soil and underground water. It has been assumed that soil bioremediation of VOCs relies upon various indigenous bacterial [3, 8, 17, 19, 27, 35, 40] and fungal populations [32].

Most VOCs are possibly evaporated from contaminated sites and are a major source for air pollution. However, major sources of VOCs are not from the natural environment, but mainly from petrochemical industries. Once the VOCs are evaporated into air, biodegradation is very difficult without a specially designed biofilter or biodegradation system [12, 14, 20]. Bacteriological purification of waste gas has earlier been reported for several air pollutants including ethylene [11], styrene [1], methyl *tert*-butylether [15], salicylic acid [31], nitrobenzene [30], and phenanthrene [18]; however, no bacteriological system for removal of VOCs including BTEX showed satisfactory operational stability and sufficient efficiency to reduce the VOCs concentration to a level near mM unit [1, 10, 12, 13, 15, 39, 28].

In this study, we used an electrical conductive carbon fiber as the biofilter matrix, to which electrical anodic potential was charged in order to test the possibility that the bacterial growth and metabolic oxidation of VOCs may be activated by the electrochemical oxidation potential formed in a biofilter matrix. Here, we report the result of studies with a biofilter-type bioreactor that was based on a petroleum-oxidizing bacterium that was self-immobilized on carbon fiber. Inorganic nutrient solution was circulated differently from the inflow pathway of air containing VOCs, and the device was operated in the trickling air biofilter mode.

MATERIALS AND METHODS

Volatile Organic Carbons

The VOCs used in this experiment were of reagent grade except kerosene. Xylene was a mixture of *ortho*- and *meta*-forms, and kerosene was purchased from a gas station (LG-Caltex).

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Organism and Growth

The *Burkholderia cepacia* was isolated from an enrichment culture system, which was incubated in a defined medium, containing soil (5 g/l) taken from areas Three-hundred ml/l/min of saturated air with a mixture of kerosene, decane, benzene, toluene, ethylbenzene, and xylene was supplied to the bacterial culture, and water vapor generated by aeration was condensed to recycle. The defined medium contained 5 g/l KH_2PO_4, 3 g/l K_2HPO_4, 10 g/l NH_4Cl, 0.2 g/l Na_2SO_4, and 2 ml/l trace mineral stock solution, which contained 0.01 g/l $MnSO_4$, 0.01 g/l $MgSO_4$, 0.01 g/l $CaCl_2$, 0.002 g/l $NiCl_2$, 0.002 g/l $CoCl_2$, 0.002 g/l $SeSO_4$, 0.002 g/l WSO_4, 0.002 g/l $ZnSO_4$, 0.002 g/l $Al_2(SO_4)_3$, 0.0001 g/l $TiCl_3$, 0.002 g/l $MoSO_4$, and 10 mM EDTA.

Identification of Isolate

An isolate was identified with a 16S rDNA sequence. 16S ribosomal DNA of the isolate was amplified by direct PCR using universal primers 5'-GAGTTGGATCCTGGCTCAG-3' and 5'-AAGGAGGGGATCCAGCC-3'. The reaction mixture consisted of 300 mM Tris-HCl (pH 8.8), 100 mM $(NH_4)_2SO_4$, 100 mM KCl, 20 mM $MgSO_4$, 20 pM each of primer, 20 mM each of dNTP, 2U Taq polymerase (Genenmed, U.S.A.), and 20 ng of template. Amplification reactions were performed using a PCR machine (T Gradient model, Biometera, Germany). The PCR products were directly sequenced with an ABI Prism 3700 Genetic analyzer upon request to a professional company (Macrogen Inc., Korea) with a DNA analysis system. The 16S rDNA sequences were analyzed using the GenBank database, and the identification was performed on the basis of 16S rDNA sequence homology.

Electric Bioreactor

Two types of bioreactor, electric bioreactor (EBR) and conventional bioreactor (CBR), were used in order to compare the effect of electrochemical oxidation potential formed in the biofilter matrix. Both bioreactors were composed of carbon fiber (biofilter matrix), titanium ring (counter electrode), porcelain ring (ion channel), VOC reservoir, and inorganic nutrient reservoir as shown in Fig. 1, but the electrical anodic potential was charged only to the carbon fiber in the EBR. The biofilter used in the EBR and CBR was named as carbon fiber anode (CFA) and carbon fiber (CF), respectively, to separate the two systems. The CO_2 traps equipped in the front and rear of bioreactor functioned as a CO_2 remover and collector, respectively. For removal of CO_2 from influx air, a calcium hydroxide trap was set between the air pump and flow meter. The CFA was electrically coupled with a titanium ring, which functioned as a counter electrode. The bioreactor volume occupied with biofilter was 500 ml. Flow rate of air containing VOCs was precisely adjusted to 100 ml/min, and 20 ml/min of inorganic nutrient was circulated through the biofilter for 15 min at 2-h intervals. The volume of the

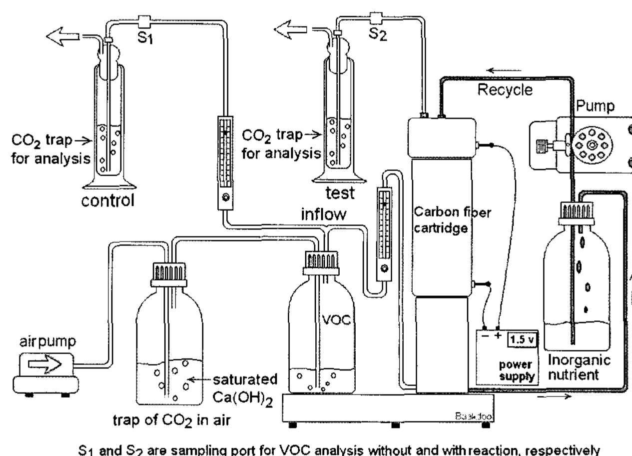

Fig. 1. Schematic diagram of the bioreactor composed of carbon fiber, VOC reservoir, inorganic nutrient reservoir, and CO_2 trap. Twenty ml/min of inorganic nutrient solution was circulated through the carbon fiber for 15 min at intervals of 2 h, and 100 ml/min of air containing VOCs was continuously flowed into the bioreactor. The air containing VOCs was not circulated but flowed out from the bioreactor. S1 and S2 were analyzed by GC equipped with a flame ionized detector and RTX-1 capillary column (30 m×0.32 mm).

inorganic nutrient solution was adjusted to 500 ml. The electrical anodic potential charged to the CFA was 1.5 volt DC. The ingredients of the inorganic nutrient solution was the same as those of the defined medium used for the enrichment culture. The bioreactor was initiated by inoculation of 100 ml of bacterial culture.

Structure of Carbon Fiber

As shown in Fig. 2, the biofilter matrix was prepared from the tightly rolled graphite felt (diameter 80 mm, surface area 0.47 m^2/g, weight 35 g, surface area 16.45 m^2, thickness 2 mm, dimension 200 mm×1200 mm, electric resistance $< 0.05\ \Omega/Cm^2$, Electrosynthesis, U.S.A.). The CFA was separated from the titanium ring (diameter 80 mm, thickness 0.5 mm, length 50 mm) by a porcelain ring (inner diameter 65 mm, outer diameter 80 mm, length 20 mm) functioning as an ion (proton) channel.

Porcelain Ring

A porcelain ring was made from 100% (w/w) white clay powder (particle size of 1–2 μm) by baking at 1,200°C for 12 h using an electric kiln (Red Corona Model 50L, U.S.A.).

Measurement of Biomass

Bacterial cells contained in the inorganic nutrient solution were measured daily by optical density at 660 nm, and viable cells contained in the nutrient reservoirs were measured with a plate-counting method at the intervals of 4 weeks, in order to compare the relationship between optical density and viable cell numbers. After measurement,

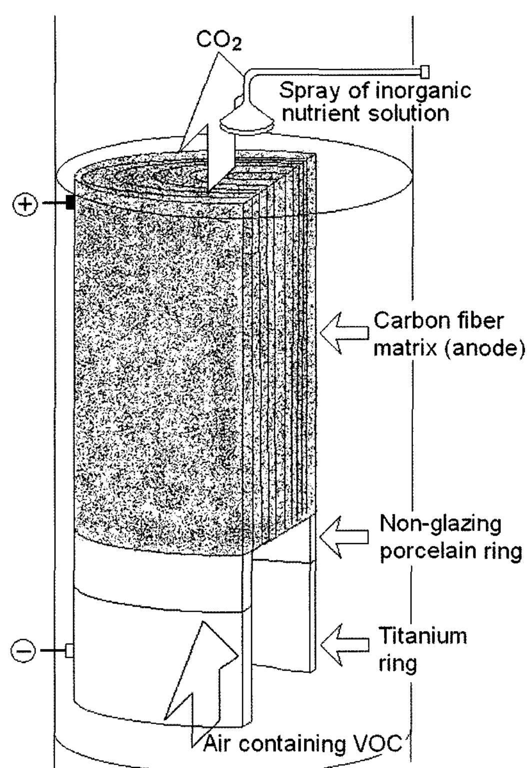

Fig. 2. Schematic diagram of a biofilter matrix composed of carbon fiber (anode).

The carbon fiber anode (CFA) was electrically linked to a titanium ring (counter electrode), but the carbon fiber (CF) was not. The porcelain ring between the carbon fiber anode and titanium ring functions as a proton channel. The bioreactor volume occupied with biofilter matrix was 500 ml. The inorganic nutrient solution was sprayed on top of the biofilter matrix and trickled down the porous fiber, and then finally returned to the reservoir.

the inorganic nutrient solution was refilled with a new solution.

Analysis of CO_2

CO_2 was analyzed by the standard titrimetric method [2]. Free CO_2 flow from the bioreactor was collected with calcium hydroxide and then titrated with 0.1 N NaOH. All experiments were repeated five times with identical results in the range below 5.0% deviation.

Analysis of VOCs

VOCs were analyzed by gas chromatography (Varian 3400 star, U.S.A.) equipped with a flame ionized detector and capillary column. RTX-1 (length 30 m, inner diameter 0.32 mm, Restek, U.K.) and DB-1 (30 m, inner diameter 0.25 mm, J&W Scientific, U.S.A.) capillary columns were used for the BTEX and kerosene analysis, respectively. Four-hundred μl of sample was separated by a gas-tight syringe from a sampling port (S_1 and S_2), as shown in Fig. 1, and injected directly into the injector of the GC without pretreatment. Injector, detector, and column temperatures were adjusted to 250°C, 300°C, and 150°C, respectively, and 99.99% of hydrogen gas was used as a carrier. All experiments were repeated five times with identical results in the range below 5.0% deviation.

Scanning Electron Microscopy

The piece (5×5 mm) of CFA and CF was cut from the biofilter matrix after operation for 3 months. The piece of biofilter was directly fixed in 4% glutaraldehyde buffered with 0.1 M sodium phosphate (pH 7.4), and following procedures for sample preparation were performed by the general method [22]. The prepared sample was examined in a JEOL JSM-6300 scanning electron microscope (Japan).

RESULTS AND DISCUSSION

Effect of Electrochemical Oxidation Potential on Bacterial Growth

The bacterial cells grown in the CFA and CF were observed by scanning electron microscopy (SEM), and the biomass

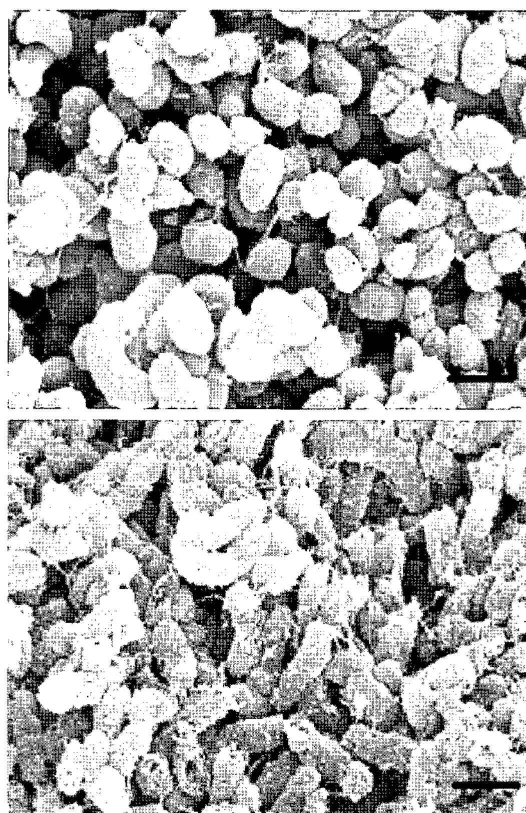

Fig. 3. SEM profile of bacterial cells growing in the CFA (upper) and in CF (lower), after 3 months.

A piece of the biofilter was cut from the bioreactor operated with ethylbenzene as the VOC species and then fixed in 4% glutaraldehyde buffered with 0.1 M sodium phosphate (pH 7.4). Procedures for sample preparation were performed by the general method. The length of the bar is 2 mM.

was determined with the inorganic nutrient solution circulating through the bioreactor and nutrient reservoir. As shown in Fig. 3, the biofilm was developed in a biofilter matrix of both CFA and CF. The SEM profile revealed that the biofilm in the CFA was loosely distributed and looked intact, but that in the CF was densely distributed and looked damaged. This is thought to be due to the fact that the electrochemical oxidation potential charged to the CFA may generate a positively charged electrostatic force on the surface of bacterial cells, thereby inducing a repulsive force between bacterial cells. Stoodely *et al.* [37] reported that the electrostatic interactions between negatively charged groups in a biofilm were a cause to expand distance among bacterial cells, and Møller *et al.* [26] reported that the biofilms formed in neutral dextran media were thicker than the biofilms in polyanionic or polycationic dextran media. These reports led us to propose the possibility that the electrochemically positive biofilter matrix might induce the bacterial surface to be unipolar, and the polarity might be the cause to protect dense distribution of bacterial cells. During operation for 6 months, the biomass in the inorganic nutrient reservoir connected to the CFA and CF was daily measured; however, the initial growth from 0 to 7 days of incubation time was not shown because the biomass between the biofilter matrix and inorganic nutrient reservoir was not equilibrated. The biomass in the inorganic nutrient reservoir was continuously increased during 4 days of initial operation and then ceased to increase. During the initial growth in the bioreactor, the bacterial cells are thought to develop a biofilm and a large population size between the fibers of the biofilter matrix. After 5 days of incubation time, the nutrient reservoir was changed daily. As shown in Fig. 4, the biomass in the inorganic nutrient reservoir connected to the CFA was higher than that connected to the CF. Based on these results, the electrochemical oxidation potential charged to the CFA appears to be a

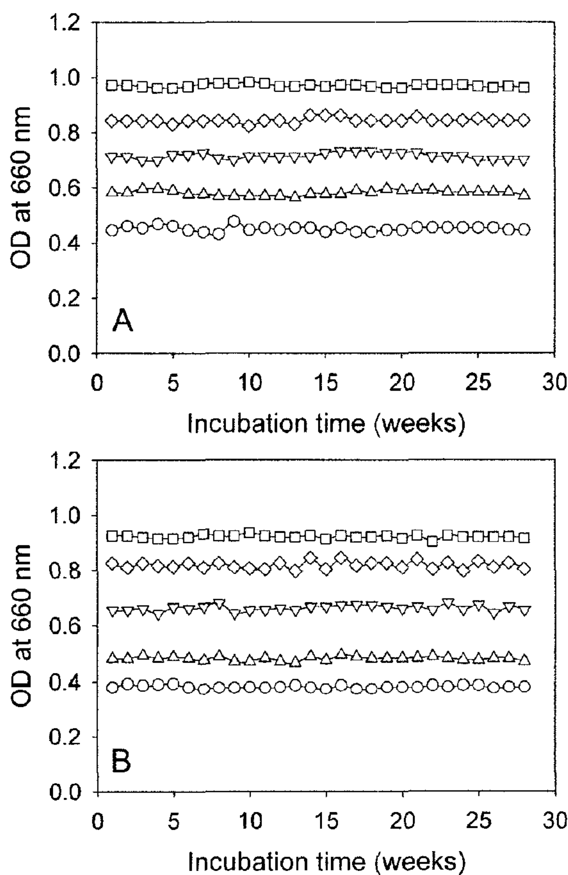

Fig. 4. Biomass variation in the inorganic nutrient reservoir connected to the bioreactor with a CFA (**A**) and CF (**B**), respectively, as the biofilter matrix.

The optical density of bacterial cells contained in the inorganic nutrient solution was measured daily. The optical density expressed in the graphs was an average obtained from values measured for one week. The bioreactor was operated with different VOCs, benzene (○), toluene (▽), ethylbenzene (□), xylene (◇), and decane (△), respectively, as the sole carbon source.

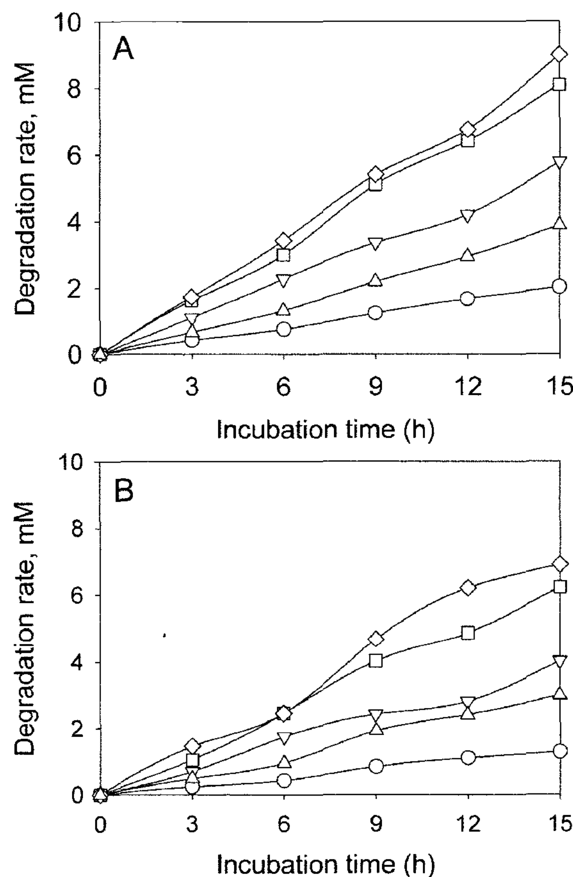

Fig. 5. Temporal biodegradation rates of benzene (○), toluene (▽), ethylbenzene (□), xylene (◇), and decane (△) by bacterial cells growing in the bioreactor with a CFA (**A**) and CF (**B**), respectively, as the biofilter matrix.

VOCs were analyzed by gas chromatography equipped with a flame ionized detector and RTX-1 (length 30 m, inner diameter 0.32 mm, Restek, U.K.) capillary column.

better environment for the population of *B. cepacia* than the CF. Overgrown cells in the biofilter matrix have to be separated and flowed out along the inorganic nutrient solution trickling through the biofilter matrix, thereby equilibrating the biomass in the biofilter matrix with that in the inorganic nutrient reservoir. Under equilibration condition of a biomass, the optical density of bacterial cells in the inorganic nutrient reservoir has to be proportional to the viable cells growing in the biofilter matrix. The viable cells contained in the reservoirs connected to the bioreactor with CFA and CF was measured with a plate-counting method at the intervals of 4 weeks, in order to compare the relationship between the optical density and viable cell numbers. The viable bacterial cells number in the reservoir connected to the bioreactor with CFA and CF were 4×10^8–7×10^8 CFU and 9×10^7–3×10^8 CFU, respectively. Consequently, it is possible that the CFA may provide a better environmental condition for bacterial growth than the CF.

Temporal Degradation Rate of VOCs

During the experiments, air containing VOCs were continuously flowed into the biofilter matrix (Fig. 2) or

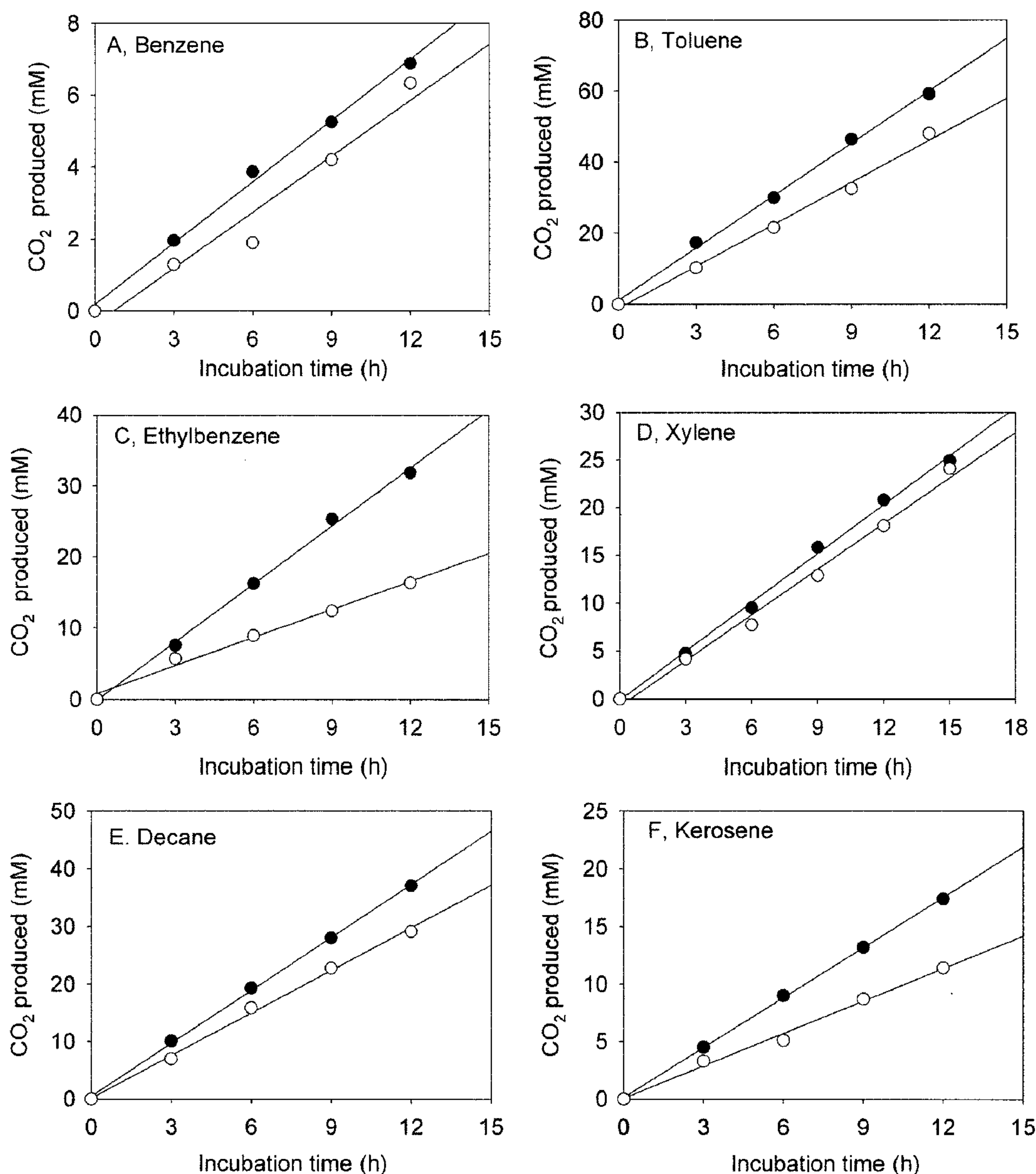

Fig. 6. Temporal CO_2 production from the bioreactor with CFA (●) and CF (○), respectively, as the biofilter matrix.
The air containing VOCs (benzene, toluene, ethylbenzene, xylene, decane, and kerosene) was flowed into the bioreactor and passed through the biofilter in which bacterial cells were growing with VOCs as the sole carbon source, and CO_2 produced from bioreactor by bacterial oxidation of VOCs was collected with calcium hydroxide.

bypassed the bioreactor in the control test (Fig. 1). The concentration of benzene, toluene, ethylbenzene, xylene, and decane contained in the air being flowed into the bioreactor was 10.55 mM/l/h (0.824 g/l), 8.94 mM/l/h (0.507 g/l), 2.88 mM/l/h (0.305 g/l), 1.99 mM/l/h (0.211 g/l), and 3.43 mM/l/h (0.489 g/l), respectively, at 25°C and the calculated retention time of VOCs containing air in the biofilter matrix was 5 min. On the basis of chromatograms obtained from the analysis of influx air into and efflux gas from the bioreactor, degradation rates of VOCs were appraised: The degradation rates of benzene, toluene, ethylbenzene, xylene, and decane in the bioreactor with CFA were about 0.131 mM/l/h, 0.401 mM/l/h, 0.535 mM/l/h, 0.602 mM/l/h, and 0.265 mM/l/h, respectively, as shown in Fig. 5A. These values were about 20–25% higher than the values obtained in the bioreactor with CF, as shown Fig. 5B. These results serve as a clue that the electrochemical oxidation potential formed in CFA may activate the bacterial growth and metabolic function for VOCs oxidation. Xu *et al.* [40] reported that a thick biofilm limited diffusion of oxygen into the inside of the biofilm and inhibited alkaline phosphatase activity of bacterial cells. This is in full accord with the difference observed in the present study in SEM image, biomass, and VOCs degradation activity between

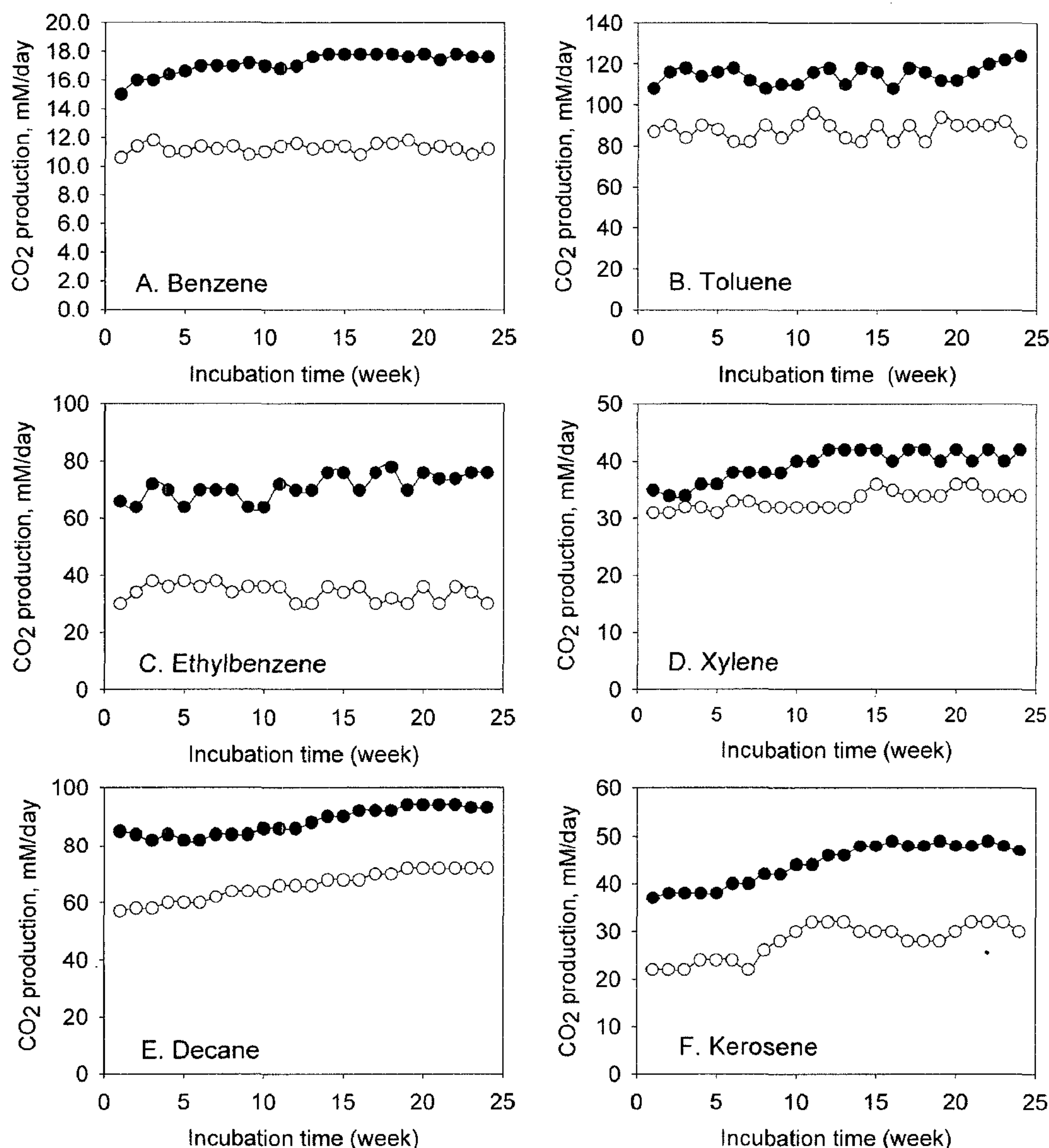

Fig. 7. The daily CO_2 production from the bioreactor with the CFA (●) and the CF (○), respectively, for six months.

The air containing VOCs (benzene, toluene, ethylbenzene, xylene, decane, and kerosene) were continuously flowed into the bioreactor for 6 months. The bacterial cells were inoculated in the biofilter matrix (CFA or CF) to start operation of the bioreactor. CO_2 was collected with calcium hydroxide from the outlet of the bioreactor and analyzed daily by titration.

bacterial cells growing in the CFA and in CF. The bacterial degradation rates of VOCs in the bioreactor with both a CFA and CF are relatively higher than the values (mM) presented in other studies [6, 7, 11, 12, 17, 19, 41]. Some of the VOCs consumed by bacterial cells in the bioreactor might have been completely oxidized to CO_2, and some of them might have been converted to building blocks for biosynthesis, because VOCs were the sole carbon and energy sources.

Effect of Electrochemical Oxidation Potential on Oxidation of VOC to CO_2

VOCs are the sole carbon and energy sources for bacterial growth, which have to be completely oxidized to CO_2 in order to produce ATP in the respiratory metabolism. Theoretically, six, seven, eight, nine, and ten moles of CO_2 can be produced from one mole of benzene, toluene, ethylbenzene, xylene, and decane, respectively. As shown in Figs. 5 and 6, however, CO_2 production was not proportional to the concentration of VOCs consumed in the bioreactor. The ratios of CO_2 produced to VOCs consumed in the bioreactor with CFA were 4.3 for benzene, 12.3 for toluene, 5.1 for ethylbenzene, 3.6 for xylene, and 12.1 for decane. Meanwhile, the ratios of CO_2 produced to VOCs consumed in the bioreactor with CF were 4.2 for benzene, 13.1 for toluene, 3.3 for ethylbenzene, 3.8 for xylene, and 12.6 for decane. The ratios of CO_2 produced to VOCs consumed in the bioreactor with CFA were similar to those in the bioreactor with CF except for ethylbenzene. The different balance of CO_2/VOCs is possibly due to a difference of bacterial metabolic affinity to VOC species and the assimilatory portion of VOC into the building blocks for biosynthesis. Generally, bacterial cells growing under a disadvantageous condition may require more free energy (ATP) for maintenance of metabolic balance [4, 25]. In the present study, CO_2 production had been measured daily in order to analyze the effect of electrochemical oxidation potential on the bacterial activity for VOCs oxidation without additional inoculation for 6 months. The oxidation-reduction potential (ORP) has been reported to influence the metabolic pathway and the gene expression of aerobic bacteria [21, 33]. As shown in Fig. 6 and Fig. 7, CO_2 production was increased in the bioreactor with CFA, providing evidence that bacterial oxidation metabolism may be activated by an extracellular oxidation potential. Riondet *et al.* [34] reported that a negative electric potential applied to *Escherichia coli* culture can convert metabolism from respiration to fermentation, but Unden *et al.* [38] reported that positive electric potential applied to *Escherichia coli* culture activates an oxidant equivalent of metabolites, which is strong evidence that the extracellular electrochemical potential can change the oxidation-reduction balance of metabolism. Little difference between the results by others and from our experiments was observed.

Effect of Electrochemical Oxidation Potential on Biodegradation of Kerosene

Kerosene is a proper model for a bacterial oxidation test of VOCs, because kerosene is a mixture of aliphatic and aromatic hydrocarbons composed of C_{11}-C_{16} [9]. We applied kerosene to the bioreactors with CFA and CF and analyzed the influx air saturated with kerosene and efflux gas from the bioreactor. As shown in Fig. 8A, the chromatogram of influx air saturated with kerosene shows that kerosene is composed of various aliphatic hydrocarbons (clustered narrow peaks) and aromatic compounds (single broad peak).

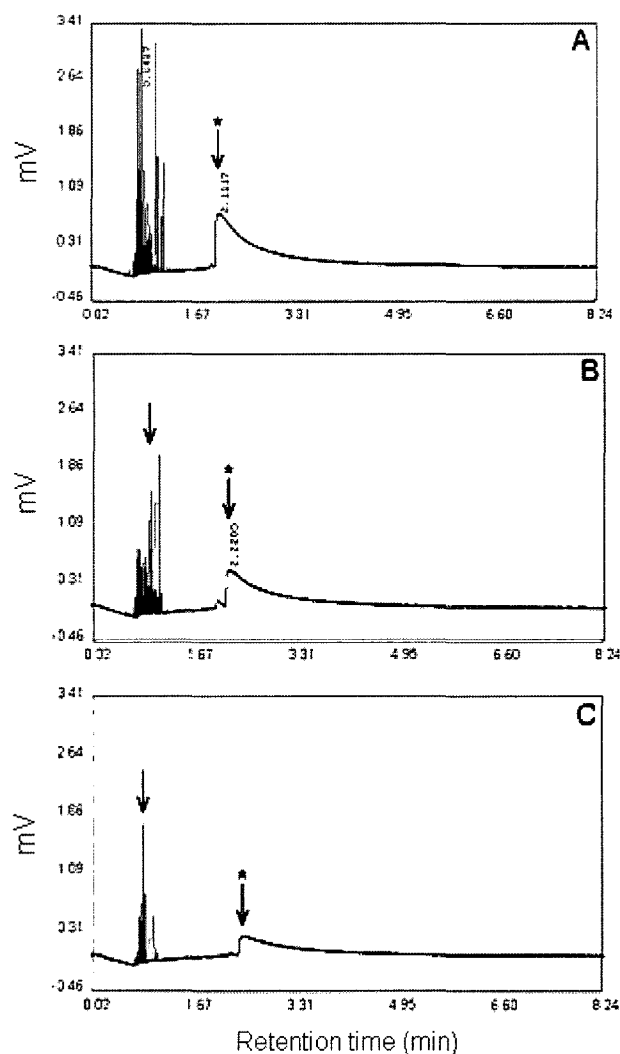

Fig. 8. Chromatographic profiles of air saturated with kerosene flowing in the bioreactor (**A**), and efflux gas from the bioreactor with the CF (**B**) and with the CFA (**C**).

The biggest peak (asterisk in **A**) was decreased (asterisk in **B**), but nearly disappeared (asterisk in **C**). Most of the clustered peaks (**A**) were also decreased (**B**) or disappeared (**C**). Gas samples were separated from a sampling port (S1 and S2 of Fig. 1) of the bioreactor by a gas-tight syringe and directly injected into the injector of the GC without pretreatment. Gas sampled from S1 and S2 was analyzed by a GC equipped with a flame ionized detector and DB-1 capillary column (30 m×0.25 mm).

In the previous test, BTEX or aromatic compounds were separated from each other by a RTX-1 capillary column, but not separated by a DB-1 capillary column. However, the DB-1 capillary column is better to analyze and separate aliphatics of kerosene, because the major components of kerosene are aliphatics. The bacterial oxidation of both aliphatic and aromatic compounds of kerosene (Figs. 8B and 8C) was more activated in the bioreactor with CFA than CF. The biofilter composed of soil, sand, or gravel column have been applied to purification of soils and waters contaminated with VOCs [1, 11, 12, 16, 24], but have been limited to constitution of an electrochemical bioreactor, because the biofilter matrices are not electrical conductive. The biofilter has to be characterized for their structural composition in order to maintain the required operational condition, and the bacterial cells have to continuously grow on a porous filter packed into columns during the removal of VOCs [29]. In the present study, *Burkholderia cepacia* was continuously grown with VOCs and inorganic nutrients in the carbon fiber of a bioreactor for more than 6 months. This is a more advantageous point of a bioelectrochemical unit system as a biofilter, which can substitute for a biofilter system made of soil, sand, gravel, and physical or chemical system for purification of air contaminated with VOCs.

In summary, the electric conductive biofilter matrix can be an innovative tool to develop a unit system for VOC removal and improve the biofilter function used in various fields, and the electrochemical oxidation potential charged to a biofilter matrix positively influences the bacterial oxidation metabolism, biofilm structure, and bacterial growth.

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