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Chemosphere 57 (2004) 697–709

CHEMOSPHERE

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Effects of cross-substrate interaction on biotrickling filtration for the control of VOC emissions

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Received 16 July 2003; received in revised form 7 June 2004; accepted 10 June 2004

Abstract

The effects of cross-substrate interaction to the performance of a gas-phase biotrickling filter for treating a mixture of volatile organic compounds (VOCs), including three structural heterologous in acetone, toluene, and trichloroethylene, were investigated. The biotrickling filter was inoculated with microbial consortium containing at least seven bacterial species utilizing either acetone or toluene, or both, as their carbon sources. In the performance study, the column operating under variable conditions typifying the waste gas emission from the microelectronics fabrication processes achieved a total hydrocarbon (THC) removal efficiency in excess of 85% and a mineralization capacity over 50% for THC mass loading as high as 36.2 g-CH_4 $m^{-3}h^{-1}$. The cross-substrate effects were examined by correlating the relative changes in the mass removal of each substrate with the biodegradative capability of the microbial consortium. The degradation of trichloroethylene was primarily due to co-metabolism by the toluene-oxidizing enzymes, but the results also indicated that trichloroethylene partially induced its own degradation. Concentration increases in acetone appeared to cause a diauxie effect that suppressed degradation of toluene and trichloroethylene, and shifted the microbial population toward the selective acetone-degraders. No irrecoverable toxicity or inhibitory effects were observed throughout the experiments. These results suggest that the relative VOC concentration in the waste gas mixture is a factor as important as the biodegradative function of the microbial consortium, and thus should be carefully evaluated to satisfy the treatment objectives.

Keywords: Biotrickling filtration; Cross-substrate; Co-metabolism; Industrial waste gas; Volatile organic compound; Bioremediation

1. Introduction

In gas-phase biological processes for the control of industrial waste air emissions, the potential effects of interaction among the mixtures of volatile organic compounds (VOCs) are important issues to the process performance. Both stimulatory and antagonistic effects

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doi:10.1016/j.chemosphere.2004.06.018

have been documented when multiple substrates (contaminants) are exposed to mono or mixed microbial cultures (Rittman and Saez, 1993). The stimulatory effects are primarily attributed to co-metabolism, which requires induction of non-specific enzymes capable of catalyzing degradation of recalcitrant compounds. These non-specific enzymes, often induced by compounds without chemically reactive functional groups such as alkanes and aromatics, bind to both the growth substrate and the co-metabolite to undergo biotransformation. In contrast, antagonistic effects generally arise from substrate competition effects that elicit a variety of responses from diauxie to concurrent utilization of multiple substrates (Maier, 1989). In diauxie the preferred substrate is utilized first, followed by a lag phase before other substrates are consumed. In general, the relative concentrations of substrates are critical to the microbial responses with respect to the substrate degradation pattern. For instance, high concentrations of target substrate normally favors diauxie pattern, provided that the concentration levels do not cause toxic inhibition. On the contrary, low substrate concentrations appear to favor concurrent utilization of several substrates. This type of competitive substrate inhibition is known to occur between structural homologous substrates binding to the same active site of an enzyme. A typical example was delineated by Burbeck and Perry (1993) in their studies for degradation of alkylbenzene mixture (toluene, benzene, ethylbenzene, xylene, and styrene) using *Mycobacterium vaccae* as a dominant species. They observed an enhanced styrene removal due to co-metabolism induced by toluene. However, the preferential degradation of toluene also delayed the benzene utilization when the two compounds were present in equimolar amounts.

Researchers have also proposed mathematical models to relate the substrate consumption and the microbial growth kinetics for homologous binary mixtures (Klečka and Maier, 1988; Bielefeldt and Stensel, 1999) or tertiary mixtures (Reardon et al., 2000). These growth kinetic models proved to be accurate by incorporating various inhibitory parameters to delineate the cross-substrate effects among the homologous substrates. However, for a body of mixture consisting of three or more structurally unrelated substrates (i.e., heterologous substrates), the metabolic role of each substrate for microorganisms in an engineering system is mostly unknown, particularly for a gas-phase biological treatment process.

To evaluate the effects of complex substrate interaction in the presence of an easily biodegradable ketonic compound in acetone, a moderately biodegradable aromatic compound in toluene, and a recalcitrant chlorinated alkene compound in trichloroethylene (TCE), a series of biotrickling filter experiments were conducted in the present study. The synthetic VOC mixture and their concentration ranges used in the study was targeted to mimic the realistic waste air emission from the microelectronics industry, such as the wafer fabrication and the printed circuit board facilities (US EPA, 1995; Coogan and Jassal, 1997; Den et al., 1999). The waste emission was typically characterized by substantial fluctuation due to the cyclical nature of the batch fabrication processes. In this type of mixture, the metabolic pathway of each VOC all appeared to be vastly different. In particular, the presence of a heavily chlorinated compound in TCE among the VOC mixtures forms a challenge to the biological system since biodegradation of the xenobiotic compound requires pathways normally not found in heterotrophic bacteria (Fetzner, 1998). Consequently, it is believed that TCE by itself cannot be utilized by heterotrophic bacteria under aerobic conditions, but may be co-metabolized in the presence of another substrate to serve as the primary carbon and energy source (Nelson et al., 1987; Shields et al., 1989; Assinder and Williams, 1990). A number of bacterial cells possessing enzymes with low substrate specificity have been demonstrated to be capable of transforming TCE via co-metabolism (Wackett and Gibson, 1988; Winter et al., 1989; Folsom et al., 1990; Hanson and Brusseau, 1994). However, the phenomena of mutual inhibition and induction of the active enzymes between toluene and TCE has also been documented (Heald and Jenkins, 1994; McClay et al., 1995). The presence of a third substrate (i.e., acetone) further complicates the question of cross-substrate interactions, because acetone is not known as an inducer for TCE-degrading enzymes but could conceivably serve as a growth substrate for the bacteria. The main objective of this study, therefore, is to examine the cross-substrate effects by correlating the biodegradative capability of the microbial consortium with the mass removal of each VOC in the gas mixtures.

2. Materials and methods

2.1. Protocol for microbial cultivation, enrichment, and biodegradation tests

The microbial seeds were originally obtained from an industrial wastewater treatment facility (Hsinchu, Taiwan) and were initially acclimated by feeding a vapor mixture of filtered air with acetone (600 ppmv), toluene (50 ppmv), and TCE (5 ppmv) into a cell fermentor containing 300-ml suspended culture in mineral salt medium of 8.5 $mg l^{-1}$ KH_2PO_4, 33.4 $mg l^{-1}$ K_2HPO_4, 17.4 $mg l^{-1}$ Na_2HPO_4, 1.7 $mg l^{-1}$ NH_4Cl, 11 $mg l^{-1}$ $MgSO_4$, augmented with 5.0 $g l^{-1}$ tryptone and 2.5 $g l^{-1}$ yeast extract. The pH of the mineral medium was neutralized before use. After 60 h of acclimation, an aliquot (1 ml) of culture suspension at three different dilution levels was transferred separately onto plate-count agars and potato-dextrose agars, followed by incubation at 30 °C for

36 h. After a series of culture enrichment and isolation procedures, the microbial cultures were purified and stored at 4 °C until further use. The bacterial identification was primarily determined by the standard Biolog procedure interfaced with the MicroLog 1 database (Biolog Inc., USA).

The growth and biodegradability studies were conducted in duplicate by shake-flask experiments. In the growth study, 150 ml of purified cultural suspensions diluted to a cell density of approximately 100 CFU ml^{-1} were separately transferred to a series of 250-ml Erlenmeyer flasks sealed with a septum device (for headspace sampling) and placed in a constant-temperature (30 °C) rotating incubator. Reagent-grade acetone (15 mM) and toluene (10 μM) were added to separate groups of cultural suspensions containing the same mineral salt medium without tryptone and yeast extract, such that the pure bacterial cultures could use acetone or toluene as their sole carbon source. A blank group containing only the cultural suspensions in the mineral medium served as the control. The headspace gas and aqueous mass concentration of the substrates can be related using Henry's law for a closed equilibrium system:

$$m_{\text{total}} = m_{\text{L}} + m_{\text{G}} \quad \text{and} \quad \frac{m_{\text{G}}}{m_{\text{L}}} = \frac{H_{\text{c}}}{R_{\text{c}}T}\left(\frac{V_{\text{f}}}{V_{\text{L}}} - 1\right) \qquad (1)$$

where m_{L} and m_{G} denote the mass of substrate in aqueous and gas phases, V_{f} and V_{L} refer to the volumes of flask and liquid suspension, respectively, H_{c} is the Henry's law constant (atm m^3 mol^{-1}), R_{c} is the gas constant (8.21×10^{-5} atm m^3 mol^{-1} K^{-1}), and T is the temperature (K). The aqueous concentrations of acetone and toluene in the suspensions provided headspace concentrations similar to those to be used in the ensuing biotrickling filter column studies. The same procedure was also followed for the biodegradability studies, except that the substrates were added as a mixture to the cultural suspensions (15 mM acetone, 60 μM toluene, 10 μM TCE). Blank controls containing sterilized solutions were used to account for the abiotic loss of the VOCs. Headspace samples were taken with a 1-ml glass syringe every 30 min for the first 4 h, and every 4 h afterward. Cell growth was determined by optical density at 600 nm (OD_{600}) on a Hach DR/4000 spectrophotometer (Hach Co., USA). The OD_{600} values were correlated with cell numbers determined by the plate-count method.

2.2. Biotrickling filter system

A bench-scale biotrickling filter (Fig. 1) comprising of two stages of acrylic column (internal diameter: 7 cm; height: 30 cm each stage) was used to evaluate the effects of VOC mixture to the overall system performance. The column was packed to a depth of 25 cm in each stage with sieved granular activated carbon (GAC, type BLP, Calgon Carbon Corps., USA) of 6×12 mesh size. Activated carbon was chosen due to a combination of favorable properties such as the uneven surface texture for microbial attachment and growth, the microporous nature for adsorption, and the structural integrity for high permeability. A total of 0.8 kg of mixed bacterial consortium (equal wt.% of each purified strain) was inoculated onto 1.4 kg of carbon for the column start-up. The pH-controlled (7.0 ± 0.5) recirculating liquid was nozzle-sprayed from the top of the filter bed at a rate of 1.5 l/min. Fresh mineral solution (500 $mg l^{-1}$ KH_2PO_4, 500 $mg l^{-1}$ K_2HPO_4, 50 $mg l^{-1}$ NH_4Cl, pH 7.0) was added daily to the recirculating liquid for replenishment of inorganic nutrients to the microorganisms. The VOC-contaminated air stream then entered from the top of the column, forming a co-current mode with the liquid flow. Gaseous samples were collected daily using 1-l Teflon® sampling bags (Alltech Inc., USA) from each of the four equidistant sampling ports for analysis.

2.3. Experimental methods

As indicated in Table 1, the biotrickling filter column experiments were broadly divided into "performance evaluation" (phases 1–6) and "cross-substrate evaluation" (phases 6–17). Prior to inoculation of microorganisms, an independent adsorption breakthrough study was conducted under the "wetted" condition (i.e., with liquid spraying) to serve as a control test for the biotrickling filter studies. For the column performance evaluation, the influent total hydrocarbon (THC) concentration, which served as a surrogate concentration for all VOCs in the mixture, was varied from 850 to 2400 ppmv (standardized as CH_4). To simulate the actual emission pattern from microelectronics manufacturing facilities (Den et al., 2003) while minimizing variation in substrate concentration ratio during the experimental phases, the composition of the three VOCs contributing to the THC was maintained such that the following conditions were satisfied: (i) the volumetric ratio (i.e., ppmv/ppmv) of acetone-to-THC was always within the range of 0.2–0.3, and (ii) the volumetric ratio of toluene-to-TCE remained close to 5. Additionally, the empty-bed-contact-time (EBCT) was maintained at 155 s except for the operation during phase 5.

The cross-substrate effects were evaluated by systematically varying the concentration of the target VOC (referred to as the "variable VOC" hereinafter) in the sequence of acetone (phases 6–9), toluene (phases 10–13), and TCE (phases 14–17). A "baseline" condition, chosen as the lower end of the VOC emission spectrum, was established as the reference to which the change in mass removal of each target VOC under the variable conditions was compared. This condition provided an influent acetone concentration of 220 ppmv, toluene of

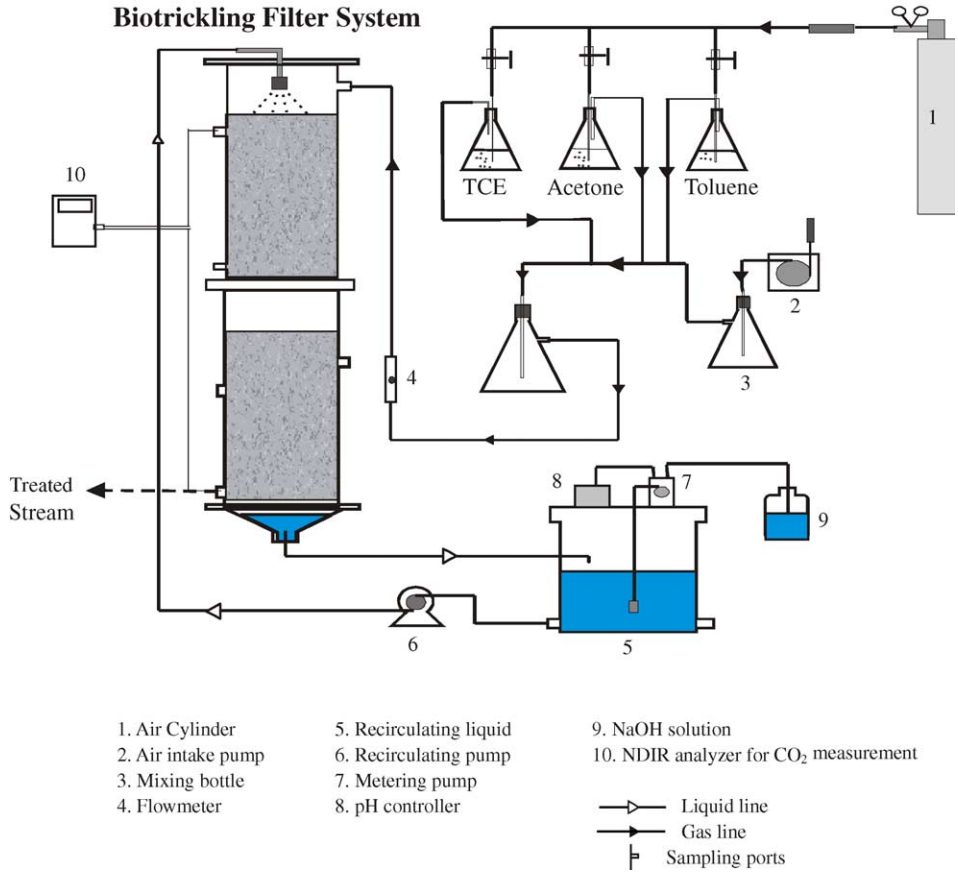

Fig. 1. Experimental setup for biotrickling filter system.

50 ppmv, and TCE of 10 ppmv, resulting in an equivalent THC concentration of approximately 850 ppmv as CH_4. When a step-change of the variable VOC was made, the mass removal corresponding to the change in the VOC was determined from the steady-state condition. The mass removal for each VOC was calculated using the following equation:

$$\bar{m}_j = \frac{Q(C_{\text{in}} - C_{\text{out}})_j f_c}{V_B} \tag{2}$$

where $\bar{m}_j$ represents the average steady-state mass removal rate ($\text{g m}^{-3}\text{h}^{-1}$) for VOC species j, Q is the volumetric gas flowrate (m^3h^{-1}), V_B is the packing volume (m^3), C_{in} and C_{out} denote to the inlet and outlet VOC volumetric concentrations (ppmv), and f_c is a conversion factor from volume-base to mass-base concentration. The relative change of mass removal at a particular mass loading condition for the non-variable VOCs deviating from those at the baseline condition was quantified using the following formula:

$$\text{Relative removal} = \frac{\bar{m}_{j,\text{ref}} - \bar{m}_j}{\bar{m}_{j,\text{ref}}} \times 100\% \tag{3}$$

where $m_{\text{ref},j}$ is the mass removal of species j at the baseline condition.

2.4. Analytical methods

The gas-phase VOC concentrations (acetone, toluene, and TCE) were determined by a thermal desorption/gas chromatograph unit (TDS-GC, GC-14B, Shimadzu Instrument Co., Japan) equipped with a flame ionization detector and a 30-m, AT-1 fused silica capillary column (Alltech Associates, Inc., USA). The THC concentration was analyzed by the same instrument, except that the analytical column was replaced by a 15-m deactivated fused silica capillary column because no compound separation was needed. The THC concentration was routinely calibrated by the standard 100 and 1000 ppmv methane gases (CH_4 in N_2, Scott Specialty Gases, USA), and therefore was defined as the CH_4-equivalent concentration. Carbon dioxide was monitored at the inlet and outlet ends of the column using a portable non-dispersive infrared analyzer (Telaire™ 1050, Telaire System Inc., USA) capable of measuring up to 2000 ppmv of CO_2 (±5%). The residual TOC in

Table 1
Loading conditions for the biotrickling filter experiments

Experimental objective	Phase	Days	Acetone concentration (ppmv)	Toluene concentration (ppmv)	TCE concentration (ppmv)	Corresponding THC concentration (ppmv as CH_4)
Column performance studies	1	0–31	220	50	10	850
	2	32–43	325	75	15	1250
	3	43–53	470	200	40	2400
	4	53–67	345	145	30	1750
	5	67–77	470	200	40	2400
	6	77–84	220	50	10	850
Cross-substrate effect studies	7	84–90	365	50	10	1250
	8	90–96	560	50	10	1750
	9	96–107	815	50	10	2400
	10	107–113	220	50	10	850
	11	113–120	220	120	10	1250
	12	120–129	220	215	10	1750
	13	129–137	220	330	10	2400
	14	137–142	220	50	10	850
	15	142–148	220	50	15	1930
	16	148–156	220	50	30	1960
	17	156–163	220	50	60	2010
	18	163–168	220	50	10	850

All experiments were performed with EBCT of 155 s except in phase 5 (310 s).

the recirculating liquid was periodically analyzed using a TOC 5000A analyzer (Shimadzu Instrument Co., Japan) with automatic injection (200 μl). Potassium hydrogen phthalate and sodium carbonate were used for the calibration of total carbon and inorganic carbon, respectively. Before analysis, 50-ml aqueous samples were directly withdrawn from the recirculating tank and treated with 0.45 μm cellulose nitrate membrane (Whatman, Inc., USA).

3. Results and discussion

3.1. Microbial characterization and biodegradability

After a series of isolation and cultivation cycles over a one-week period, a total of seven bacterial strains capable of aerobically degrading acetone, toluene, or TCE were isolated and identified, including three *Pseudomonas* strains (designated as B1–B3) possessing distinct morphological and/or biochemical characteristics as determined by the Biolog method. As shown in Table 2, all seven strains were capable of growing on acetone as the sole carbon source, and five of the seven strains also grew on toluene. These bacteria generally exhibited an exponential growth phase lasting 20–24 h before entering their stationary phases, and demonstrated rapid cell growth over eight orders of magnitude during the exponential phase. The observed lag phase typically did not extend beyond 10 h except for the *Acetobacteriaceae* species (~18 h).

The rapid microbial growth on acetone and toluene indicated that these bacterial strains were efficient degraders of the target VOCs. The specific growth rate (μ) of each bacterial culture was calculated for the growth on acetone (15 mM) or toluene (10 μM) as the sole carbon source. Data were interpreted only during their exponential growth phases. The numerical values of the specific growth rate can be also be estimated from the doubling time of cell number ($k = \ln 2/t_{1/2}$, where $t_{1/2}$ is the doubling time), as it was done in this study as a confirmatory procedure (Bustard et al., 2000). As shown in Table 2, all seven species efficiently utilized acetone as their carbon and energy source, exhibiting a broad range of μ values between 0.49 and 1.03 h^{-1}. In contrast, the five species (B1–B5) that also grew on toluene showed relatively narrow range of μ values between 0.34 and 0.46 h^{-1}. As a general observation, the three *Pseudomonas* strains (B1–B3) were not as efficient to grow on acetone as the other species, whereas species B4 and B5 were relatively efficient to grow on both acetone and toluene.

The biodegradability experiments were conducted in the shake-flasks at 30 °C to correlate the specific growth rates with the actual substrate consumption rates. The bacterial strains from the growth experiments were individually transferred to a set of fresh mineral medium solutions and adjusted to a cell density (from OD_{600} measurement) of approximately 10^8 cells ml^{-1}. The cultures were then exposed to a mixture of acetone, toluene, and TCE. The substrate degradation rate was calculated based on the maximum rate of concentration

Table 2
Characteristics and substrate biodegradability of the microbial consortium

No.	Identification[a]	Gram staining	Specific growth rate (μ)[b], h^{-1}		Substrate degradation rate (R)[c], $mM\,h^{-1}\,10^{-8}$ cell		
			Acetone	Toluene	Acetone	Toluene	TCE
B1	*Pseudomonas*	G−	0.49	0.35	0.12	1.0×10^{-3}	1.1×10^{-4}
B2	*Pseudomonas*	G−	0.59	0.36	0.09	7.1×10^{-4}	5.4×10^{-5}
B3	*Pseudomonas*	G−	0.51	0.34	0.14	1.2×10^{-3}	1.0×10^{-4}
B4	*Sphingomonas*	G−	0.62	0.36	0.08	5.0×10^{-4}	2.8×10^{-5}
B5	*Bacillus* sp.	G+	0.73	0.46	0.02	1.3×10^{-4}	NA
B6	*Acetobacteriaceae*	G−	1.0	NA	0.21	NA	NA
B7	*Mycobacterium* sp.	G+	0.79	NA	0.24	NA	NA

"NA" indicates "no significant biodegradation".

[a] Similarity index > 75% of taxonomic pattern using the Biolog procedure and database.

[b] Specific growth rate on either acetone (15 mM) or toluene (10 mM) at 30 °C.

[c] Substrate consumption rate normalized with the starting cell number in the batch study. Each cultural suspension was exposed to a mixture of acetone, toluene, and TCE at initial aqueous concentrations of 15, 60, and 10 mM, respectively.

reduction in the headspace. Since the maximum substrate degradation rates occurred in the first 4 h of the experiments, the headspace air and the initial dissolved oxygen (DO) in the microbial suspensions was presumably sufficient to prevent DO depletion during the study. As shown in Table 2, the acetone degradation rate displayed a similar tendency as previously seen in the specific growth rates, with the exception for B5 having high value of specific growth rate but relatively low degradation rate. Contrarily, the degradation rates of toluene did not correlate well with the corresponding specific growth rates for the tested concentration, possibly due to the metabolic interference from the presence of acetone in the mixture. All three *Pseudomonas* species showed significant TCE degrading capability, and the *Sphingomonas* species also degraded TCE to a much lower extent. It has been demonstrated that *Sphingomonas* species share physiological similarities with the Pseudomonads (Fredrickson et al., 1995), although the enzymatic mechanism associated to its TCE degradation has not been clarified. In principle, for the four TCE-degrading species (B1–B4), the TCE degradation rates seemed to follow closely to their respective toluene degradation rates, implying a strong biodegradative correlation between the two substrates.

3.2. Removal of VOC mixture under variable loading conditions

As described earlier in Section 2.3, the biotrickling filter column experiments were arranged into two parts; namely the "performance evaluation" (phases 1–6) and the "cross-substrate evaluation" (phases 6–17). The transient column response obtained from the former part has already been extensively presented elsewhere (Den et al., 2003). However, since the latter part was a continuation of the column operation, it would be pertinent to reiterate some of the important findings in order to substantiate the discussion on the occurrence of cross-substrate effects due to the presence of the three heterologous VOCs.

The step-increase pattern of influent THC mass loading, along with the effluent THC and CO_2 production profiles, are jointly presented in Fig. 2 for experimental phases 1–6. During the start-up period (phase 1) with an influent mass loading of 12 g-CH_4 $m^{-3}h^{-1}$ (equivalent to a THC concentration of 850 ppmv as CH_4), the low production of carbon dioxide suggested that the high THC removal efficiency was primarily due to carbon adsorption. As the microorganisms gradually acclimated to the loading conditions, biodegradation became the predominant removal mechanism, as manifested by the steady increase in the CO_2 evolution profile. The carbon balance calculation showed that approximately 70% of the organic carbon was recovered as CO_2 at the end of phase 1, when an apparent steady state was reached. Subsequent increases in mass loading to 18 g-CH_4 $m^{-3}h^{-1}$ (1250 ppmv, phase 2) and then to 36 g-CH_4 $m^{-3}h^{-1}$ (2400 ppmv, phase 3) appeared to deteriorate the column performance. As a result, the THC removal efficiency at the peak loading of 36 g-CH_4 $m^{-3}h^{-1}$ not only reduced the removal efficiency to as low as 85%, but also diminished the carbon mineralization (into CO_2) ratio to the vicinity of 50%. Furthermore, for each of the step-increases, a distinct "hump" was observed in the effluent profile, indicating the occurrence of a lag phase between THC mass removal and CO_2 evolution in response to the changes of influent mass loading. This phase delay was most probably due to the time needed for microbial adaptation to the new loading conditions. The duration of these phase delays appeared to lengthen at higher mass loading, extending from roughly two days at 18 g-CH_4 $m^{-3}h^{-1}$ to nearly four days at 36 g-CH_4 $m^{-3}h^{-1}$.

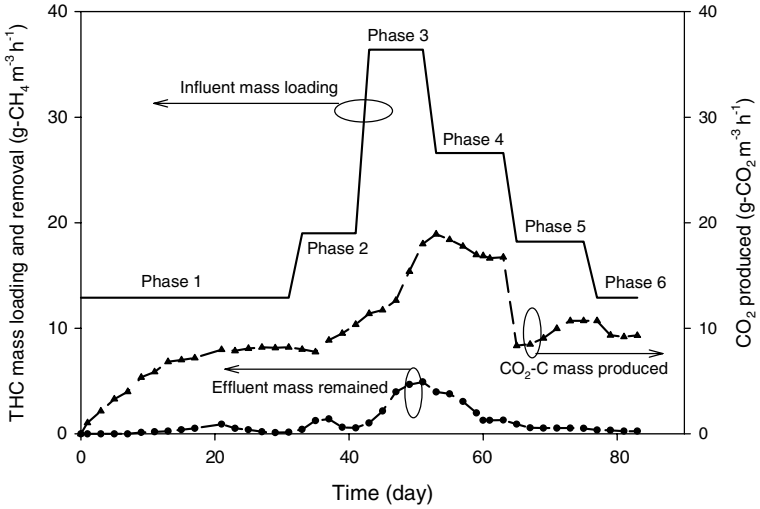

Fig. 2. Total hydrocarbon (THC) mass loading and removal profiles in the biotrickling filter. The corresponding carbon dioxide production is also shown in the figure.

Due to the observed column performance deterioration at a THC loading of 36 g-CH_4 $m^{-3}h^{-1}$, two different procedures were attempted to decrease the influent mass loading; namely, by lowering the influent THC concentration (phase 4), and by reducing the gas flowrate in half (phase 5). As shown in Fig. 2, reduction of influent loading from 36 to 27 g-CH_4 $m^{-3}h^{-1}$ (phase 4) immediately led to improved removal efficiency as well as mineralization capability. At this juncture, the gas flow rate was reduced in half, whereas the influent THC concentration was raised to the same level as in phase 3 to provide an equivalent mass loading of only 18 g-CH_4 $m^{-3}h^{-1}$ (phase 5). This change did not result in significant improvement of THC removal from phase 4. However, a noticeable increase in CO_2 production was observed during this period, indicating that the THC concentration was not inhibitory to biodegradation. This result showed that the reduction of THC removal efficiency and mineralization ratio experienced in phase 3 was likely caused by the large mass loading (i.e., combination of high influent concentration and high flow rate), rather than by the high THC influent concentration alone.

As noted, approximately 70–80% of the organic carbon was mineralized into CO_2 when the THC loading was below 27 g-CH_4 $m^{-3}h^{-1}$, with no consistently detectable intermediate products based on GC analyses. Since biomass assimilation typically accounts for 5–10% of the organic carbon depending on the biomass yield efficiency of the carbon sources (Weber and Hartmans, 1996; Zhu et al., 1996), as much as 20% of the organic carbon was still unaccounted for in the carbon balance. It was therefore assumed that the VOCs—particularly for acetone, a highly soluble compound—were partially absorbed into the liquid stream in the biotrickling filter and carried into the circulation tank. TOC analyses periodically performed on the liquid phase in the circulating tank were always rather low (<5 mgl^{-1}), indicating that biodegradation occurring in the liquid phase was highly possible in this study. Nevertheless, because the tank liquid was either partly or fully replaced by fresh mineral medium solution, no further investigations were made on the extent of aqueous phase biodegradation.

Considering that the system was operated under nutrient-rich conditions (C/N $\approx$ 4) as compared to the theoretical nutrient requirement (C/N $\approx$ 6) for cell synthesis, depletion of DO in the excessively grown biofilm could conceivably occur. However, cell enumeration regularly measured from the top, middle, and bottom of the filter demonstrated a sharp reduction in the cell counts by more than three orders of magnitude (from start-up cell count of $\sim 10^9$ g^{-1} GAC) after 15 days of operation. For operation of a biotrickling filter, the wash-out of excess cells by the shear force of the continuous liquid flow has been reported in a number of studies (Diks et al., 1994; Weber and Hartmans, 1996). This mechanism helps stabilize the thickness of biofilm, which can be reflected by measuring the number of viable cells. In the present study, relatively steady cell counts in the order of magnitude of 10^7 cells g^{-1} GAC were consistent obtained after 30 days of operation. The results of the controlled biofilm thickness, coupled with the sufficiently high ratio of conversion from organic carbon to CO_2, suggest that oxygen depletion was not a significant factor within the VOC concentration range tested in the study. Furthermore, although the pressure

drop across the filter bed was not measured, no direct evidence (e.g., liquid flooding) associating with microbial clogging was observed throughout the study.

3.3. Effects of variation of influent VOC concentration

In contrast to the experimental strategy employed for the column performance study, the subsequent experiments (phases 6–17) were designed to investigate the response in the removal of each VOC by step-changing the concentration of the variable VOC, while maintaining the others constant. Specifically, the influent loading for each of the VOCs was varied in the order of acetone (220–850 ppmv), toluene (50–330 ppmv), and TCE (10–60 ppmv). It was noted that the removal data under each "base condition" (phases 6, 10, 14, 18) were somewhat different due to the varying conditions prior to returning to these base levels. Nevertheless, they were comparable enough to give a fair reference to the extent of VOC removals under the ensuing experimental conditions. Typically, it took more than four days to reach a steady-state removal after returning to the base levels.

Fig. 3 shows the mass removal profiles of all three VOCs in response to the change of influent acetone concentration from 220 to 850 ppmv (phases 6–9, corresponding to the acetone mass loading of 12 g-acetone $m^{-3}h^{-1}$ up to 44 g-acetone $m^{-3}h^{-1}$) as denoted by the dotted lines in the figure. It can be observed that, with each step-increase in the acetone mass loading, the mass removal efficiency of acetone itself progressively decreased, reaching a valley of approximately 90% at the peak loading of 44 g-acetone $m^{-3}h^{-1}$. The extents of mass removal for toluene and TCE also decreased markedly from 4.2 g-toluene $m^{-3}h^{-1}$ and 1.3 g-TCE $m^{-3}h^{-1}$ (at the baseline loading) to 3.8 g-toluene $m^{-3}h^{-1}$ and 1.1 g-TCE $m^{-3}h^{-1}$ (at the peak acetone loading), respectively. The rate of reduction was particularly noticeable when the acetone loading exceeded 30 g-acetone $m^{-3}h^{-1}$.

The removal profiles of the VOCs in response to the step-increase in the toluene loading (phases 10–13) are compiled in Fig. 4. In contrast to the removal patterns observed when acetone was the variable VOC, the extent of toluene mass removal only marginally lessened when the influent loading of 4.5 g-toluene $m^{-3}h^{-1}$ was step-increased to 29 g-toluene $m^{-3}h^{-1}$. It was also noted that TCE removal was not significantly influenced by the influent toluene loading, as its steady-state mass removal remained at the proximity of 1.2 g-TCE $m^{-3}h^{-1}$. The removal of acetone, however, did show a gradual decline from 12 to 10 g-acetone $m^{-3}h^{-1}$. For both TCE and acetone profiles, noticeable "dips" were again observed immediately after each step-increase in toluene concentration, apparently due to the lag-phase phenomenon discussed earlier. In the similar manner, Fig. 5 shows the removal profiles of the VOCs when TCE was the variable VOC, whose influent loading was increased from 1.2 up to 7.5 g-TCE $m^{-3}h^{-1}$ (phases 14–17). In general, the removal patterns somewhat resembled those observed in Fig. 3 because all three VOCs exhibited a declining manner with respect to their removal capabilities. The extent of the reductions in their removals, however, appeared to be less substantial than that when acetone was the variable VOC.

The different removal patterns in response to each variable VOC clearly suggest a number of possible cross-substrate interactions involved in the biotrickling filtration of the VOCs. It is noted, however, that the extents of removal reduction (or enhancement) were only moderate, suggesting that the biodegradation of the

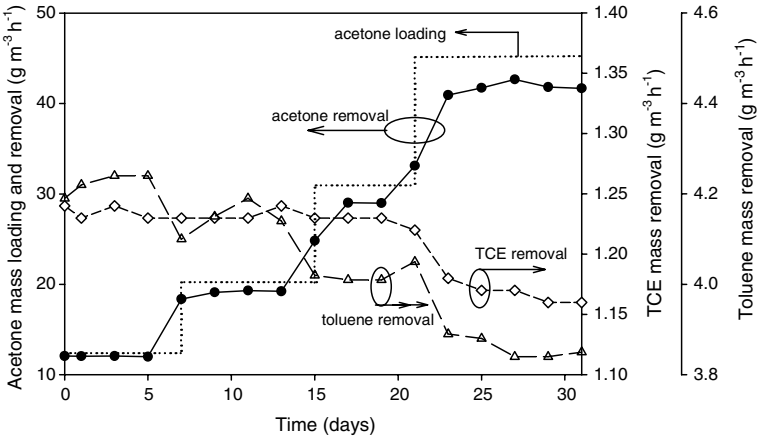

Fig. 3. Mass removal profiles for acetone, toluene, and TCE with step-increases in influent acetone loading (dotted stairlines). The mass removal of acetone (●) is indicated by the single arrow corresponding to the left scale, and mass removal of TCE (◇) and toluene (△) are indicated by the single and double arrows corresponding to the right-inner and right-outer scale, respectively.

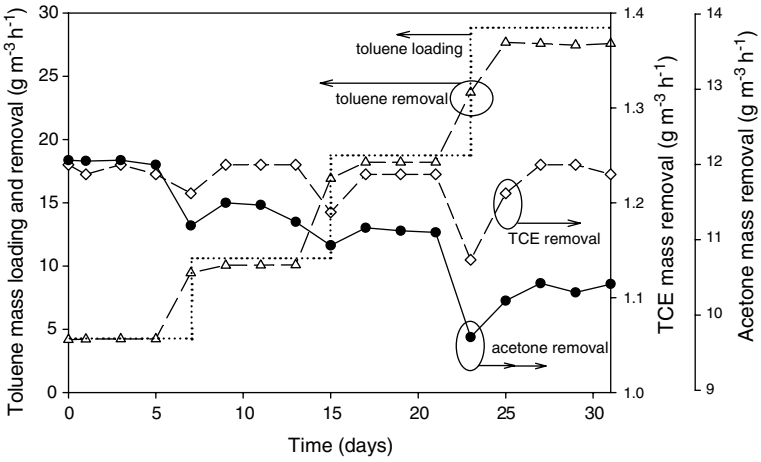

Fig. 4. Mass removal profiles for acetone, toluene, and TCE with step-increases in influent toluene loading (dotted stairlines). The mass removal of toluene (△) is indicated by the single arrow corresponding to the left scale, and mass removal of TCE (◇) and acetone (●) are indicated by the single and double arrows corresponding to the right-inner and right-outer scale, respectively.

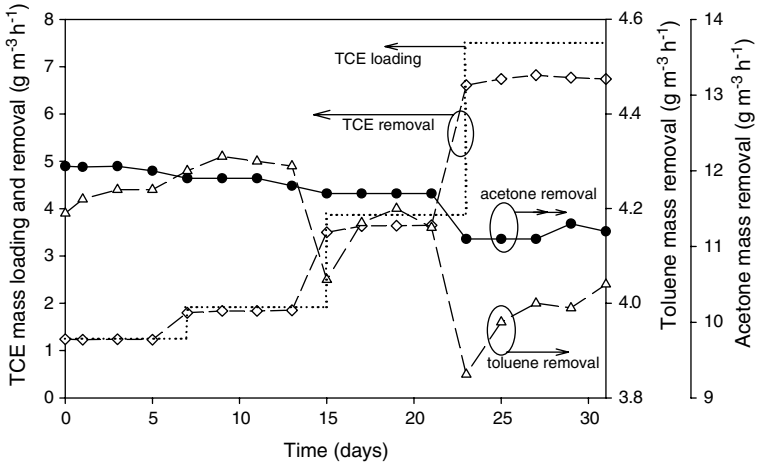

Fig. 5. Mass removal profiles for acetone, toluene, and TCE with step-increases in influent TCE loading (dotted stairlines). The mass removal of TCE (◇) is indicated by the single arrow corresponding to the left scale, and mass removal of toluene (△) and acetone (●) are indicated by the single and double arrows corresponding to the right-inner and right-outer scale, respectively.

variable VOCs were still efficient. These results effectively eliminate the occurrence of self-toxicity that generally leads to irreversible or chronic recovery of microorganisms when they were exposed to excessively high concentrations. Therefore, the observed reductions in the removal efficiency of the variable VOCs were primarily due to overloading rather than toxic effect (Chou and Wu, 1999). To present a clearer picture on the potential cross-substrate effects, the relative removal defined in Eq. (3) was calculated and graphically shown in Fig. 6. The variable VOC and its loading increases (in concentration) were indicated in the categorical vertical scale, and the relative changes of mass removal (in %) of the other VOC species deviating from the baseline condition were indicated in the horizontal scale. As seen in the figure, when the influent toluene concentration was increased from 50 to 330 ppmv, the mass removal of TCE neither showed much improvement nor significant deterioration. Since TCE is known to be biodegraded via co-metabolism by toluene-induced oxygenases, the results of the sustained TCE removal clearly suggested the occurrence of toluene-induced co-metabolism. For a direct comparison, Cox et al. (1998) reported that continuous supply of toluene at 25 ppmv led to sustained

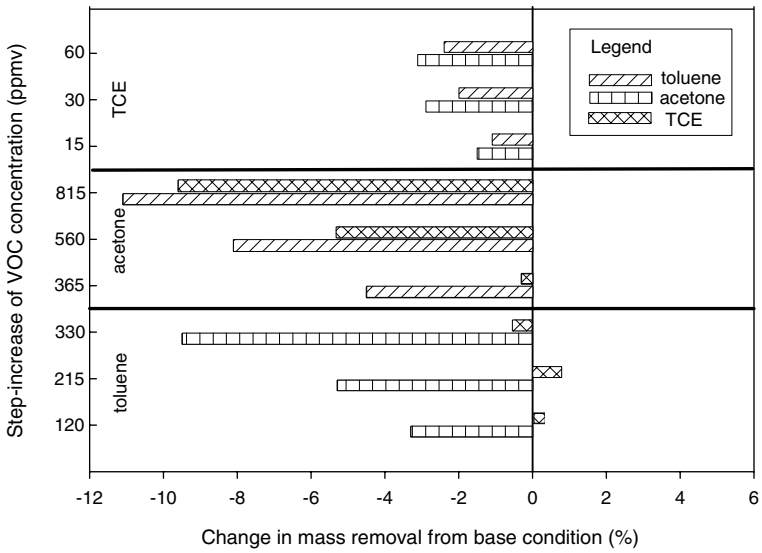

Fig. 6. The relative changes (in %) in the steady-state mass removal of the non-variable VOCs in response to the step-increases in the influent mass loadings (concentration) of the variable VOC. The relative change was calculated using Eq. (3) in the text.

TCE degradation (influent concentration of 11 ppmv) when *Pseudomonas putida* F1 was inoculated as a pure culture in their gas-phase, binary-component biofilter studies. However, increase in the TCE concentration caused temporary toxicity effect that reduced the toluene degradation efficiency, and increase in the toluene concentration led to a competitive advantage of toluene that inhibited TCE degradation by the same system of active enzymes. Their findings presented an ambiguity between toxicity (by TCE) and competitive inhibition (by toluene) when both substrates co-existed in the same waste stream. In the present study, the fact that no deterioration of TCE removal was observed even at high toluene loading implied that the competitive inhibition was not a factor within the tested range of toluene-to-TCE concentration ratios. Moreover, the possibility that the consorts in the mixed culture were responsible for the sustained TCE degradation should not be overlooked. The presence of toluene-degrading (but without co-metabolic function) bacteria can alleviate the effect of competitive inhibition on the bacteria co-metabolizing TCE. In that scenario, however, the microbial population carries a risk of shifting towards toluene degraders that do not degrade TCE over a long period of operating time (Mars et al., 1998).

When TCE concentration was increased from 10 ppmv (baseline level) to 15 ppmv, the removal of toluene was slightly enhanced. Further increase to 30 and 60 ppmv resulted in progressive decline of toluene removal to a limited extent (less than 4%). The toxicity inhibition of TCE or its intermediate products apparently was not in effect, as opposed to the observation by Cox et al. (1998) using the mono-culture of *Pseudomonas putida* F1. The lack of toxicity effect could also be ascribed to the use of microbial consortium, which generally manifests greater efficiency and stability for biodegradation of recalcitrant compounds through sequential degradation of the partial oxidation products.

A more interesting result, however, was that the degradation of TCE also increased with its own influent concentration (shown in Fig. 5), even with the fixed concentration of toluene. This result is contrary to the presumption that toluene-oxidizing oxygenases cannot be induced by TCE, and that TCE transformation cannot be enhanced without inducing more toluene-oxidizing enzymes. To verify whether the TCE removal was due to biotic or non-biotic causes, the biofilm-coated carbon granules directly taken from the top-quarter of the column packing were washed and re-suspended in the mineral solution augmented with yeast extract. Without re-separating the microorganisms, the microbial suspension was prepared by following the same protocol used for the batch biodegradation test, with TCE (5 μM) as the sole substrate. The headspace analysis showed that TCE removal was significant (≈30%) over a 24-h period, after which the rate of removal appeared to slow down (<10% removal for the next 24-h period). This result provided evidence that TCE degradation was possible without toluene as an inducer for the microbial consortium, and is partially consistent with the previous finding of Leahy et al. (1996). These researchers reported that TCE or its oxidation product not only can act as an inducer of toluene-oxidation activity with several *Pseudomonas* strains previously grown on toluene and

TCE, but can also enhance its own degradation in the absence of an aromatic inducer. Although the mechanism activating the pre-induced toluene-oxidizing oxygenases for TCE degradation without the continuous presence of toluene has not been clarified, it is hypothesized that the mechanism requires toluene-oxidizing cells to be previously grown in the presence of TCE.

Increase in acetone influent concentration from 220 up to 815 ppmv progressively reduced the removal of toluene and TCE. Comparing with the baseline condition, the mass removal of toluene was reduced by nearly 5% as the influent acetone concentration was raised to a level at 365 ppmv, and by over 11% at 815 ppmv. The TCE mass removal also deteriorated with the similar pattern. Acetone is oxidized by either acetone monooxygenase or methylhydroxylase into products of acetol and methyl acetate, and then into pyruvate via dehydrogenation (Taylor et al., 1980). Subsequently, these intermediate products enter the common tricarboxylic acid (TCA) cycle that cleaves the acetone skeleton into C1 or C2 fragments for further mineralization (Mendz et al., 1994). Toluene metabolism, on the other hand, is initiated by the insertion of oxygen atoms by toluene mono- or dioxygenases into the aromatic ring to form catechols. Enzymatic ring fission of catechols then leads to major intermediate products such as pyruvate and acetaldehyde that also enter the TCA cycle (Ellis and Wackett, 1995). Therefore, acetone and toluene degradations do not share common enzymatic reaction for their initial decomposition, but their oxidation products may merge into common degradation pathways. The apparent differences in the active enzymatic reaction effectively eliminated the possibility of competitive inhibition between acetone and toluene (or TCE) for the same active site of enzyme. A more plausible explanation to this antagonistic effect is the occurrence of diauxie, in which five of the acetone-degraders also capable of oxidizing toluene (B1–B5) preferably utilized acetone over toluene as the primary substrate. Moreover, when the acetone concentration was high enough to become the predominant substrate (as in the case where acetone concentration was increased up to 815 ppmv), the competitive balance may be lost in the structure of the microbial consortium. This result can be supported by the observation that strains B6 and B7, two strains that are highly selective for acetone degradation over other toluene- and TCE-degrading bacteria, prevailed over other bacteria in the microbial population at the end of the experiments. Consequently, the acetone removal remained highly efficient, whereas the removal efficiencies of toluene and TCE were significantly suppressed.

The use of activated carbon as the packing medium inevitably contributed to a certain extent of VOC removal by adsorption. Consequently, there exists an experimental difficulty to completely discriminate the contribution of adsorption from the biodegradation of the VOCs. However, a number of studies have also shown that, while adsorption plays a prominent role in the initial stage of bioactive carbon systems, its adsorptive property would gradually diminish due to the growth and maturation of biofilm (Schwarz et al., 1999; Kim et al., 2000). In support of these findings, one can observe that the adsorption breakthrough eventually occurred after 20 days of operation (phase 1, Fig. 2), revealing the gradual exhaustion of the adsorption capacity of the bioactive carbon. Furthermore, in a water-saturated system, water molecules tend to gain competitive advantage for the adsorption sites over organic molecules, leaving lesser chances of direct adsorption of VOCs after an extended period. Therefore, while adsorption may contribute to the beneficial "buffering" effects during transient response to a step-increase in mass loading, it would be much less likely to be a factor for direct removal of gas-phase VOCs under a steady-state condition. In view of these notions, the effect of adsorption was neglected from the analysis for the steady-state removal of the VOCs.

4. Conclusions

The transient and steady-state performance of a biotrickling filter for the control of a synthetic waste gas mimicking the emission from the microelectronics industry was studied. The waste gas mixture contained three representative VOCs in acetone, toluene, and TCE over a range of concentrations, allowing the examination of the possible cross-substrate interactions by relating the variation of mass loading with the functional characteristics of the microbial consortium. A threshold capacity for the column appeared to be reached when the THC concentration was 2400 ppmv with a 155-s gas retention time, but no evidence of irrecoverable inhibition was observed. Influent mass loading below this threshold loading typically resulted in THC removal efficiencies over 95% and carbon mineralization ratios over 70%. Microbial characterization revealed that the consortium contained at least seven bacterial strains capable of degrading acetone and/or toluene and TCE. The collective analysis using the "relative change" in mass removal against a variable VOC manifested some interactions of practical importance to a biological treatment system. Concentration increases in acetone, a readily biodegradable compound, appeared to cause a diauxie effect that suppressed degradation of toluene, and a population shift that favored acetone-degraders (strains B6 and B7). Competitive substrate inhibition was unlikely a factor since the degradation of acetone and toluene follows different enzymatic mechanisms. The interactions between toluene and TCE showed a mutual induction of toluene-oxidizing oxygenases that resulted in enhanced TCE removal at fixed concentration of toluene. Taken

together, this study demonstrated that the relative VOC concentration in the waste gas mixture is a factor as important as the biodegradative function of the microbial consortium, and much more information concerning the cross-substrate interactions needs to be gathered before biological treatment of organic mixtures can be truly considered as a matter of design.

Acknowledgment

This study was partially supported by the National Nano Device Laboratories (NDL), Taiwan, Republic of China.

References

Assinder, S.J., Williams, P.A., 1990. The Tol plasmids—Determinants of the catabolism of toluene and xylenes. Adv. Microbiol. Physiol. 31, 1–69.

Bielefeldt, A., Stensel, H.D., 1999. Modeling competitive inhibition effects during biodegradation of BTEX mixtures. Water Res. 33, 707–714.

Biolog, 1993. Biolog microStation system, v. 3.5, Biolog Inc., Hayward, Calif., USA.

Burbeck, B.L., Perry, J.J., 1993. Biodegradation and biotransformation of groundwater pollutant mixtures by *Mycobacterium vaccae*. Appl. Environ. Microbiol. 59, 1025–1029.

Bustard, M.T., McEvoy, E.M., Goodwin, J.A.S., Burgess, J.G., Wright, P.C., 2000. Biodegradation of propanol and isopropanol by a mixed microbial consortium. Appl. Microbiol. Biotechnol. 54, 424–431.

Chou, M.-S., Wu, F.-L., 1999. Treatment of toluene in an air stream by a biotrickling filter packed with slags. J. Air Waste Manage. Assoc. 49, 386–398.

Coogan, J.J., Jassal, A.S., 1997. Silent discharge plasma (SDP) for point-of-use (POU) abatement of volatile organic compound (VOC) emissions: Final report (ESHCOO3). SEMATECH, Technology Transfer #97023244-ENG, Austin, Texas, USA.

Cox, C.D., Woo, H.J., Robinson, K.G., 1998. Cometabolic biodegradation of trichloroethylene (TCE) in the gas phase. Water Sci. Tech. 37, 97–104.

Den, W., Pirbazari, M., Huang, C., 1999. Biofiltration for treatment of VOC-contaminated waste air by a semiconductor manufacturing facility. In: Proceedings of the 16th Annual Conference on Air Pollution Control Technology, Chinese Institute of Environmental Engineering, paper CO1-4.

Den, W., Huang, C., Li, C.-H., 2003. Biotrickling filtration for control of volatile organic compounds from microelectronics industry. J. Environ. Eng. 129, 610–619.

Diks, R.M.M., Ottengraf, S.P.P., Vrijland, S., 1994. The existence of a biological equilibrium in a trickling filter or waste gas purification. Biotechnol. Bioeng. 44, 1279–1287.

Ellis, L.B.M., Wackett, L.P.A., 1995. Microbial biocatalysis database. Soc. Ind. Microb. News 45, 167–173.

Fetzner, S., 1998. Bacterial dehalogenation. Appl. Microbiol. Biotechnol. 50, 633–657.

Folsom, B.K., Chapman, P.J., Pritchard, P.H., 1990. Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4. Kinetics and interactions between substrates. Appl. Environ. Microbiol. 56, 1279–1295.

Fredrickson, J.K., Balkwill, D.L., Drake, G.R., Romine, M.F., Ringelberg, D.B., White, D.C., 1995. Aromatic-degrading *Sphingomonas* isolates from the deep subsurface. Appl. Environ. Microbiol. 61, 1917–1922.

Hanson, R.S., Brusseau, G.A., 1994. Biodegradation of low-molecular-weight halogenated compounds by aerobic bacteria. In: Chaudry, G.R. (Ed.), Biological Degradation and Bioremediation of Toxic Chemicals. Dioscorides Press, Portland, Oreg., USA, pp. 227–297.

Heald, S., Jenkins, R.O., 1994. Trichloroethylene removal and oxidation toxicity mediated by toluene dioxygenase of *Pseudomonas putida*. Appl. Environ. Microbiol. 60, 4634–4637.

Kim, B.R., Adams, J.A., Klaver, P.R., Kalis, E.M., Contrera, M., Griffin, M., Davidson, J., Pastick, T., 2000. Biological removal of gaseous VOCs from automotive painting operations. J. Environ. Eng. 126, 745–753.

Klečka, G.M., Maier, W.J., 1988. Kinetics of microbial growth on mixtures of pentachlorophenol and chlorinated aromatic compounds. Biotechnol. Bioeng. 31, 328–335.

Leahy, J.G., Byrne, A.M., Olsen, R.H., 1996. Comparison of factors influencing trichloroethylene degradation by toluene-oxidizing bacteria. Appl. Environ. Microbiol. 62, 825–833.

Maier, W.J., 1989. Kinetics-dynamics of biodegradation of potentially toxic organic chemicals. In: 43rd Purdue Industrial Waste Conference Proceedings. Lewis Publishers, Chelsea, Michigan, USA, pp. 257–265.

Mars, A.E., Prins, G.T., Wietzes, P., de Koning, W., Janssen, D.B., 1998. Effect of trichloroethylene on the competitive behavior of toluene-degrading bacteria. Appl. Environ. Microbiol. 64, 208–215.

McClay, K., Streger, S., Steffan, R.J., 1995. Induction of toluene oxidation activity in *Pseudomonas mendocina* KR1 and *Pseudomonas* sp. Strain ENVPC5 by chlorinated solvents and alkanes. Appl. Environ. Microbiol. 61, 3479–3481.

Mendz, G.L., Hazell, S.L., van Gorkom, L., 1994. Pyruvate metabolism in *Helicobacter pyloi*. Arch. Microbiol. 162, 187–192.

Nelson, M.J., Montgomery, S.O., Mahaffey, W.R., Pritchard, P.H., 1987. Biodegradation of trichloroethylene and involvement of an aromatic biodegradative pathway. Appl. Environ. Microbiol. 53, 949–954.

Reardon, K.F., Mosteller, D.C., Bull Rogers, J.D., 2000. Biodegradation kinetics of benzene, toluene, and phenol as single and mixed substrates for *Pseudomonas putida* F1. Biotechnol. Bioeng. 69, 385–400.

Rittman, B.E., Saez, P.B., 1993. In: Levin, J.M., Gealt, M. (Eds.), Biotreatment of Industrial and Hazardous Wastes. McGraw-Hill, New York, NY, USA, pp. 113–136.

Shields, M.S., Montgomery, S.O., Chapman, P.J., Cuskey, S.M., Pritchard, P.H., 1989. Novel pathway of

toluene catabolism in the trichloroethylene-degrading bacterium G4. Appl. Environ. Microbiol. 55, 1624–1629.

Schwarz, B.C.E., Devinny, J.S., Tsotsis, T.T., 1999. Degradation of PCE in an anaerobic waste gas by biofiltration. Chem. Eng. Sci. 54, 3187–3195.

Taylor, D.G., Trudgill, P.W., Cripps, R.E., Harris, P.R., 1980. The microbial metabolism of acetone. J. Gen. Microbiol. 118, 159–170.

US Environmental Protection Agency, 1995. EPA Office of Compliance Sector Notebook Project: Profile of the Electronics and Computer Industry; EPA/310-R-95-002; US Governmental Printing Office, Washington, DC.

Wackett, L.P., Gibson, D.T., 1988. Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with *Pseudomonas putida* F1. Appl. Environ. Microbiol. 54, 1703–1708.

Weber, F.J., Hartmans, S., 1996. Prevention of clogging in a biological trickle-bed reactor removing toluene from contaminated air. Biotechnol. Bioeng. 50, 91–97.

Winter, R.B., Yen, K.-M., Ensley, B.D., 1989. Efficient biodegradation of trichloroethylene by a recombinant *Escherichia coli*. Biotechnology 7, 282–285.

Zhu, X., Rihn, M.J., Suidan, M.T., Kim, B.J., Kim, B.R., 1996. The effect of nitrate on VOC removal in trickling bed biofilters. Water Sci. Tech. 34, 573–581.