ELSEVIER

Journal of Hazardous Materials B131 (2006) 131–136

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Assessment of ethylene removal with *Pseudomonas* strains

Jongo Kim *

Department of Environmental Education, Mokpo National University, Muan-gun, Chungkye-myon, Chonnam 534-729, Republic of Korea

Received 5 November 2004; received in revised form 30 August 2005; accepted 10 September 2005
Available online 10 October 2005

Abstract

This study investigated the biological removal of ethylene by *Pseudomonas* strains in a batch test and a biofilter column. In the batch test, no removal of ethylene was found in the absence of inoculated system, whereas more than 50% of the ethylene in the presence of inoculated system was degraded within 17 h, and completely removed after 25 h. The biofilter, packed with activated carbons, was capable of achieving ethylene removal efficiency as much as 100% at a residence time of 14 min and an inlet concentration of 331 mg m^{-3}. Under the same conditions, carbon dioxide with a concentration of up to 1097 mg m^{-3} was produced. It was found that carbon dioxide was produced at a rate of 87 mg day^{-1}, which corresponded to a volume of 0.05 L day^{-1}. During operation with an inlet ethylene of 331 mg m^{-3}, the maximum elimination capacity of the biofilter was 34 g C_2H_4 m^{-3} day^{-1}. This biological system could reduce the ethylene concentration to levels below the threshold limit for the plant hormonal response (0.01 mg m^{-3}), and provide an attractive treatment technology in horticultural storage facilities.

Keywords: Activated carbon; Biofilter; Elimination capacity; Ethylene; *Pseudomonas*

1. Introduction

Ethylene (C_2H_4) is one of the major constituents in petrochemical products. It is also reported that ethylene is produced by biosynthesis in soils [1]. Ethylene, as a gaseous pollutant, has an effect on plant physiological processes such as ripening, senescence, and aging. Accumulation of ethylene in plants might occur in horticultural storage facilities due to the endogenous production by the plant material [2]. In addition, ethylene causes the serious air pollution problem producing ozone (O_3) as a result of photochemical reaction. In order to remove ethylene, scrubbers are widely used in storage facilities [3]. Disadvantages of the scrubbers include high operation costs and replenishing the ethylene-removing agent. Ethylene is an extremely volatile and gaseous compound at room temperature. It is very difficult to treat ethylene by adsorption methods [4]. Because of the limitations of the previously mentioned treatment technologies of scrubbers and adsorption, a new approach is needed in order to treat ethylene efficiently.

Elsgaard [5] studied ethylene removal using a peat/soil biofilter with an immobilized pure culture. After starting the operation of the biofilter with 134 mg m^{-3} of ethylene, the compound was reduced to 0.05 mg m^{-3}. Biofilers have been widely applied to the treatment of organic off-gases containing biodegradable organic compounds [6]. Biofiltration technology has been known to be a reliable and cost-effective technology for the treatment of odor and organic compounds. Various types of biofilters, based on different filter media, have been used. Soil biofilters have a small surface area, low permeability, and limited sorption capacity resulting in poor performance [7]. The disadvantages of compost and peat biofilters include the replacement of filter media and the requirement of a large installation space [8].

The objective of this study was to assess the ethylene biodegradation from a batch test and a biofilter study. This process involved the use of granular activated carbon (GAC) and the employment of ethylene-degrading microorganisms, *Pseudomonas* strains. The activated carbon biofilter was introduced because GAC provided several advantages, such as greater surface area and greater porosity [8]. In addition, the estimated carbon dioxide production, the maximum elimination capacity, and ethylene concentration profile according to sampling depth, were presented.

* Tel.: +82 614502792; fax: +82 614534843.
E-mail address: jongokim@mokpo.ac.kr.

0304-3894/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2005.09.019

2. Materials and methods

2.1. Microorganisms

In order to isolate new strains, ethylene-degrading microorganisms were obtained from a raw wastewater at the Nam-Hae Wastewater Treatment Plant in the City of Mokpo, South Korea. The microorganisms were continuously acclimated to ethylene as its sole source of carbon under the conditions of 25 °C with a minimal medium in a cultivation reactor (29.2 cm i.d. and 50 cm long) under the aerobic condition for 3 weeks. For the physiological and biochemical analyses, the microorganisms were grown on the LB agar medium (Luria-Bertani; 1% tryptone, 0.5% yeast extract, 1% NaCl, and adjusted to pH 7.0 with 5 N NaOH). The NFT-API 20 NE and API 20 E (API BioMerieux SA, France) were used as identification kits based on the Bergey's Manual [9]. After the analyses, dominant strains in the ethylene-degrading microorganisms were identified as *Pseudomonas putida* (gram negative) and *P. fluorescens* (gram negative). The minimal medium had the following components: 50 mg NaH_2PO_4, 85 mg KH_2PO_4, 165 mg K_2HPO_4, 100 mg NH_4Cl, 0.1 mg $MgSO_4{\cdot}7H_2O$, 0.12 mg $FeSO_4{\cdot}7H_2O$, 0.036 mg $MnSO_4{\cdot}H_2O$, 0.03 mg $ZnSO_4{\cdot}7H_2O$, 0.01 mg $CoCl_2{\cdot}6H_2O$, 0.1 mg $CaCl_2{\cdot}2H_2O$, and 0.5 mg yeast extract in 1 L of distilled water.

2.2. Activated carbon

In order to pack the biofilter with filter media, granular activated carbon was obtained from Shin-Ki Chemical, South Korea. Before the carbon was transferred into the biofilter, the carbon was washed with tap water, graded with USA standard no. 8 (2.36 mm opening) and no. 35 (0.5 mm opening) sieves, and dried at a room temperature. Surface area and bulk density of the carbon were from 900 to 1100 $m^2\ g^{-1}$ and 0.4 to 0.5 $g\ mL^{-1}$, respectively.

2.3. Batch study

A preliminary batch study on ethylene degradation was conducted in the presence and absence of inoculated system in a 250 mL amber bottle and a 40 mL vial. For the batch study, the microorganisms were acclimated in the cultivation reactor for 3 weeks. One hundred milliliters of the cultivated solution was dispensed into the bottle sealed with a Teflon-lined septum and cap, whereas 25 mL of the solution was transferred to the vial. The ethylene standard gas was injected into the bottles and vials. The bottle and vial were placed on a shaking incubator set (Vision Scientific, South Korea) at 220 rpm (31–33 °C). The analysis of each bottle was initiated by injecting 0.8 mL of a 6N-HCl solution to lower the pH to 1.2 and to stop microbial activity [10]. During the course of the experiment, the bottle and vial was periodically used for analyzing ethylene, carbon dioxide, and volatile suspended solids (VSS) at each sampling time. The concentrations of ethylene and carbon dioxide were measured by taking samples from the headspace through the septum.

2.4. Column study

Fig. 1 shows a schematic diagram of lab-scale biofilter column. The biofilter housing was made of PVC (6.7 cm i.d. and 62.5 cm long), and operated in room temperature. The biofilter was packed with activated carbon as a filter media and inoculated with *Pseudomonas* strains. The depth of the activated carbon in the column was 24.4 cm, and its weight was 500 g. The initial VSS concentration of cultivated solution was 40 $mg\ L^{-1}$ before they were fed into the biofilter.

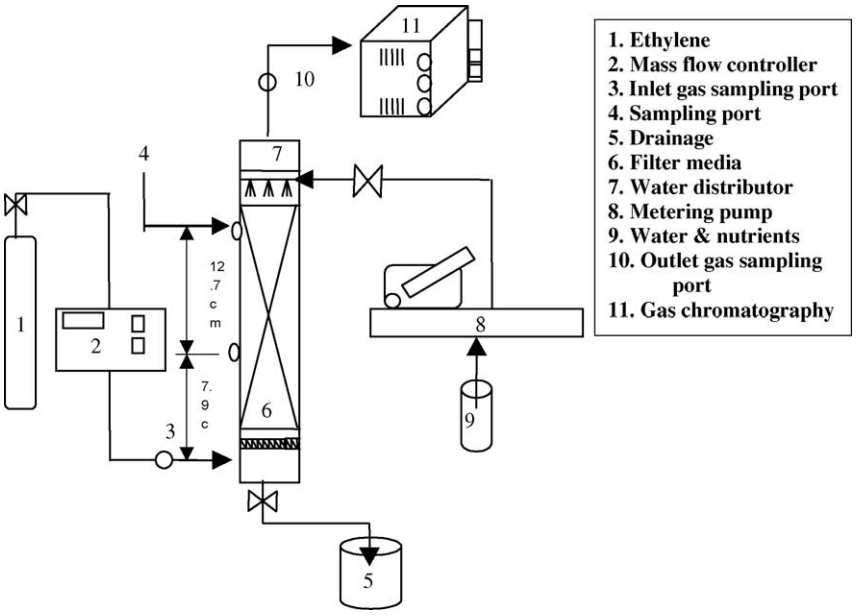

Fig. 1. Schematic diagram of a biofilter column.

Ethylene, as an inlet source, was purchased from a specialty gas company in South Korea. The inlet ethylene concentrations of 113, 331, and 517 mg m^{-3} were fixed with pure air (carbon dioxide concentration = 0 mg m^{-3}), a mixture of oxygen and nitrogen. Ethylene was fed into the biofilter at the bottom using a 1/4 in. o.d. Teflon tubing and fittings. Inlet ethylene flow was maintained through a mass flow controller. The nutrient solution in distilled water was introduced at the top of the biofilter through a water distributor using a Cole-Parmer metering pump (USA) at a rate of 400 mL day^{-1}. The solution was provided for the maintenance of 40–47% moisture content of carbon in the biofilter.

The maximum elimination capacity (EC) is a design parameter for a biofilter. It is a measurement of the highest removal of ethylene per unit volume of filter media. It is measured in grams of compound removed per cubic meter of media per hour (g m^{-3} h^{-1}) and is represented by the following equation:

$$\mathrm{EC} = (C_{\mathrm{in}} - C_{\mathrm{out}})\, Q/V = C_{\mathrm{in}}\, \mathrm{RE}\, Q/V$$

where EC is the elimination capacity (g m^{-3} h^{-1}), C_{in} the inlet concentration (g m^{-3}), C_{out} the outlet concentration (g m^{-3}), Q the flow rate (m^3 h^{-1}), RE the highest removal efficiency, and V is the volume of filter media (m^3).

2.5. Gas sampling and analysis

As shown in Fig. 1, inlet and outlet samples were collected using 1.6 L tedlar gas sampling bags (Cole-Parmer, USA) with on–off and septum valves connected to the inlet and outlet gas sampling ports. The on–off valve was used for collecting gaseous samples, and the septum valve for needle injections. Sampling was conducted over a 20 min period to collect the gaseous samples. Before every sampling, the bags were filled with air and cleaned out by an air pump several times. For the ethylene concentration measurement at biofilter height, a 1 mL Pressure-Lok gas syringe was directly injected into sampling ports sealed with the septum of the biofilter. Twice sample injections and a standard curve ($r^2 = 0.99$) were used for all data analyses.

Ethylene and carbon dioxide were analyzed with a GC (Shimadzu 14A, Japan) fitted with a thermal conductivity detector (TCD) and a Porapak-Q column (Supelco, USA). Temperatures for column, injector, and detector were 90, 120, and 100 °C, respectively. The column temperature was maintained for 3 min at 90 °C, and then heated to 130 °C at a rate of 8 °C/min. Helium was used as a carrier gas at a flow rate of 30 mL min^{-1}. The samples were directly injected into the injector port using a 1 mL Pressure-Lok gas syringe (Series A-2) with a push-button valve.

3. Results and discussion

3.1. Batch study

Before *Pseudomonas* strains were inoculated into the biofilter column, this batch study on ethylene degradation was performed. First, 100 mL of cultivated solution containing ethylene-degrading microorganisms was transferred to a bottle, and then standard ethylene was injected into the bottle. The ethylene can be redissolved and degraded in the bottle solution in a well-mixed system. The VSS concentration of the cultivated solution was 40 mg L^{-1}. Fig. 2 shows the degradation of ethylene during a period of 26 h. The initial concentration of ethylene in the presence and absence of the inoculated system was 184 mg m^{-3}. Ethylene concentration in the absence of the inoculation was measured as a control experiment in order to investigate ethylene removal without the microorganisms. No removal of ethylene was found without inoculated system, whereas more than 50% of the ethylene with inoculation was degraded within 17 h.

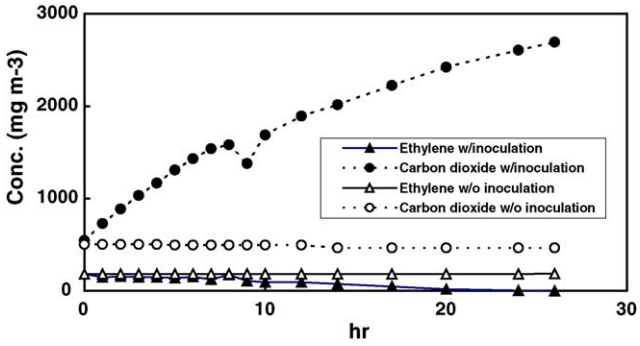

Fig. 2. Removal of ethylene in a batch study.

Ethylene was slowly degraded in the beginning of the experiment because it might have been resistant to biodegradation. Thereafter, a relatively fast degradation rate was observed. Approximately 98% of the ethylene was degraded in 24 h. Eventually, ethylene, as a substrate, was completely utilized by the inoculation. However, no ethylene degradation in the absence of the inoculated system was found. As shown in Fig. 2, carbon dioxide production in the presence of the inoculation was increased due to microbial activity, whereas no variation in carbon dioxide concentration in the absence of the microorganisms was found. It was of interest that more carbon dioxide than theoretical calculation was produced in the presence of inoculation. It is likely that the microorganisms were forced to metabolize their own cells because the ethylene available was limited in the bottle. Study on the mass balance of carbon dioxide production could not be attempted due to cell metabolism.

For the reliability of batch study, another batch experiment was conducted in the presence and absence of inoculation in a 40 mL vial. Twenty-five milliliters of the cultivated solution was dispensed into the vial, and ethylene degradation was monitored at an initial concentration of 106 mg m^{-3} during a period of 12 h. Like the previous batch result, no removal of ethylene was found without inoculation, whereas more than 75% of the ethylene with inoculation was degraded within 10 h. A similar removal rate was obtained between both experiments.

3.2. Column study

After the strains were inoculated into the column, a study based on different inlet concentrations and empty bed residence time (t) was conducted. The gas flow rate was 61 mL min^{-1} through the column, resulting in an empty bed residence time of 14 min. The evaluation of biofilter performance in terms of

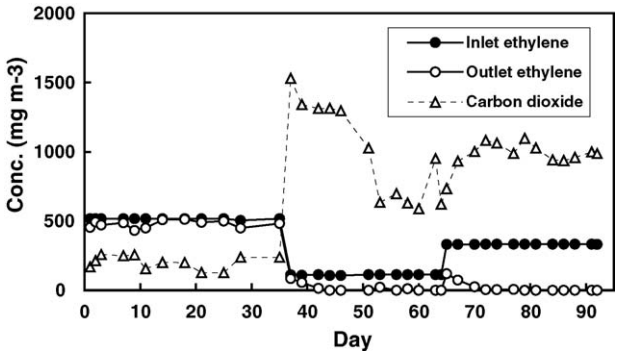

Fig. 3. Ethylene removal and carbon dioxide production according to different inlet concentrations.

different inlet concentrations: 517, 331, and 113 mg m^{-3}, was evaluated. Fig. 3 shows variations of inlet and outlet concentrations of ethylene during 92 days of operation. Low removal efficiency, 10–15%, was observed because the ethylene was not well adsorbed onto the activated carbon, resulting in poor biodegradation at the relatively higher concentration. After changing the inlet concentration to 113 mg m^{-3} while maintaining the same residence time, the ethylene removal efficiency increased to 90% in 6 days, as shown in Fig. 3. Thereafter, outlet ethylene was gradually decreased and 100% of ethylene was degraded. With an inlet concentration of 331 mg m^{-3}, 100% removal efficiency was also observed.

The removal efficiency is determined by the concentration of ethylene removed by the biofilter and expressed as a percentage of the inlet ethylene concentration. In this study, the removal efficiency of the biofilter was lowest at an inlet concentration of 517 mg m^{-3}. As previously mentioned, the ethylene was difficult to degrade biologically at the relatively higher concentration. Because ethylene is an extremely volatile and slowly adsorbed compound, sufficient time for microbial adaptation to the ethylene might be required. The inlet concentration needed to be lowered because of poor removal efficiency. With the lower inlet concentrations, all of ethylene was completely removed by the inoculated system as it was adsorbed onto biofilm and degraded. Improved performance in subsequent days might have occurred because the necessary microbial enzymes were induced and the initially small populations of ethylene-degrading microorganisms grew. Typically, a reactor performance in the absence of the inoculated system was measured as a control experiment in order to investigate ethylene removal without inoculation. With an inlet ethylene concentration of 331 mg m^{-3}, the outlet ethylene concentrations were 331 mg m^{-3} for 28 days.

In a comparable study, van Ginkel et al. [11] reported the removal of ethylene by a compost biofilter inoculated with *Mycobacterium* strain E3. With an inlet concentration of 2.3 mg m^{-3}, 87% removal efficiency was achieved during the operation for 8 weeks. Elsgaard [5] employed a peat–soil biofilter inoculated with ethylene-degrading bacterial strain RD-4 to remove ethylene and obtained 99% removal efficiency at a residence time of 9.4 min and an inlet concentration of 134 mg m^{-3}. With a relatively high concentration of ethylene used in this study, 100% was removed. The biofilter study was evaluated in terms of the ethylene elimination capacity, defined as the amount of ethylene degraded per unit of reactor volume and time. With an inlet ethylene of 331 mg m^{-3}, it was found that the maximum elimination capacity of this study was 34 g C_2H_4 m^{-3} day^{-1}, whereas the capacity in Elsgaard's study was 21 g C_2H_4 m^{-3} day^{-1}. This capacity was slightly higher than that calculated for Elsgaard's biofilter study. This could be due to the selection of activated carbon and *Pseudomonas* strains.

A short residence time is an important design consideration in a field facility because the highest possible flow of polluted gas should be treated. The relatively high concentration was applied to the biofilter because it is frequently encountered in industrial emission sources. The selection of appropriate filter media and microorganisms are important operation factors for the biofiltration process of ethylene removal. This study showed that using an activated carbon biofilter inoculated with *Pseudomonas* strains provides an alternative and more attractive treatment technology for ethylene removal at relatively high concentrations. This study suggested that ethylene from industrial point sources or horticultural storage facilities could be reduced to the low range of concentration when a biofilter is applied.

3.3. Carbon dioxide production from column

Fig. 3 also shows the concentrations of carbon dioxide produced in the biofilter with different operation conditions. An increase in the carbon dioxide concentration of up to 1532 mg m^{-3} was found at an inlet concentration of 113 mg m^{-3}, usually 589–636 mg m^{-3} was detected at a steady state. With an inlet concentration of 331 mg m^{-3}, the carbon dioxide concentrations of 735–1097 mg m^{-3} were investigated. Carbon dioxide concentrations ranging from 128 to 251 mg m^{-3} were produced due to low removal efficiency (10–15%) at an inlet concentration of 517 mg m^{-3}.

Carbon dioxide and water vapor are produced as a result of ethylene degradation with ethylene-degrading microorganisms. Ethylene degradation might have resulted in the growth of biofilm and a mineralization of ethylene in the biofilter. With the lower inlet concentration, almost all of the ethylene was degraded because the biofilter was capable of removing ethylene quickly. Higher carbon dioxide production rates were obtained at the lower inlet concentrations due to the high removal efficiency of ethylene. With an inlet concentration of 331 mg m^{-3} at a residence time of 14 min, it was found that carbon dioxide was produced at a rate of 87 mg day^{-1}, which corresponded to a volume of 0.05 L day^{-1}.

It is difficult to calculate mass balance of carbon dioxide production because of inconsistent microbial activity in the biofilter. But a rough mass balance of carbon using stoichiometry was attempted. Theoretically, for each molecule of ethylene, two molecules of carbon dioxide are produced:

$$C_2H_4 + 3O_2 \rightarrow 2CO_2 + 2H_2O$$

As summarized in Table 1, different carbon dioxide concentrations between the measured and the calculated mass balances

Table 1
Mass balance of carbon in column

Inlet concentration ($mg\,m^{-3}$)	Measured outlet CO_2 concentration at steady state ($mg\,m^{-3}$)	Calculated outlet CO_2 concentration ($mg\,m^{-3}$)	Removal (%)
Ethylene			
113	589–36	359	100
331	735–1097	1042	100
517	128–251	180	10–15

were observed due to inconsistent microbial activity. However, the carbon dioxide concentrations of the measured mass balances were similar to those of the calculated. With an inlet concentration of 113 $mg\,m^{-3}$, more carbon dioxide was measured because the previously biosorbed ethylene at an inlet concentration of 517 $mg\,m^{-3}$ may have been degraded. Some of ethylene may be adsorbed on the biofilm rather than on the carbon. Because it is not clear why high carbon dioxide was produced in the beginning of 113 $mg\,m^{-3}$ (inlet concentration), two possibilities were assumed. First, it is likely that some of previously biosorbed ethylene may have effected on increase in carbon dioxide production. At the steady state, however, the carbon dioxide production was rapidly decreased. Hodge et al. [12] hypothesized gaseous pollutants may be directly adsorbed from gas phase to the surface of the microorganisms (biosorption). Second, it was assumed that the microorganisms in the biofilter were forced to metabolize their own cells because the ethylene available was reduced after changing to 113 $mg\,m^{-3}$.

3.4. Concentration profile versus column depth

The ratio between the outlet (C) and inlet (C_0) ethylene concentration is presented in Fig. 4. After operating with an inlet concentration of 113 $mg\,m^{-3}$ in 5 days, ethylene concentrations at different heights in the biofilter were measured. About 27% of the inlet ethylene was degraded in the biofilter height between the inlet port (0 cm) and 7.9 cm, whereas 22% of the ethylene was degraded between 20.6 cm and outlet port. With an inlet ethylene concentration of 331 $mg\,m^{-3}$ in 15 days, about 67% of the ethylene was degraded in the biofilter height between 0 and 7.9 cm.

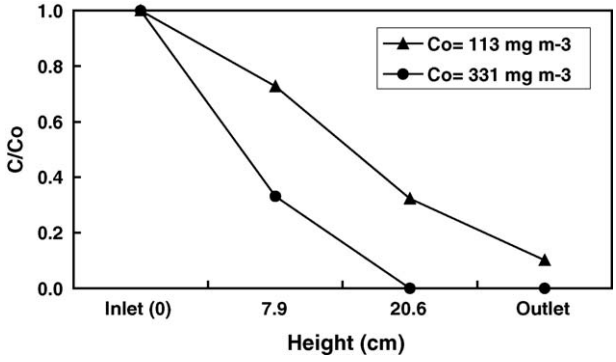

Fig. 4. Concentration profiles according to biofilter height.

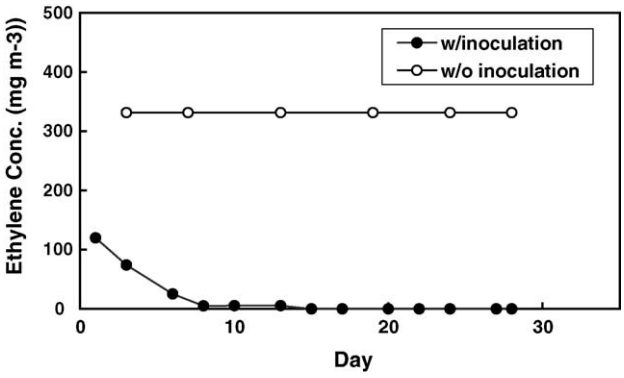

Fig. 5. Outlet ethylene concentrations in the presence and absence of inoculation.

The presence of ethylene appeared to decrease in the higher layer of the biofilter. More ethylene was removed in the lower and middle layers of the biofilter due to the more active microbial growth. This implies that sufficient substrate may induce more microbial activity in the lower layer of the biofilter. As a result of increase in microbial populations in the lower layer, a biofilm could have grown and the biofilm thickness may have increased. Ergas et al. [13] also reported that acclimatization of microbial population to dichloromethane took place according to biofilter height over a period of 10 weeks. The dichloromethane degradation moved progressively toward to lower layer of the biofilter bed.

3.5. Adsorption study

In order to investigate the ethylene adsorbed on activated carbon in a biofilter, a column was typically operated in the absence of microbial inoculation at an inlet ethylene concentration of 331 $mg\,m^{-3}$. This experiment was the same as the biofilter study except for the inoculated system. As shown in Fig. 5, in the absence of inoculation, ethylene was not removed, whereas the ethylene in the presence of inoculation was continuously removed over a period of days. This experiment implied that the activated carbon could not have adsorbed the ethylene.

4. Conclusions

The biological removal of ethylene by *Pseudomonas* strains was assessed in a batch test and a biofilter study. As a result of the batch study, ethylene was completely utilized by the inoculated system, whereas no ethylene degradation was found in the absence of the inoculation.

The selection of appropriate filter media and strains may be important operation factors for the biofiltration process of ethylene removal. It was found that appropriate design variables for the residence time and inlet concentration could be 14 min and 331 $mg\,m^{-3}$, respectively. Under the conditions, the biofilter, packed with activated carbons and inoculated with the *Pseudomonas* enrichment culture, was capable of achieving ethylene removal efficiency as much as 100%. While operating with an inlet ethylene of 331 $mg\,m^{-3}$, it was found that the maximum elimination capacity of the biofilter in this study was

34 g C_2H_4 m^{-3} day^{-1}, and that carbon dioxide was produced at a rate of 87 mg day^{-1} with a volume of 0.05 L day^{-1}.

This study suggested that ethylene from horticultural storage facilities or industrial point sources could be reduced to low ranges when a biofilter inoculated with *Pseudomonas* strains is applied. Especially, this biological system could reduce the ethylene concentration to levels below the threshold limit for the plant hormonal response (0.01 mg m^{-3}). The biofilter could be a cost-effective and environmentally benign alternative to incineration and sorption technologies.

Acknowledgement

This work was supported by grant no. R05-2001-000-01275-0 from the Basic Research Program of Korea Science and Engineering Foundation, Republic of Korea.

References

[1] S. Zechmeister-Boltenstern, K.A. Smith, Ethylene production and decomposition in soils, Biol. Fertil. Soils 26 (1998) 354–361.
[2] F.B. Abeles, P.W. Morgan, M.E. Saltweit Jr., Ethylene in Plant Biology, 2nd ed., Academic Press, Inc., San Diego, CA, USA, 1992.
[3] M. Sherman, Control of ethylene in the postharvest environment, HortScience 20 (1985) 57–60.
[4] K.A. Smith, J.M. Bremner, M.A. Tabatabai, Sorption of gaseous atmospheric pollutants in soils, Soil Sci. 116 (1973) 313–319.
[5] L. Elsgaard, Ethylene removal by a biofilter with immobilized bacteria, Appl. Environ. Microbiol. 64 (1998) 4168–4173.
[6] S.P.P. Ottengraf, Exhaust gas purification, in: H.-J. Rehm, G. Reed (Eds.), Biotechnology, vol. 8, VCH, Weinheim, 1986, pp. 427–452.
[7] W.H. Prokop, H.L. Bohn, Soil bed system for control of rendering plant odors, Air Pollut. Contr. Assoc. 35 (1985) 1332–1338.
[8] C. Lith, G. Leson, R. Michelsen, Evaluating design options for biofilters, Air Waste Manage. Assoc. 47 (1997) 37–48.
[9] J.G. Holt, N.R. Krieg, P.H.A. Sneath, J.T. Stanley, S.T. Williams, Bergey's Manual of Determinative Bacteriology, 9th ed., Williams and Wilkins, Baltimore, USA, 1994.
[10] L. Alvarez-Cohen, P.L. McCarty, Product toxicity and cometabolic inhibition modeling of chloroform and trichloroethylene transformation by methanotrophic resting cell, Appl. Environ. Microbiol. 57 (1991) 1031–1037.
[11] C.G. van Ginkel, H.G.J. Welten, J.A.M. de Bont, Growth and stability of ethene-utilizing bacteria on compost at very low substrate concentrations, FEMS Microbiol. Ecol. 45 (1987) 65–69.
[12] D.S. Hodge, V.F. Medina, R.L. Islander, J.S. Devinny, Treatment of hydrocarbon fuel vapors in biofilters, Environ. Technol. 12 (1991) 655–662.
[13] S.J. Ergas, K. Kinney, M.E. Fuller, K.M. Scow, Characterization of a compost biofiltration system degrading dichloromethane, Biotechnol. Bioeng. 44 (1994) 1048–1054.