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Assessment of ethylene removal with *Pseudomonas* strains

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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.

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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.

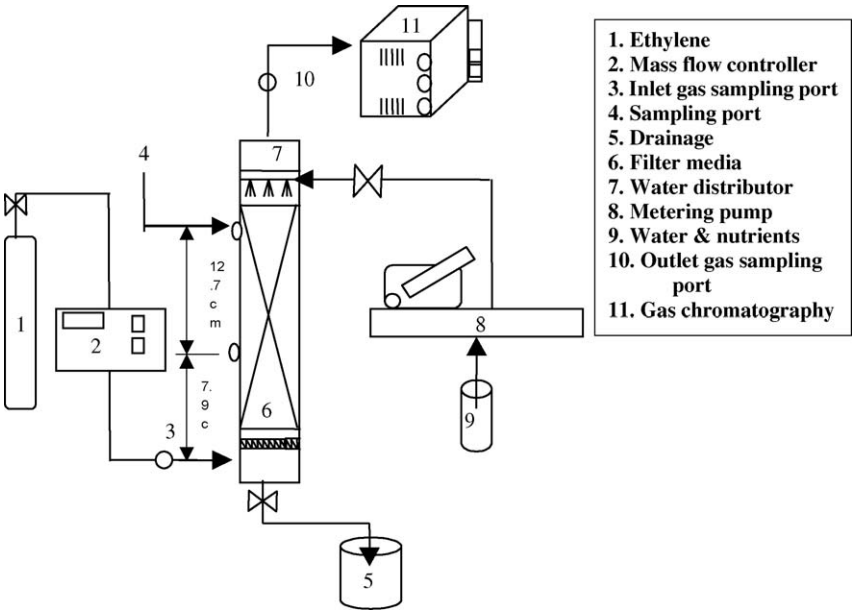

Fig. 1. Schematic diagram of a biofilter column.