ISSN 0039-5471
Suo 51(3): 181–187

Use of peat-soil for biological purification of ethylene contaminated air

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The gaseous plant hormone and air pollutant ethylene (C_2H_4) has a strong effect on plant physiological processes, such as ripening and senescence, and its removal is often required from contaminated air. This study tested the efficiency of indigenous microorganisms in horticultural peat-soil to purify C_2H_4 contaminated air under biofilter conditions. Peat-soil, acclimated to C_2H_4 removal, was placed in a biofilter (687 cm^3) and subjected to an air flow (73 mL min^{-1}) with ~117 ppm C_2H_4 (ppm, parts per million; equivalent to µL L^{-1}). C_2H_4 was removed to a lowest level of 0.034 ppm after operation of the biofilter for 12 days at 26°C. This corresponded to a C_2H_4 removal efficiency of > 99.9% and a specific C_2H_4 removal rate of 6.4 µg C_2H_4 g^{-1} dry wt soil h^{-1} (wt, weight). However, this efficient C_2H_4 removal was only transient (4 days), and during day 16 to 21, the C_2H_4 removal efficiency decreased to 51%. In contrast to this result, it was previously found that, under comparable biofilter conditions, cultivated ethylene-oxidizing bacteria were able to survive and efficiently remove C_2H_4 for at least 75 days. Thus, prolonged and efficient purification of highly C_2H_4 contaminated air by horticultural peat-soil under biofilter conditions apparently depended on bacterial inoculation.

Key words: Bacteria, biofilter, ethylene, microbiology, soil

INTRODUCTION

In Europe, including Russia, approximately 37.5 million m^3 of peat is annually processed for horticultural purposes, such as growing media for ornamental plants (Schmilewski 1996). The best substrate is weakly decomposed *Sphagnum* peat, which has a range of chemical, physical and biological properties, that are advantageous to plant growth (Reinikainen 1996).

A biological aspect of horticultural peat-soil, that has recently been studied, is the capacity to remove the gaseous plant hormone and air pollutant C_2H_4 (Elsgaard 1998, Elsgaard & Andersen 1998). C_2H_4 has a strong effect on physiological processes, such as ripening and senescence (Abeles et al. 1992), and C_2H_4 removal is often required from storage facilities for horticultural produce and from industrial gas emissions (Knee et al. 1985, Sherman 1985, de Heyder et al. 1994, Jack et al. 1997). Previously, a peat-soil biofilter was described (Elsgaard 1998), which removed C_2H_4 from the 100-ppm-range to concentrations near the threshold level for plant hormonal activity (0.01 to 0.1 ppm). This biofilter was based on ethylene-oxidizing bacteria, that were grown in mass culture and immobilized (inoculated) on horticultural peat-soil. Other studies have shown, however, that indigenous microorganisms in peat-soil may similarly be efficient C_2H_4 removers (Els-

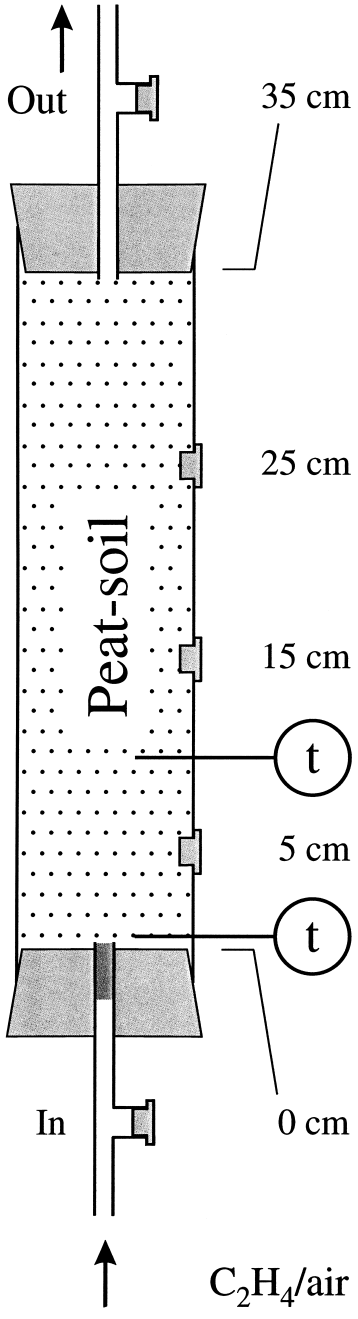

Fig. 1. Biofilter setup where C_2H_4 contaminated air was passed through a biofilter with acclimated, C_2H_4 consuming peat-soil. Gas samples for measurement of C_2H_4 were sampled through butyl rubber stoppers at the inlet (i.e., 0 cm soil depth), at 5, 15 and 25 cm soil depth, and at the outlet (i.e., 35 cm soil depth). Temperatures were measured by temperature probes (t) permanently inserted at 1 and 10 cm soil depth.

gaard & Andersen 1998). In the present study, it was tested whether bacterial inoculation of peat-soil was necessary to obtain an efficient C_2H_4 removal under biofilter conditions or if a similar performance could be accomplished by indigenous microorganisms in the peat-soil. The performance of the biofilter was tested at a high C_2H_4 level (~117 ppm), which could occur, for example, in industrial waste gases.

MATERIALS AND METHODS

Acclimated peat-soil

Four hundred grams of fresh horticultural peat-soil (Pindstrup Blend 2, Pindstrup Mosebrug, Denmark) was mixed with 0.4 L of water, and the indigenous soil microorganisms were acclimated to C_2H_4 removal. This was done by incubation of the soil (final dry matter content, 23.1%) in a gas-tight 5.5-L glass bottle with ~500 ppm C_2H_4 in the headspace air (*cf.* Elsgaard & Andersen 1998). Through a butyl rubber stopper, gas samples (0.5 mL) for C_2H_4 analysis were withdrawn regularly during incubation at room temperature (~ 20°C). After depletion of the C_2H_4 pool, new C_2H_4 (~500 ppm) was added for seven successive depletions. Hereafter the soil was stored at 2°C for 10 weeks until used. Before the biofilter experiment the depletion of new C_2H_4 (~500 ppm) was tested with a soil sample equivalent to 103 g dry wt.

Biofilter experiment

The acclimated peat-soil was loosely packed (~ 0.13 g dry wt cm^{-3}) in a biofilter (height, 35 cm, diameter 5 cm; see Fig. 1), that was subjected to a constant flow of air (73 mL min^{-1}) with ~117 ppm C_2H_4. The gas flow was controlled by two mass flow controllers and, before reaching the biofilter, the gas was humidified by being bubbled through distilled water (Elsgaard 1998). The pseudo-residence time of the biofilter was 9.4 min.

During operation for 21 days at 26°C (in a thermostatted incubator), the C_2H_4 removal efficiency was determined from consecutive measurements of C_2H_4 concentrations at the biofilter

inlet and outlet. Generally, three gas samples (20 mL) were withdrawn from the biofilter inlet and outlet at each sampling occasion. Additional gas sampling at biofilter soil depth of 5, 15 and 25 cm was done regularly (in duplicate) through inserted butyl rubber stoppers (Fig. 1). During operation, temperatures in the center of the biofilter (at 1 and 10 cm soil depth) were verified with two permanently installed digital thermometers with stainless steel penetration probes (Fig. 1). Further details of the biofilter operation have been described elsewhere (Elsgaard 1998).

Ethylene analysis

C_2H_4 was quantified using a Shimadzu GC-14B with a flame ionization detector. Gases were separated on a Poropak Q (100–120 mesh) column operated at 95°C. During acclimation of peat-soil, gas samples of 0.5 mL were injected by use of a 1-mL gas-tight syringe. During the biofilter experiments, gas samples were injected through a 2.5-mL sample loop, that was purged with a sample volume of 20 mL. With the latter configuration the C_2H_4 detection limit was 0.013 ppm for a signal-to-noise ratio of 3.

Calculations

Specific C_2H_4 removal rates (i.e., per g dry wt soil) during the acclimation of peat-soil were calculated according to:

$$SR = HR \times V \times \rho(C_2H_4) \times M^{-1} \quad (1)$$

where SR is the specific C_2H_4 removal rate (μg C_2H_4 g^{-1} dry wt soil h^{-1}), HR is the headspace removal rate (μL C_2H_4 L^{-1} h^{-1}), V is the headspace volume (~5 L), $\rho(C_2H_4)$ is the density of C_2H_4 at 20°C (1.16 μg μL^{-1}), and M is the soil dry wt (103 g).

C_2H_4 removal efficiencies (RE) during biofilter operation was calculated as:

$$RE = (1 - [C_{out}/C_{in}]) \times 100\% \quad (2)$$

where C_{out} and C_{in} are the biofilter outlet and inlet C_2H_4 concentrations, respectively.

Specific C_2H_4 removal rates for the biofilter, and the individual biofilter segments (i.e., 0–5 cm, 5–15 cm, 15–25 cm and 25–35 cm), were calculated as:

$$SR(s) = \Delta C_2H_4(s) \times F \times \rho(C_2H_4) \times M(s)^{-1} \quad (3)$$

where SR(s) is the specific C_2H_4 removal rate by a given segment (s), $\Delta C_2H_4(s)$ is the difference between the inlet and outlet C_2H_4 concentration of the segment (μL C_2H_4 L^{-1}), F is the flow rate (4.4 L h^{-1}), $\rho(C_2H_4)$ is the density of C_2H_4 at 26°C (1.14 μg μL^{-1}), and M(s) is the soil dry wt of the segment (26.1 g per 10-cm segment).

Finally, the elimination capacity of the biofilter (i.e., the amount of C_2H_4 removed per unit of reactor volume and time) was calculated according to:

$$EC = (C_{in} - C_{out}) \times F \times \rho(C_2H_4) \times V_r^{-1} \quad (4)$$

where EC is the elimination capacity (g C_2H_4 m^{-3} day^{-1}), C_{in}, C_{out}, F and $\rho(C_2H_4)$ are as defined above and V_r is the biofilter reactor volume (687×10^{-6} m^3).

RESULTS

Acclimated peat-soil

In the fresh horticultural peat-soil, C_2H_4 removal proceeded after an acclimation period of ~11 days, as defined as the time required for a 10% reduction in the initial C_2H_4 concentration (Fig. 2). New C_2H_4 was depleted without further acclimation and, after eight successive C_2H_4 additions, the depletion proceeded at a specific rate of 1.13 μg C_2H_4 g^{-1} dry wt soil h^{-1} (data not shown). After storage of the acclimated soil at 2°C, a reacclimation period of ~3 days preceded the C_2H_4 depletion, which then occurred at a headspace rate of ~8 ppm h^{-1} (Fig. 2). This corresponded to a specific C_2H_4 removal rate of 0.45 μg C_2H_4 g^{-1} dry wt soil h^{-1}.

Biofilter experiment

During the biofilter experiment (21 days), the flow rate ranged from 71.4 to 73.9 mL min^{-1} with a mean ± SD of 72.6 ± 0.8 mL min^{-1} (n = 15). The inlet C_2H_4 concentration ranged from 112 to 123 ppm with a mean ± SD of 117 ± 3 ppm (n = 15).

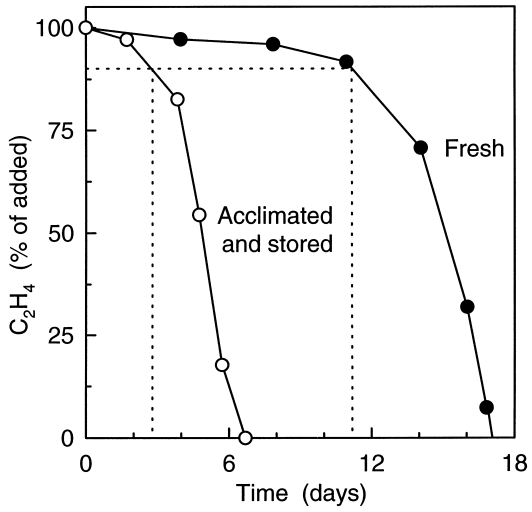

Fig. 2. Removal of ~500 ppm C_2H_4 in fresh horticultural peat-soil (●) and in acclimated peat-soil after storage at 2°C (○). Dotted lines indicate the acclimation time required for removal of 10% of the initial C_2H_4 concentration.

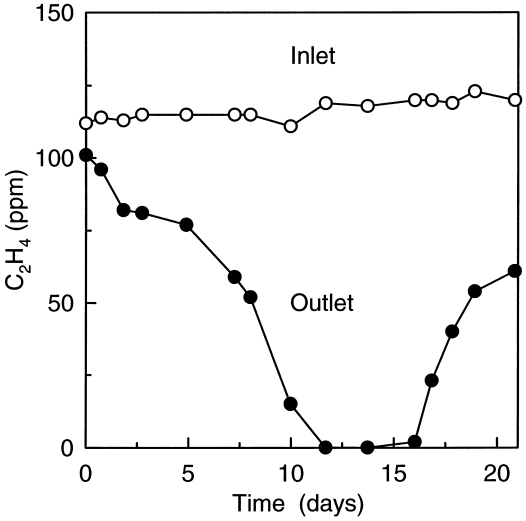

Fig. 3. C_2H_4 concentration at the biofilter inlet (○) and outlet (●) during operation for 21 days with contaminated air (~117 ppm C_2H_4). Data are the mean of three samples. Standard deviations ranged from 0.03 to 1.01 ppm C_2H_4.

Temperatures at 1-cm depth in the biofilter ranged from 25.8 to 26.0°C (n = 15) and temperatures at 10–cm depth ranged from 26.1 to 26.3°C (n = 15).

Measurements of the outlet C_2H_4 concentration after 1 h of operation (101 ppm) showed that 10% of the incoming C_2H_4 was initially removed (Fig. 3). Then, during 12 days, the outlet concentration gradually decreased to only 0.034 ppm, corresponding to a C_2H_4 removal efficiency of more than 99.9%. For the entire biofilter, this was equal to an average specific C_2H_4 removal rate of 6.4 µg C_2H_4 g^{-1} dry wt soil h^{-1} and an elimination capacity of 20 g C_2H_4 m^{-3} day^{-1}. However, after this efficient C_2H_4 removal for two days, the biofilter gradually lost the capacity and the outlet concentration started to increase (Fig. 3). Thus, at the end of the experiment (21 days), the outlet C_2H_4 concentration was 61 ppm, corresponding to a removal efficiency of 51%, a specific removal rate of 3.4 µg C_2H_4 g^{-1} dry wt soil h^{-1}, and an elimination capacity of 11 g C_2H_4 m^{-3} day^{-1}.

C_2H_4 measurements at different biofilter soil depth, showed that, during the first 10 days, the C_2H_4 concentration decreased linearly along the length of the biofilter (Fig. 4). This demonstrated that all soil layers contributed equally to the C_2H_4 removal and, therefore, the specific C_2H_4 removal rates for the individual segments were similar during this phase (Fig. 5). At day 14, when the biofilter removal efficiency was more than 99.9%, most of the incoming C_2H_4 was removed within the first 15 cm of the biofilter (Fig. 4). This was due to an increase in the specific C_2H_4 removal rates in the first two segments (15 cm) of the biofilter (Fig. 5), which, due to the depletion of C_2H_4, caused a decrease in the C_2H_4 removal rate in the subsequent biofilter segments (15–35 cm). At day 16 to 21, when the efficiency of the biofilter started to decrease, the C_2H_4 concentration again decreased linearly along the length of the biofilter (Fig. 4) and the specific C_2H_4 removal rates were rather similar for the individual segments (Fig. 5).

DISCUSSION

When peat-soil was sterilized (autoclaved on two consecutive days) no removal of C_2H_4 occurred in acclimation experiments. This showed that microorganisms were responsible for the C_2H_4 removal. Also, previous studies have shown that soil microorganisms may act as sinks of atmospheric C_2H_4 and notably reduce the emission of C_2H_4 produced in soil (Abeles et al. 1971, Smith et al. 1973, Sawada & Totsuka 1986, Zechmeister-Boltenstern & Smith 1998). Dynamics of C_2H_4 in

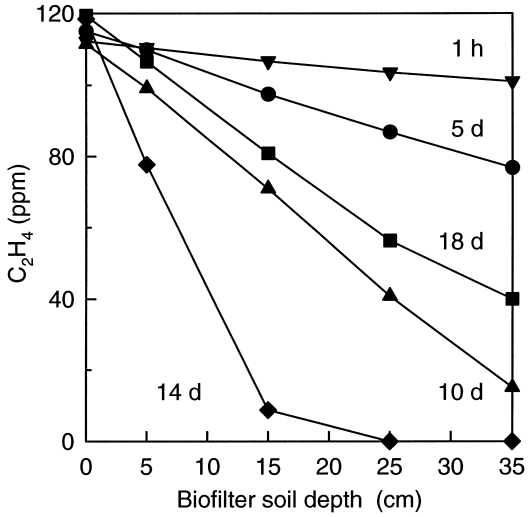

Fig. 4. C_2H_4 concentration at different depths of the biofilter after operation with ~117 ppm C_2H_4 for 1 h (▼), 5 days (●), 10 days (▲), 14 days (◆) and 18 days (■). Soil depths of 0 and 35 cm represent the biofilter inlet and outlet, respectively. Data are the mean of two or three samples. Standard deviations ranged from 0.03 to 2.92 ppm C_2H_4.

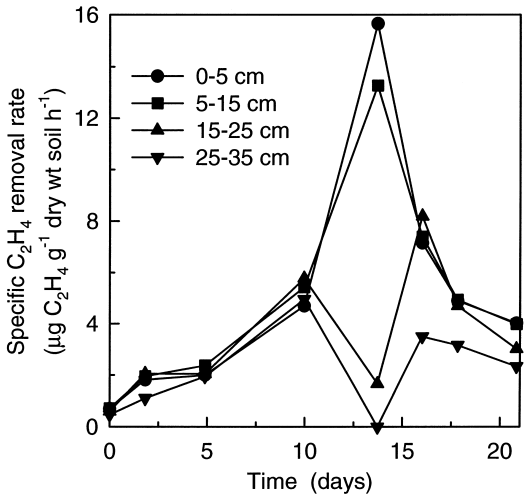

Fig. 5. Specific C_2H_4 removal rates by individual segments of the biofilter, i.e., 0–5 cm (●), 5–15 cm (■), 15–25 cm (▲) and 25–35 cm (▼), during operation for 21 days with ~117 ppm C_2H_4.

natural peatlands have not been studied so far, however, but C_2H_4 removal has been reported for soil amended with peat (Frye et al. 1992) and peat-based growing media for horticulture (Turner et al. 1988, Elsgaard & Andersen 1998).

During the present experiments, the specific C_2H_4 removal rates in the acclimated peat-soil (1.13 μg C_2H_4 g^{-1} dry wt soil h^{-1}) were within the same order of magnitude as the rates (0.37 to 0.45 μg C_2H_4 g^{-1} dry wt soil h^{-1}) reported by Elsgaard and Andersen (1998). The lower C_2H_4 removal rate (0.45 μg C_2H_4 g^{-1} dry wt soil h^{-1}), and the re-acclimation period of 3 days, that was observed after 10 weeks of storage at 2°C, showed that the activity of the ethylene-consuming soil microorganisms decreased (but was not eliminated) after such C_2H_4 starvation.

Under biofilter conditions, the acclimated peat-soil could be adapted to efficient removal (> 99.9%) of highly C_2H_4 contaminated air. The time course of this adaptation, and the resulting specific C_2H_4 removal rates, were in reasonable agreement with previous results obtained with an inoculated peat-soil biofilter that was operated under similar conditions (Elsgaard 1998). However, a major difference between the two biofilter experiments was the low operational stability that was presently found for the C_2H_4 removal in the acclimated peat-soil. Thus, Elsgaard (1998) previously showed that for more than 75 days of constant operation, the inoculated biofilter was able to reduce an inlet C_2H_4 concentration of 117 ppm to less than 0.04 ppm C_2H_4 at the outlet.

During the first 10 days of the present experiment all soil layers increased the specific C_2H_4 removal rate to a similar extent. This indicated a stable growth throughout the soil column of ethylene-consuming bacteria, which may derive their energy and carbon from oxidation of C_2H_4 (Hartmans et al. 1989). After 10 days of operation, however, the indigenous microorganisms in the first 15-cm segment of the present biofilter showed an increased C_2H_4 removal rate, which indicated a preferential growth of ethylene-consuming bacteria in these soil layers. Yet, the increased C_2H_4 removal rate was transient and only persisted for few days. It is not clear which factors caused the observed decrease in the specific C_2H_4 removal rate in the first 15-cm soil layer after 16 days of operation. Depletion of (unknown) specific nutrients supplied by the peat-soil could be one possibility. If so, this would reflect that such growth requirements were more pronounced for the indigenous ethylene-consuming microflora than for

the bacteria used in the experiment with inoculated peat-soil (Elsgaard 1998). Changes in the moisture content of the peat-soil could possibly also influence the C_2H_4 removal efficiency. Yet, no adverse effects of such changes were seen during the previous studies with inoculated peat-soil under similar biofilter conditions (Elsgaard 1998). Another possibility was that a stable microbial community structure was not reached during the present experiments, or possibly product inhibition could occur if all C_2H_4 was not completely oxidized to CO_2. The development of anoxic zones in the biofilter, which could impede the oxidation of C_2H_4, was unlikely because the studies with an inoculated biofilter demonstrated that O_2 levels of 15.2% were present in the outlet air (Elsgaard 1998).

The use of peat-soil for environmental purposes, such as cleaning of waste water and biological air purification, has previously been described (Mutka 1996). Likewise, waste gas purification by biofiltration has been applied for removal of several air pollutants including C_2H_4 (Leson & Winer 1991, Ottengraf & Diks 1992, Elsgaard, 1999). However, these biofilter techniques have generally been based on cultivated bacteria inoculated on various support materials (e.g., van Ginkel et al. 1986, De Heyder et al. 1994). Thus, there are only few reports on C_2H_4 biofilters based on activation of indigenous microbial soil populations. Van Ginkel et al. (1987) showed that ethylene-consuming bacteria in compost could be enriched under biofilter conditions. The acclimation time ranged from ca. 1 to 4 weeks during biofilter operation with 2, 50 and 200 ppm C_2H_4 in the inlet air. Under biofilter conditions, the maximal C_2H_4 removal efficiency was ca. 80% and the maximal elimination capacity corresponded to 20 g C_2H_4 m^{-3} day^{-1}, as calculated from the data of van Ginkel et al. (1987). Frye et al. (1992) reported the removal of various hydrocarbons in a sandy loam soil amended with sand, peat and compost. When 0.2 ppm C_2H_4 was injected into aquaria with acclimated soil-bed reactors, a C_2H_4 removal efficiency of 99.1 to 100% was obtained during 4 days of air circulation (Frye et al. 1992). This corresponded to an elimination capacity of ~0.06 g C_2H_4 m^{-3} day^{-1} (Frye et al. 1992). Although the performance of soil-bed reactors can only be directly compared if they have been operated under similar conditions (e.g., C_2H_4 inlet concentration and biofilter volume-to-flow ratio), it was remarkable that a similar elimination capacity was obtained with the present biofilter and the bioreactor of van Ginkel et al. (1987). Stable C_2H_4 removal efficiencies were not reached during operation periods of 3 to 8 weeks in the study of van Ginkel et al. (1987), but on the other hand no decrease in the removal efficiencies was indicated.

In conclusion, the present study demonstrated that indigenous peat-soil microorganisms could be acclimated to complete removal of ~117 ppm C_2H_4 under biofilter conditions. Yet, this capacity persisted only for a brief period of a few days. The cause of the low operational stability remained uncertain and, although the observed behavior may be exceptional, the experiment demonstrated an unreliable performance of the acclimated peat-soil biofilter. In contrast to this result, it was previously found that, under similar biofilter conditions, cultivated ethylene-oxidizing bacteria were able to survive and efficiently remove C_2H_4 for an extended period of at least 75 days (Elsgaard, 1998). Therefore, efficient and stable purification of highly C_2H_4 contaminated air by use of peat-soil biofilters seemed to depend on bacterial inoculation.

ACKNOWLEDGEMENTS

I appreciate the excellent technical assistance of Gitte Hastrup Andersen and I thank Sophie Zechmeister-Boltenstern and two journal reviewers for critically reviewing the manuscript.

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Received 1.10.1999, accepted 27.1.2000