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TOLUENE BIOFILTRATION AS AFFECTED BY RYEGRASS ROOTS

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Abstract

Gaseous toluene biofiltration as affected by ryegrass (*Lolium perenne* L.) roots was investigated in this study. The results revealed that the populations of bacteria, actinomycetes were larger in the ryegrass rhizosphere than in the bulk soil since ryegrass provided root exudates as major nutrients available for the microorganisms. An increase in the microbial populations in a ryegrass-growing biofilter would significantly stimulate toluene biodegradation in comparison with a vegetation-free biofilter. The stimulation of toluene biofiltration by ryegrass roots increased with enhancing temperature in the range of 5-35°C. Elevating light intensity can increase toluene removal efficiency in the ryegrass-growing biofilter while the efficiency in the vegetation-free biofilter was invariable with the change of light intensity.

Key words: biofiltration, microorganisms, ryegrass, toluene

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1. Introduction

With the rapid development of industry, a great amount of volatile organic compounds (VOCs) are emitting into atmosphere. Since these VOCs are directly harmful to human health and convert into atmospheric aerosols further playing a role in air quality and climate change, much attention is paid to treatment technology for VOCs removal. Of these VOCs, toluene is widely released from fuels, solvents and industrial products at a rate of millions of tons per year (Huang et al., 2016; Zilli et al., 2001). Inhalation exposure to toluene for humans may cause central nervous system depression, and damages liver, kidneys, and lungs (Singh et al., 2010). Thus, toluene removal from air has been attempted by many different methods (Delhoménie et al., 2002; Kong et al., 2013; Moussavi and Khosravi, 2017; Popova et al., 2009; Tham et al., 2011). Among these treatment methods for toluene-contaminated air, biological treatment is regarded as a cost-effective and environment-friendly technique as toluene is

completely degraded into harmless final products in the treatment method.

Widely reported studies indicated that high removal efficiencies attained for toluene biodegradation with high volumes and low concentrations (Dixit et al., 2012; Park and Jung 2006; Rahul et al., 2013; Rene et al., 2012). Compared with these biodegradation methods, a new biofiltration of gaseous toluene by microorganisms associated with plant roots has been reported by Xu et al. (2013), which demonstrated that toluene biofiltration rate was enhanced by ryegrass (*Lolium perenne* L.) planted in the packing materials. Although plant-aided bioremediation includes some mechanisms: phytoextraction, phytopumping, phytostabilization, phytotransformation, phytovolatilization, and rhizodegradation (Susarla et al., 2002), the rhizodegradation is a major contributor to the removal of biodegradable organic pollutants (Xu et al., 2010, 2011). The symbiotic relationships between plants and microorganisms result in high microbial activities in the rhizosphere since plants moderate the rhizosphere

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to ideal conditions for microorganisms growing and root exudates provide nutrients to stimulate microbial activities (Susarla et al., 2002).

Ryegrass with a dense fibrous root system, which provides a larger surface area for the colonization of microorganisms, is often selected as the model plant for the treatment of organic pollutants (Rezek et al., 2008; Tang et al., 2010; Xie et al., 2012). The purpose of this study was to investigate the effect of ryegrass planted in the packing materials on toluene biofiltration, and toluene biofiltration in response to temperature and illumination was also involved. Bacteria, fungi and actinomycetes populations in the packing materials were further investigated to provide a reasonable explanation for the effect of ryegrass roots on toluene biofiltration.

2. Material and methods

2.1. Toluene biofiltration

In this study two treatments were constructed: an unplanted treatment containing microorganisms without ryegrass, and a planted treatment with ryegrass and microorganisms. Both biofilter systems consisted of two major components: a biofilter and a gaseous toluene generation system (Biofilter A, Fig. 1). Each biofilter was made of stainless steel column with an inner diameter of 10 cm and a total height of 70 cm, equipped with a 30 cm high plexiglass top. Two ports were set 0 and 2.5 cm above each biofilter

bottom for water drainage and toluene-polluted air release, respectively. A mixture of lava rock, peat and compost (2/2/1, w/w/w) packing to 60 cm high was supported by a stainless steel screen at 5 cm above each biofilter bottom. The packing materials had a dry density of $464 \text{ kg}\cdot\text{m}^{-3}$, a pH of 7.1. Ryegrass was planted in a biofilter at ca. 2000 seedlings per square meter. Four sampling ports were located at 0, 20, 40, 60 cm bed heights, respectively. Each sampling port was connected to a perforated stainless steel tube with an inner diameter of 1 cm and a length of 10 cm, crossing the biofilter horizontally as a gas sampling pipe. Both biofilters were put into an illumination incubator (GXZ-280B, Ningbo Jiangnan Instrument Factory, China), which controlled the light intensity and the temperature during the whole study.

A low flow rate of nitrogen gas carrying toluene vapor from a toluene liquid vessel to a homogenization vessel was adequately mixed with a high flow rate of air, which was humidified to 50-60% before mixing. The target toluene concentration was obtained by regulating both the nitrogen flow and the air flow with mass flow meters. In this study, the total gas flow rate for a biofilter was 2 L min^{-1} , corresponding to an empty bed residence time (EBRT) of 2.4 min. After the ryegrass seeds were sown in the biofilter, both biofilters were running with 0.02 g m^{-3} influent toluene for 90 days to acclimatize microorganisms to the new process conditions. Then the packing materials were loosened for toluene biofiltration.

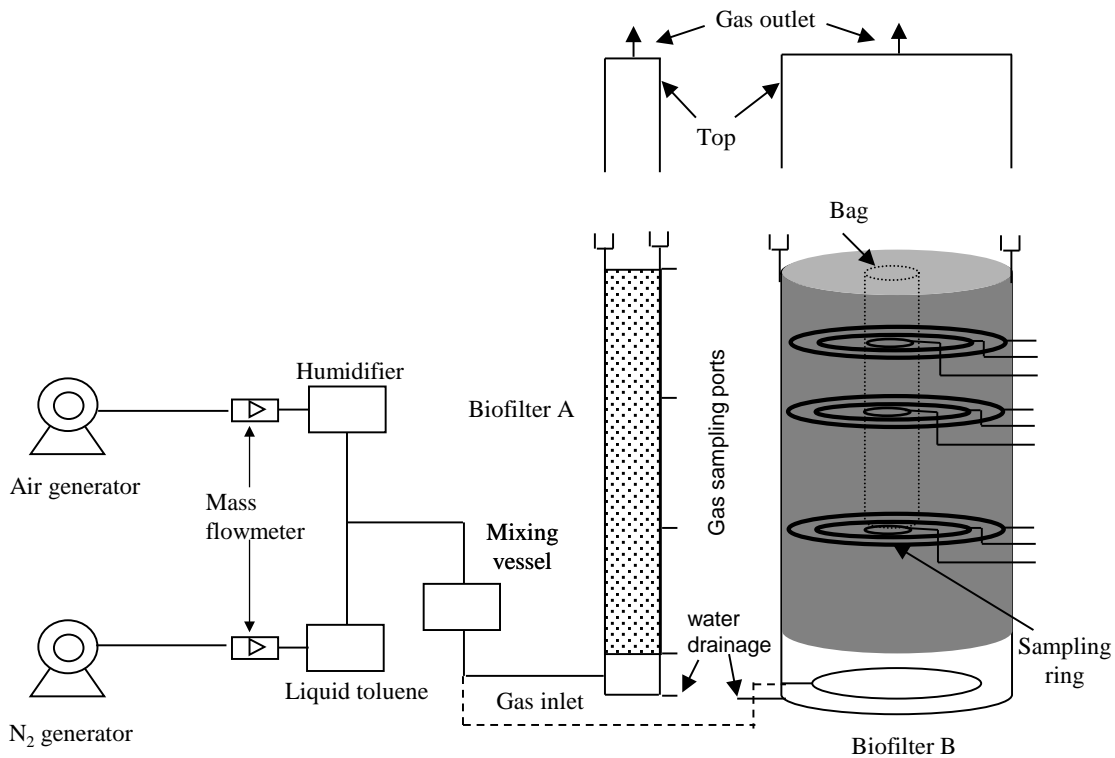


Fig. 1. Schematic diagram of the biofilter system. Biofilter A for the toluene biofiltration experiment and Biofilter B for microorganism measurement. The unplanted biofilter without any vegetation and the planted biofilter planted with ryegrass in the bed

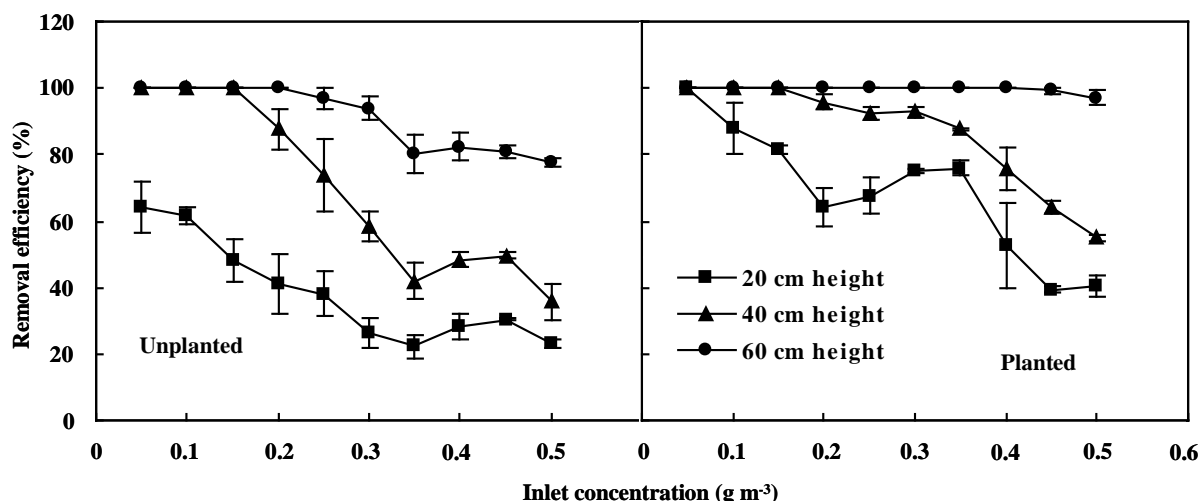


Fig. 2. Toluene removal efficiencies for the unplanted and the planted biofilters at 20, 40 and 60 cm high beds with inlet toluene concentrations. The vertical bars are standard errors for three runs of experiments

During the whole study, 150 mL modified Hoagland nutrient solution (945 mg L⁻¹ Ca(NO₃)₂, 506 mg L⁻¹ KNO₃, 80 mg L⁻¹ NH₄NO₃, 136 mg L⁻¹ KH₂PO₄, 493 mg L⁻¹ MgSO₄) was applied in each biofilter every week, and enough deionized water was sprayed into the biofilter to hold ca. 35% volumetric moisture content in the packing materials every two days.

Firstly, toluene removal was measured at 25°C and 12 000 Lx light intensity (12h in light) within 0.02-0.5g·m⁻³ inlet concentrations after the preincubation. Secondly, the effect of temperature on toluene removal was investigated at 0.5g·m⁻³ inlet concentration and 12 000 Lx light intensity (12h in light) within 5-35°C. Subsequently, the effect of illumination on toluene removal was investigated at 0.5g·m⁻³ inlet concentration and 25°C within 4 000-12 000 Lx light intensity (12 h in light). The three experiments have been conducted for three runs, respectively. The pressure drop across each biofilter bed was less than 120 Pa during each experiment. The incubation lasted 3-5 days to obtain a steady-state in each biofilter during each conditional test. Toluene concentration at each gas sampling port was determined daily with a gas chromatograph (Younglin ACME 6100 series, Korea) equipped with a flame ionization detector.

2.2. Microorganism measurement

A biofilter was made of cylindrical plexiglass chamber with an inner diameter of 40 cm and a total height of 70 cm, equipped with a 30 cm high plexiglass top (Biofilter B, Fig. 1). A plexiglass screen was placed 5 cm above the biofilter bottom and the mixed materials were packed to 60 cm high. A cylindrical bag (made of 500-mesh nylon net) with a diameter of 10 cm and a height of 40 cm was placed in the center of the biofilter, from 20 cm to 60 cm bed height. The bag was filled with the same packing materials as those in the biofilter. Ryegrass was planted in the bag at ca. 2000 seedlings per square

meter. The zones in the bag, in the radius range from 5 cm to 10 cm and in the radius range from 10 cm to 20 cm were considered as rhizosphere, transition zone and bulk soil, respectively. Three sampling rings made of perforated Teflon pipes with an inner diameter of 1 cm were horizontally set 20, 40, 50 cm bed heights in each zone, respectively. The gaseous toluene generation system, the property of packing materials, nutrient and water applications, the EBRT and the preincubation were the same as Biofilter A described in section 2.1. The biofilter was also put into an illumination incubator (GXZ-280B, Ningbo Jiangnan Instrument Factory, China), which controlled the light intensity and the temperature during the whole study.

Toluene concentrations in the rhizosphere, the transition zone and the bulk soil were measured at 25°C and 12 000 Lx light intensity (12h in light) within 0.35-0.55 g m⁻³ inlet concentrations after the preincubation for three experimental runs. At the end of this experiment, 1 g of packing material samples were collected from each zone at 20, 40, 50 cm bed heights. 9 mL sterile water solution containing 0.8% NaCl was added to each sample. This suspension was shaken vigorously for 15 min and serially diluted with sterile 0.8% NaCl solution for bacteria, actinomyces and fungi determination. Beef extract peptone agar media, Gause's No. 1 synthetic agar media and Martin's agar media were used for bacteria, actinomyces and fungi, respectively (Xue et al., 2013). Petri dishes were incubated for 3-7 days at 28 °C prior to counting the number of microorganisms. The enumeration of viable microbial population was recorded as the number of colony forming units (cfu) per gram of dry packing materials. The incubation was performed in triplicate.

3. Results

3.1. Toluene biofiltration with inlet concentration

Toluene removal efficiencies for the unplanted and the planted biofilters with inlet toluene concentrations are shown in Fig. 2.

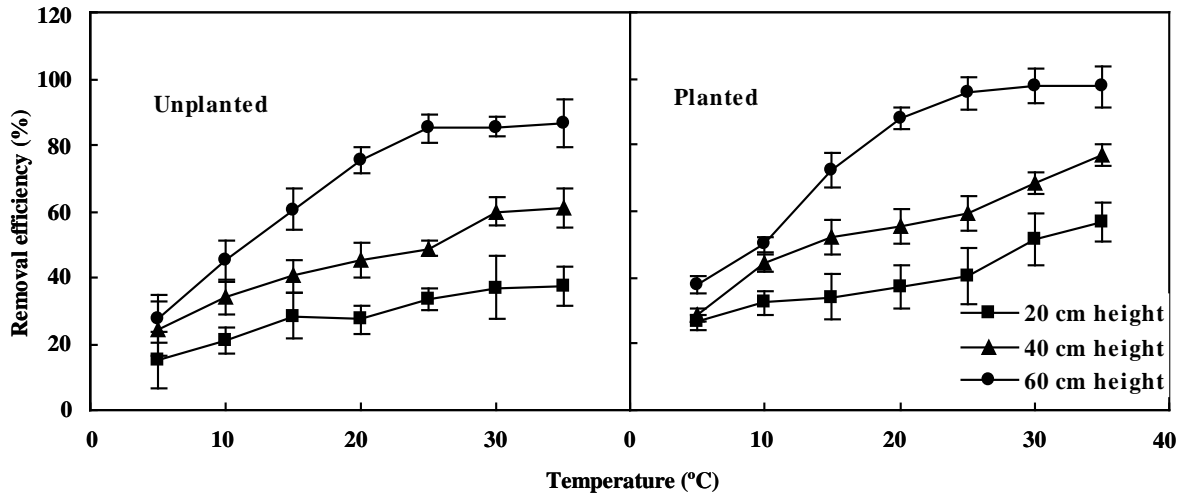


Fig. 3. Toluene removal efficiencies for the unplanted and the planted biofilters at 20, 40 and 60 cm high beds with temperature. The vertical bars are standard errors for three runs of experiments

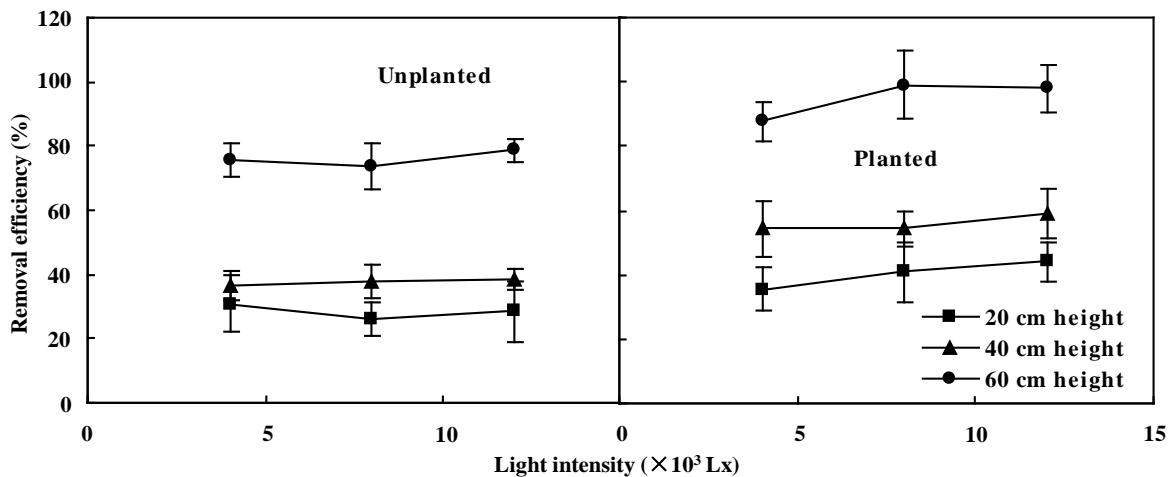


Fig. 4. Toluene removal efficiencies for the unplanted and the planted biofilters at 20, 40 and 60 cm high beds with light intensity. The vertical bars are standard errors for three runs of experiments

Toluene was completely removed by the unplanted and the planted biofilters in less than 0.2 and 0.4 g·m⁻³ inlet concentrations, respectively. At the same inlet concentration, the removal efficiency increased with enhancing the bed height for both biofilters. At the same bed height, toluene removal efficiency decreased with enhancing the inlet concentration for both biofilters. Toluene was mainly removed by the bottom parts of both biofilter beds in low inlet concentrations, e.g., ca. 50% and 80% toluene removal efficiencies for the 20 cm bottom beds of the unplanted and the planted biofilters in less than 0.15 g·m⁻³ inlet concentration, respectively. In contrast, toluene removal efficiencies for the 20 cm top beds of the unplanted (42%) and the planted biofilters (43%) were more than those for the respective 20 cm bottom parts at 0.5 g·m⁻³ inlet concentration. Compared with the unplanted biofilter, toluene removal efficiencies for the corresponding bed parts of the planted biofilter were higher ($P < 0.05$) in all inlet concentrations. At 0.5 g·m⁻³ inlet concentration, especially, the planted biofilter

removed almost all toluene while the unplanted biofilter removed 77% toluene. Additionally, the decrease in toluene removal efficiency for the unplanted biofilter was sharper than that for the planted biofilter when inlet concentration was gradually enhanced.

3.2. Toluene biofiltration in response to temperature

Toluene biofiltration in response to temperature at 0.5 g·m⁻³ inlet concentration is presented in Fig. 3.

Toluene biofiltration is very sensitive to temperature. The unplanted and the planted biofilters removed 27% and 38% toluene at 5 °C while both biofilters removed 86% and 98% toluene at 35 °C, respectively. When enhancing temperature from 5 to 35 °C, toluene removal efficiencies for the top parts of both biofilters increased more than those for the bottom parts. At the same temperature, toluene removal efficiencies for the planted biofilter were significantly ($P < 0.01$) higher than those for the unplanted biofilter.

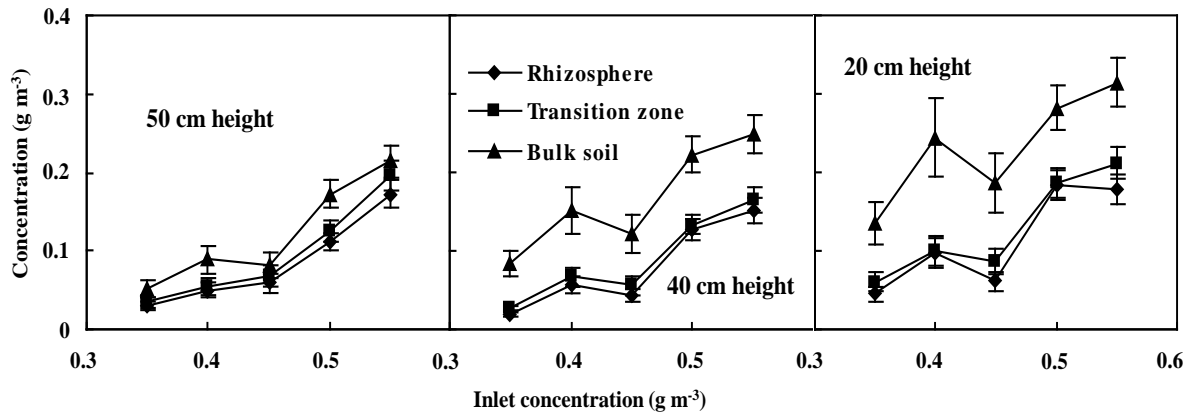


Fig. 5. The vertical and horizontal distributions of toluene concentration in Biofilter B in 0.35-0.55 g·m⁻³ inlet toluene concentrations. The vertical bars are standard errors for three runs of experiments

3.3. Toluene biofiltration in response to illumination

The illumination from 4000 to 12 000 Lx light intensity in daytime hardly affected toluene removal efficiency for the unplanted biofilter (Fig. 4). Elevating light intensity from 4 000 to 8 000 Lx significantly ($P < 0.05$) increased toluene removal efficiency from 87% to 99% for the planted biofilter. Since nearly all toluene was removed for the planted biofilter at 8 000 Lx light intensity, the difference in toluene removal could not be observed when light intensity was elevated from 8 000 to 12 000 Lx.

3.4. Microorganisms in biofilters in response to ryegrass roots

The vertical and horizontal distributions of toluene concentration during the biofiltration were given in Fig. 5. Generally, the distributions of toluene concentration around ryegrass roots followed a same order of the bulk soil > the transition zone > the rhizosphere in the three layers of packing materials in 0.35-0.55 g·m⁻³ inlet concentrations. Toluene concentration gradually decreased along the height of packing materials, and the differences of toluene concentration among the bulk soil, the transition zone and the rhizosphere decreased along the height of packing materials. At 20 and 40 cm bed height, toluene concentration in the rhizosphere was significantly ($P < 0.01$) lower than those in the transition zone and the bulk soil. At 50 cm bed height, toluene concentration in the rhizosphere was significantly ($P < 0.05$) lower than that in the bulk soil, but not significantly lower than that in the transition zone.

Bacteria were the dominant species among the investigated bacteria, actinomycetes and fungi in the packing materials (Fig. 6). The number of bacteria in the rhizosphere was significantly ($P < 0.01$) more than those in the transition and bulk soils at the three heights of 20, 40 and 50 cm. The number of bacteria in the transition zone was also significantly ($P < 0.05$) more than those in the bulk soil at the height of 20 cm, 40 and 50 cm. The number of actinomycetes at the heights of 20, 40 and 50 cm all followed an order of

the rhizosphere > the transition zone > the bulk soil ($P < 0.05$). The number of fungi at the height of 50 cm followed an order of the rhizosphere > the transition zone > the bulk soil ($P < 0.01$) while the most fungi dwelled in the transition zone in contrast with the rhizosphere and bulk soil at the heights of 20 and 40 cm.

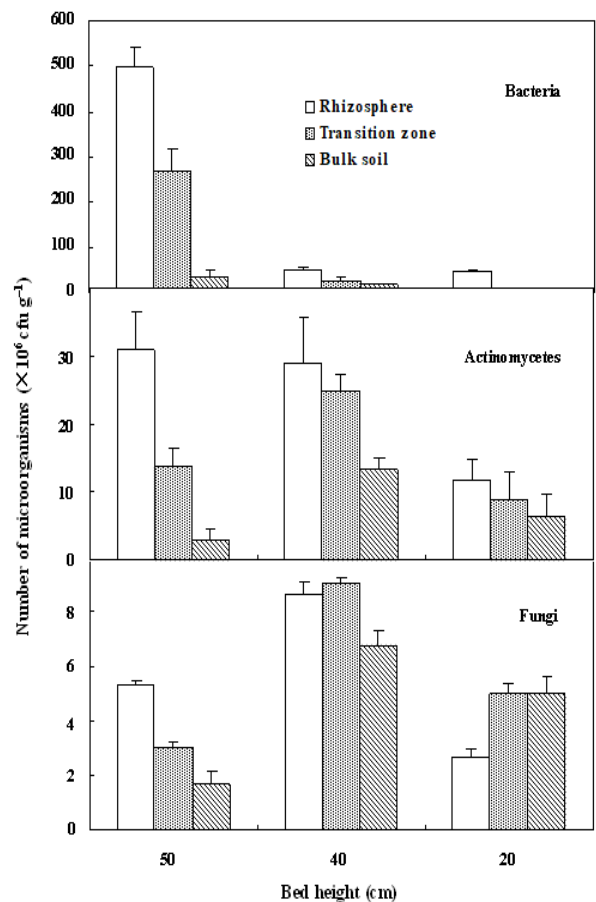


Fig. 6. The vertical and horizontal distributions of bacteria, actinomycetes and fungi in Biofilter B. The vertical bars are standard errors for three runs of experiments

As seen in Figs. 5 and 6, toluene concentration was negative related to the number of bacteria and actinomycetes in the packing materials. This

correlation was not observed for fungi dwelling in the packing materials.

4. Discussion

4.1. Toluene biofiltration stimulated by ryegrass roots

Numerous studies have reported that plant roots increased the soil microbial number and activity (Kaimi et al., 2006; Lynch and Whipps, 1990; Walker et al., 2003). Firstly, root surfaces can provide adhering space for microorganism growth, resulting in the enhanced biodegradation of organic pollutants in the rhizosphere (Banks et al., 2003). Most importantly, modified microbial composition and enhanced microbial activity occur in the rhizosphere since plants provide root exudates as nutrients available for microorganisms (Bowen and Rovira, 1999; Donnelly et al., 1994; Siciliano et al., 2003). The higher microbial activity in the rhizosphere would cause more hydrocarbon removal in comparison with the bulk soil (Günther et al., 1996). Admittedly, more toluene consumption in the rhizosphere in this study was also due to higher microbial degradation in the rhizosphere. The number of bacteria was on average 10-fold and 20-fold more than those of actinomycetes and fungi in the packing materials, respectively (Fig. 6), indicating that bacteria were the dominant flora in the biofilter. In fact, bacteria can dominate in biofilter beds since they grow more rapidly and are able to degrade more organic compounds in comparison with actinomycetes and fungi (Devinny et al., 1998; Smith, 1990). At the same height of biofilter bed, lower toluene concentration concurrent with higher bacteria and actinomycetes densities demonstrated that toluene removal from the biofilter depended mainly on bacteria and actinomycetes.

Several studies demonstrated that the loss of hydrocarbon in soils through direct plant-uptake was negligible (Kaimi et al., 2006; Reilley et al., 1996). Our previous studies also indicated that the removal of gaseous organic pollutants was mainly due to microbial decomposition rather than phytoextract when the combination of plant and microorganisms is used for the purification of gaseous organic pollutants (Xu et al., 2010, 2011). In this study, a reasonable explanation for the significant higher toluene removal in the planted biofilter than in the unplanted biofilter is that the significant larger microbial populations were in the planted biofilter since ryegrass roots nourished microorganisms, such as bacteria and actinomycetes.

As seen in Fig. 7, toluene elimination capacity for the planted biofilter was significantly ($P < 0.01$) higher than that for the unplanted biofilter within 6-13 $\text{g m}^{-3} \text{h}^{-1}$ loading rates. This phenomenon suggests that the ryegrass roots stimulated toluene degraders in the whole biofilter to remove more toluene in the planted biofilter.

Since toluene biofiltration enhanced by ryegrass is based on the stimulation of toluene degraders by ryegrass roots rather than direct

phytoextract by ryegrass, a planted biofilter can be used to remove other degradable gaseous organic pollutants. Greater specific surface area and water holding capacity in a planted biofilter may result in higher toluene removal compared to an unplanted biofilter, which need to be tested in further experiments. It should be noted that the maximum of toluene loading rate in this study is lower than those in literature (Delhoménie et al., 2002; Singh et al., 2010; Zilli et al., 2001). When an outlet toluene concentration achieves $10 \text{ mg} \cdot \text{m}^{-3}$, its concentration approaches $80 \text{ mg} \cdot \text{m}^{-3}$ at 50 cm bed height. This high concentration of toluene is phytotoxic to the roots of ryegrass. Avoiding a damage to ryegrass, the maximum of inlet toluene concentration was set at ca. $0.5 \text{ g} \cdot \text{m}^{-3}$.

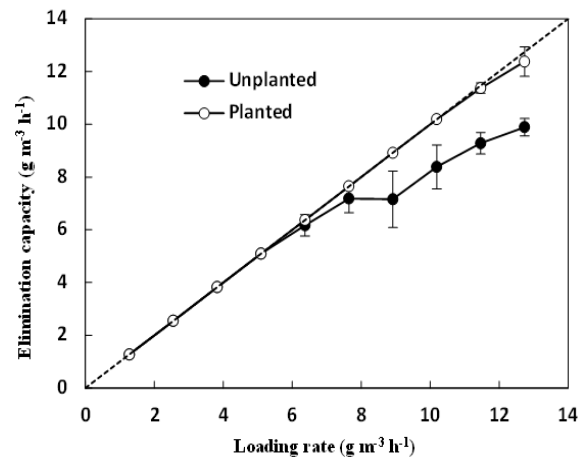


Fig. 7. Toluene elimination capacities for the unplanted and the planted biofilters. The vertical bars are standard errors for three runs of experiment

4.2. Effects of temperature and illumination on toluene biofiltration

It was reported that an increase in the biofiltration efficiencies of various pollutants with enhancing temperature (less than $40 \text{ }^\circ\text{C}$) was due to the increase in the activities of microorganisms (Kiared et al., 1997; Jorio et al., 2000; Lee et al., 2002; Vergara-Fernández et al., 2007). In this study, toluene biofiltration stimulated by enhancing temperature was also observed in the unplanted and the planted biofilters. However, significantly higher toluene removal efficiencies in the planted biofilter than in the unplanted biofilter at the same temperature indicated that toluene removal by microorganisms was aided by ryegrass roots. Vergara-Fernández et al. (2007) argued that the coefficient of Henry's Law for toluene increases with temperature, and this is associated with a reduction in the solubility of toluene in the aqueous phase and, consequently, the toluene is less readily available for the microorganisms. In the unplanted biofilter, toluene removal efficiencies for the 20, 40 and 60 cm high beds were stable at ca. 37%, 61% and 86 % in a temperature range of $30\text{--}35 \text{ }^\circ\text{C}$, respectively.

This might imply that the higher microbial activity was offset by the lower solubility of toluene with enhancing temperature in 30-35°C. In the planted biofilter, a stable toluene removal in response to the temperature in 30-35°C could not be affirmed for the 60 cm high bed since almost all toluene removed by the whole bed. However, toluene removal efficiencies for the 20 and 40 cm high beds increased continually in the temperature of 5-35°C, indicating an obvious positive effect of ryegrass on toluene biofiltration especially in a relatively high temperature.

Toluene degraders like most microorganisms are insensitive to light because they are heterotrophs, while ryegrass like other plants is sensitive to light because it is an autotroph relying on photosynthesis. Moreover, most toluene degraders dwelled inside a biofilter bed avoiding light. Therefore, a change in illumination would not obviously affect microbial densities as a result of unalterable toluene removal efficiency for the unplanted biofilter. An increase in plant photosynthesis by enhancing light intensity can result in more photosynthetically fixed organic compounds transferring to the rhizosphere through root exudates as nutrients available for microorganisms (Walker et al., 2003). Thus, the planted biofilter removed more toluene under higher light intensity (8 000 and 12 000 Lx) than under lower light intensity (4 000 Lx).

5. Conclusions

This study investigated the effect of ryegrass roots on gaseous toluene biofiltration. The results showed ryegrass roots stimulated the growths of bacteria and actinomycetes, resulting in more toluene removal in a ryegrass-growing biofilter than in a vegetation-free biofilter. When the temperature rose in the range of 5-35°C, the increase of toluene removal in the planted biofilter was higher than that in the unplanted biofilter due to nutrition of ryegrass roots for microorganisms. Elevating light intensity would stimulate ryegrass photosynthesis, resulting in more root exudates around the rhizosphere. Thus, the planted biofilter removed more toluene under higher light intensity (8 000 and 12 000 Lx) than under lower light intensity (4 000 Lx) while toluene removal in the unplanted biofilter was unchanged. This study implies that the plant-aided biofiltration might an improved way for VOCs removal.

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