Bioresource Technology 100 (2009) 5656–5663

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Removal of benzene and toluene in polyurethane biofilter immobilized with *Rhodococcus* sp. EH831 under transient loading

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ARTICLE INFO

Article history:
Received 25 April 2009
Received in revised form 9 June 2009
Accepted 10 June 2009
Available online 5 July 2009

Keywords:
Biofilter performance
Transient loading
Rhodococcus sp.
Benzene
Toluene

ABSTRACT

The performance of a polyurethane (PU) biofilter inoculated with *Rhodococcus* sp. EH831 was evaluated under different transient loading conditions, such as shutdown, intermittent and fluctuating loading. A mixture of benzene and toluene vapors was employed as model pollutants. When the biofilter was restarted after a 2 week-shutdown, during which neither clean air nor water was supplied, the benzene and toluene removal capacities were rapidly restored after a re-adaptation period of only 1 day. A comparison of the removal capacity under continuous and intermittent loading revealed that constant and periodic loading (8 h on/16 h off per day) and a 2 day-shutdown did not significantly influence the biofilter performance, although the removals of benzene and toluene were relatively unstable and lower under intermittent loading during the initial week. The result of quantitative real-time PCR showed that *Rhodococcus* sp. EH831 could be maintained during transient loading periods (10^{10}–10^{11} CFU/g-dry PU) irrespective of the different operating conditions.

1. Introduction

Many volatile organic compounds (VOCs) are classified as hazardous air pollutants (HAPs), and have been emitted from various industrial complexes (Elmrini et al., 2001; Qi and Moe, 2006). Many applicable techniques have been developed for the control of VOCs, including biofiltration, which has been spotlighted as a promising and alternative method for the control of VOCs as well as odor, even though it was initially designed for the control of odors emitted from wastewater treatment plants, compost facilities and industrial complexes (Kown et al., 2003; Shim et al., 2006). VOCs could be degraded H_2O and CO_2 by passing through biological active media within a biofilter (Moe and Qi, 2005).

Biofilters have been widely studied, but most studies have been conducted under steady-state conditions, focused on the influence of operating conditions, such as biofilter design and structure, packing materials, composition and concentration of pollutant, empty bed residence time, microbial capacity, pH, temperature, moisture content and pressure drop in the filter bed, etc. (Irvine and Moe, 2001; Kim et al., 2005a; Wright et al., 2005). However, the application of biofiltration has been limited to industries due to unstable loading rates and a variety of pollutant compositions (Fitch et al., 2002; Popov et al., 2004; Littlejohns and Daugulis, 2008; Baquerizo et al., 2009; Shareefdeen et al., 2009). In addition, biofilters operating in industry have generally been exposed to non-use periods, such as the shutdown of equipment for repair or during weekends and holidays. Information exists on biofilter performance under unsteady-state or transient loading conditions, such as shutdown, periodic feed on/off, starvation and shock loading, etc. (Irvine and Moe, 2001; Kim et al., 2005b); however, more quantitative and qualitative information on biofilter performance under transient loading is required to ensure effective performance (Wright et al., 2005).

In our previous study, a polyurethane (PU) biofilter, employing PU as the packing material, was demonstrated, which could overcome the problems of clogging caused by excess biomass growth and low treatment capacities of conventional biofilters (Kown et al., 2003; Ryu et al., 2008). Moreover, *Rhodococcus* sp. EH831, isolated from an oil-contaminated soil, has been proved to have excellent capacity for the degradation of multiple hydrocarbons, including BTEX (Lee and Cho, 2009). In this study, the performance of a PU biofilter inoculated with *Rhodococcus* sp. EH831 was evaluated under different transient loading conditions: periodic feed on/off (8 h on/16 h off per day); short-term shutdown (2 days off per week); long-term shutdown (2 weeks off); shock loading (fluctuating high and low loading). A mixture of benzene and toluene vapors, two common solvents extensively employed in industry (Gentry, 2007), served as model pollutants for this study. The removal efficiencies and capacities of benzene and toluene in the PU biofilter were compared, with the dynamics of *Rhodococcus* sp. EH831 monitored using quantitative real-time PCR (qRT-PCR) during transient loading. Furthermore, the bacterial community

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doi:10.1016/j.biortech.2009.06.036

structures within the biofilters operating under different conditions were compared using 16S rDNA-denaturing gradient gel electrophoresis (DGGE) fingerprinting analysis.

2. Methods

2.1. Packing material and inoculum

PU foam cubes (Seilsponge, Korea), with an average size of 3 × 3 × 3 mm, were used as the packing material. The bulk density, water holding capacity, porosity, average pore size and surface area of PU were 0.015 g/cm^3, 57 g-H_2O/g, 98.8%, 0.8 mm and 76.81 m^2/g, respectively (Kown et al., 2003). *Rhodococcus* sp. EH831, isolated from a petroleum-contaminated soil (Lee and Cho, 2009), was inoculated in 2.5 L of Bushnell–Haas (BH) medium (Shim et al., 2006) where the mixture of benzene and toluene (1:1, v/v) was supplemented to be final concentration of 103 mM as the sole carbon and energy sources. The broth was incubated at 30 °C and 180 rpm, and then harvested by centrifugation at 7600 *g* for 15 min. The collected cells were resuspended in 0.5 L of BH medium. The immobilized filter materials were then prepared by soaking in the concentrated cell suspension.

2.2. Biofilter setup and experimental conditions

Five laboratory-scale biofilters were constructed from cylindrical acrylic resin columns. The height and diameter of each column were 500 and 100 mm, respectively. The immobilized filter materials prepared above were packed into each column; the working height and total volume were 250 mm and approximately 2 L, respectively.

Compressed air was allowed to flow through a volatilization chamber before entering the biofilter. The chamber was made of a stainless-steel tube (20 mm internal diameter × 100 mm) connected to a liquid injection system, which consisted of a peristaltic pump and the storage bottle containing the liquid mixture of benzene and toluene (1:1, v/v). The mixed benzene and toluene vapor was generated by injecting the mixed liquid, at 0.001–0.5 mL/min, into the volatilization chamber air stream using a M930 peristaltic pump (Young-Lin Instrument Co. Ltd., Korea). The desired vapor concentration was obtained by adjusting the injection rate of the solution using the peristaltic pump (Shim et al., 2006).

All five biofilter were operated for 3 weeks with the benzene and toluene mixture (200 ± 50 ppmv of each compound) at a space velocity (SV) of 50 h^{-1} to establish steady-state conditions. For the removal of volatile organic compounds such as BTEX, relatively low space velocity (50–100 h^{-1}) is used in practical operation because their solubility is lower than those of malodorous gases (Mathur et al., 2007). After 3 weeks, the PU packing materials were removed from the five biofilter columns, mixed manually, and re-packed into the five columns for transient loading tests. One gram of the mixed packing material was sampled for microbial analysis. Experimental conditions for transient loading are summarized in Fig. 1: Biofilter 1 (BF1; the control) was operated under continuous and constant loading (200 ± 50 ppmv each of benzene and toluene, SV 50/h); Biofilter 2 (BF2) was operated under continuous and constant loading (200 ± 50 ppmv each of benzene and toluene, SV 50/h) after a 2-week long-term shutdown; Biofilter 3 (BF3) was operated under discontinuous and constant loading (loading for 8 h and unloading for 16 h on weekdays, 200 ± 50 ppmv each of benzene and toluene, SV 50/h) and shutdown at weekends (2 days shutdown/week); Biofilter 4 (BF4) was operated under discontinuous and fluctuating loading (loading for 8 h and unloading for 16 h on weekdays, 700 ± 300 ppmv each of benzene and toluene, fluctuating 300–400 ppmv for 2 h and 700–900 ppmv for 6 h, SV 50/h) and shutdown at weekends (2 days shutdown/week); Biofilter 5 (BF5) was operated under continuous and fluctuating loading (high loading for 8 h and low loading for 16 h on weekdays, 700 ± 300

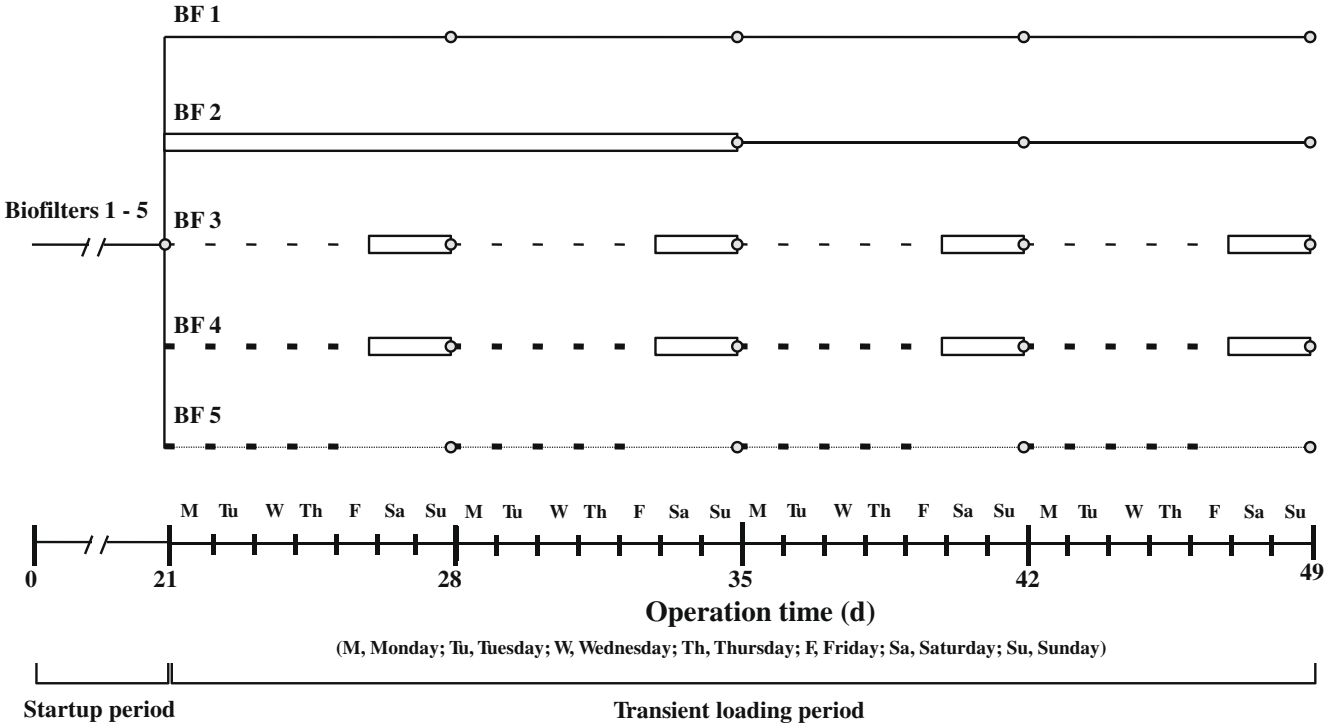

Fig. 1. Operating conditions of the five kinds of biofilter. (——) Continuous and constant loading; toluene/benzene = 200 ± 50 ppmv. (– – –) Discontinuous (8 h on/16h off) and constant loading; toluene/benzene = 200 ± 50 ppmv. (▬ ▬ ▬) Discontinuous (8 h on/16h off) and fluctuating loading; toluene/benzene = 700 ± 300 ppmv. (▬·····▬·····▬) Continuous and fluctuating loading; toluene/benzene for high loading = 700 ± 300 μl/L, for low loading = 75 ± 25 ppmv. (▭) Shutdown. (●) Sampling point of packing materials for bacterial analysis.