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Short Communication

Degradation kinetics and metabolites in continuous biodegradation of isoprene

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HIGHLIGHTS

- Isoprene degradation kinetics in the bioreactor has been evaluated.
- Biofilter unit has shown better degradation efficiency than the bioscrubber unit.
- Major portion of inlet isoprene (62–75%) gets converted to carbon dioxide.
- Metabolites analysis has shown the oxidative cleavage of double bond of isoprene.
- The isoprene degradation pathway in *Pseudomonas* sp. has been elucidated.

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ABSTRACT

The kinetic parameters of isoprene biodegradation were studied in a bioreactor, comprising of bioscrubber and polyurethane foam packed biofilter in series and inoculated with *Pseudomonas* sp., using a Michaelis–Menten type model. The maximum elimination capacity, EC_{max}; substrate constant, K_s and EC_{max}/K_s values for bioscrubber were found to be 666.7 g m^{-3} h^{-1}, 9.86 g m^{-3} and 67.56 h^{-1}, respectively while those for biofilter were 3333 g m^{-3} h^{-1}, 13.96 g m^{-3} and 238.7 h^{-1}, respectively. The biofilter section exhibited better degradation efficiency compared to the bioscrubber unit. Around 62–75% of the feed isoprene got converted to carbon dioxide, indicating the efficient capability of bacteria to mineralize isoprene. The FTIR and GC–MS analyses of degradation products indicated oxidative cleavage of unsaturated bond of isoprene. These results were used for proposing a plausible degradation pathway for isoprene.

1. Introduction

Isoprene (2-methyl-1,3-butadiene) is the most abundant non-methane volatile organic compound (NMVOC) emitted in the atmosphere. Various biogenic and anthropogenic sources lead to its global emission of 450–700 TgC yr^{-1} (Ashworth et al., 2010). It readily photo-oxidizes to generate tropospheric ozone, secondary organic aerosols and carbon monoxide. Its exposure affects skin and respiratory system of humans and it is also reported to possess carcinogenic properties (IARC, 1994). Hence, its removal from the contaminated environment is imperative. The pioneer work on isoprene biodegradation was carried out by Hou et al. (1981) using Methanotrophs and Xanthobacter. This was followed by identification of other isoprene degrading bacteria (Alvarez et al., 2009). The information on biochemical pathway for isoprene degradation is rather in infancy. Vlieg et al. (1999) reported isoprene degradation through epoxidation by *Rhodococcus* AD45. In another study, dioxygenase was shown to be responsible for degradation of isoprene by *Pseudomonas* sp. (Boyd et al., 2000). Studies on biofiltration of isoprene are scanty (Yoon et al., 2002; Srivastva et al., 2015) and lack information on the biodegradation kinetics.

In our previous study, efficacy of *Pseudomonas* sp. (NCBI accession number: KM226326) for isoprene biodegradation in shake flasks and a bioscrubber-cum-biofilter unit packed with polyurethane foam were evaluated (Srivastva et al., 2015). In this follow up study, the biodegradation kinetics in the bioreactor system is studied and the relevant kinetic parameters are evaluated using a Michaelis–Menten type model. The various metabolites produced during biodegradation are analyzed and correlated with earlier reports to propose a more complete and plausible degradation pathway for isoprene.

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2. Methods

2.1. Biodegradation of isoprene

The bioscrubber-cum-biofilter units mounted vertically and connected in series were inoculated with *Pseudomonas* sp., for investigating biodegradation in continuous mode. The lower part of the bioreactor was operated as the bioscrubber and the upper part packed with polyurethane foam as the conventional biofilter. Details of these units are given elsewhere (Srivastva et al., 2015). Isoprene loaded air (isoprene concentration: 0.03–17.4 g m^{-3}) was fed to the bottom of the bioscrubber at four different flow rates. The outlet from this served as feed to the biofilter. The biodegradation was carried out for a total period of 130 days and performance of the bioreactor system was assessed during operation by measuring the concentration of isoprene at the inlet of bioscubber and the oulets of bioscubber and filter sections.

2.2. Analysis of degradation products

2.2.1. Carbon balance

For carbon balance the concentration of carbon in the inlet was taken as 100% and the carbon in other fractions were estimated in reference to this value. The inlet and outlet concentrations of isoprene were determined by gas chromatography (GC) as described earlier (Srivastva et al., 2015). Carbon dioxide in the outlet was also determined chromatographically using GC (Agilent Technologies, 7820A GC system, California, USA) as per manufacturer's method. The analysis of total organic carbon content of the leachate was performed using a TOC analyzer (Analytik Jena Multi N/C 2100, UK) as per manufacturer's protocol. Biomass carbon was calculated by subtracting the sum of outlet isoprene carbon, leachate carbon and CO_2-C from the inlet isoprene carbon using equation proposed by Lu et al. (2002):

$$C_{inlet} = C_{outlet} + C_L + C_{CO_2} + C_{bio} \quad (1)$$

Here, C_{inlet} is the percentage of carbon in inlet gas stream, C_{outlet} is percentage of carbon in outlet gas stream, C_L is total organic carbon percentage in leachate, C_{CO_2} is percentage of carbon in carbon dioxide and C_{bio} is the carbon percentage in biomass.

2.2.2. Fourier transform infrared spectroscopy (FTIR)

The leachate sample was centrifuged at 10000 rpm for 10 min to remove the bacterial cells and the supernatant (1 mL) was used for FTIR analysis (ALPHA, Bruker Optics, Billerica, MA) in the mid IR region of 500–4000 cm^{-1}. Same volume of pure isoprene (Merck Company, Germany) was used as control. The peaks interpretation was carried out using IR Pal 1.0 software.

2.2.3. Gas chromatography–mass spectrometry (GC–MS)

The metabolites were extracted from the leachate (10 mL) in n-pentane as described earlier (Srivastva et al., 2015). One μL of the extracted sample was injected into Shimadzu GCMS-QP2010 Ultra (Serial No. O205249, USA) equipped with Rxi® 5 ms capillary column (30 m × 0.25 mm × 0.25 μm), using a Hamilton gas-tight syringe (Model: 701 N). Same volume of pure isoprene (Merck Company, Germany) was used as control. Helium (99.99% purity) was used as the carrier gas at 100 kPa pressure and 11.9 mL min^{-1} flow rate. The injector temperature was set at 250 °C and the oven temperature at 40 °C for 5 min and then ramped to 90 °C at the rate of 5 °C min^{-1} for 2 min followed by ramping to 130 °C at the rate of 10 °C min^{-1} for 2 min and finally to 260 °C at the rate of 10 °C min^{-1} for 2 min. Split ratio was chosen as 4. The MS was operated in full scan mode with m/z ranging from 35 to 200. Ion source and interface temperatures were set at 250 and 300 °C, respectively. Mass spectra of the peaks were compared with compounds present in NIST database (NIST08.LIB). Compounds with >90% similarity were selected.

3. Results and discussion

3.1. Biodegradation kinetics

The experimentally achieved elimination capacity (EC) remained almost constant with the increasing inlet loading rate during Phases II and III of the bioscrubber. While in the biofilter section, the experimentally calculated EC increased with increase in the inlet loading rate during those phases (Srivastva et al., 2015). This indicated the absence of inhibition in the bioreactor system during the operation. Hence, the Michaelis–Menten type model:

$$\frac{V}{Q(C_{in} - C_{out})} = \frac{1}{EC} = \frac{K_s}{EC_{max}} \frac{1}{C_{ln}} + \frac{1}{EC_{max}} \quad (2)$$

modified for continuous system (Mathur et al., 2006) was used for evaluating the kinetic parameters from the steady state experimental data of Phases I to III. Here, V is the working volume (m^{-3}), Q is the volumetric flow rate (m^{-3} h^{-1}), C_{in} is the inlet concentration (g m^{-3}), C_{out} is the outlet concentration (g m^{-3}), EC is the elimination capacity (g m^{-3} h^{-1}), EC_{max} is the maximum elimination capacity (g m^{-3} h^{-1}), K_s is the saturation constant of substrate (isoprene) (g m^{-3}) and $C_{ln}\{= \frac{C_{in} - C_{out}}{ln\frac{C_{in}}{C_{out}}}\}$ is the logarithmic average of inlet and outlet concentrations of isoprene.

The equations corresponding to the best fit straight line for $\frac{1}{EC}$ vs $\frac{1}{C_{ln}}$ plots for bioscrubber and biofilter were generated using the least-squares method (Fig. 1). The EC_{max} and K_s for bioscrubber were found to be 666.7 g m^{-3} h^{-1} and 9.86 g m^{-3}, respectively (Fig. 1A) while those for biofilter were 3333 g m^{-3} h^{-1} and 13.96 g m^{-3}, respectively (Fig. 1B). The value of EC_{max}/K_s obtained for bioscrubber and biofilter units were 67.56 h^{-1} and 238.7 h^{-1}, respectively.

It has been established that the biofilter operates in the plug-flow regime while the bioscrubber exhibits the well-mixed behavior (Yadav et al., 2014). The EC_{max} for the bioscrubber obtained from the analysis of the data is comparable to the EC (567 g m^{-3} h^{-1}) obtained experimentally (Srivastva et al., 2015) which indicates that the bioscrubber is operating at the near optimum condition. Further, the lower K_s value compared to that for batch mode (Srivastva et al., 2015) indicates that this unit is more efficient than the batch operation. This can be attributed to the absence of oxygen limitation and better mixing due to bubbling of isoprene loaded air. Further, the bioscrubber is also advantageous in two ways – it acts as humidifier and prevents organic overloading of biofilter. The degradation capability of the biofilter, as evident from the kinetic parameter – EC_{max}/K_s, however, is better than the bioscrubber. This ratio reflects the efficiency of substrate degradation by bacterial community and is considered as an useful index for the enzymatic reaction. This kinetic study is also consistent with the previous report (Srivastva et al., 2015) where removal efficiencies of biofilter (89% in Phase II and 66% in Phase III) were found to be better than those of bioscubber (31% in Phase II and 17% in Phase III). Schlegelmilch et al. (2005) also reported 29% removal in the bioscrubber and 97–99% for the bioscrubber/biofilter combination. The EC_{max} value for the biofilter unit obtained through analysis of data is around 2.6 times greater than the highest EC value (1256 g m^{-3} h^{-1}) observed experimentally (Srivastva et al., 2015). This shows that the biofilter could be operated at even higher inlet loading rates.

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