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Characterization of bacterial isolates from rubber dump site and their use in biodegradation of isoprene in batch and continuous bioreactors

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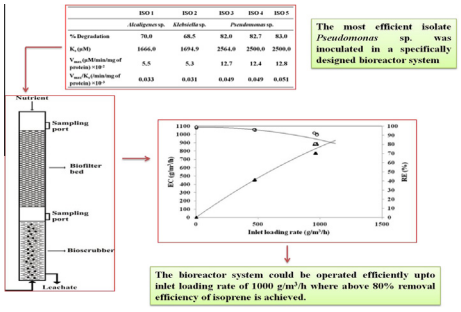
HIGHLIGHTS

- Five bacterial isolates have been isolated from isoprene contaminated soil.
- *Pseudomonas* sp. have been found to degrade up to 83% isoprene in batch mode.
- Kinetic constants have been used to explain the efficiency of isolates.
- Degradation is better (71–100% RE) in continuous mode.
- Use of bioscrubber enhances the performance of biofilter.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacterial isolates from contaminated soil of a waste rubber dumping site were isolated and characterized using biochemical and molecular approaches. Isoprene degradation kinetics in batch mode (isoprene concentration: 100–1000 ppm) revealed the degradation efficiency of isolates as: *Pseudomonas* sp. (83%) > *Alcaligenes* sp. (70%) > *Klebsiella* sp. (68.5%). The most efficient isolate *Pseudomonas* sp. was finally inoculated in a specifically designed bioreactor system comprising a bioscrubber and a biofilter packed with polyurethane foam connected in series. The bioscrubber and biofilter units when operated in a series showed more than 90% removal efficiency up to the inlet loading rate (IL) of 371.1 g/m^3/h. Maximum elimination capacity (EC) of biofilter was found to be an order of magnitude greater than that for bioscrubber. Oxidative cleavage of the double bond of isoprene has been revealed through IR spectra of the leachate.

1. Introduction

Isoprene (2-methyl-1,3-butadiene) is the highest emitted non-methane volatile hydrocarbon in the atmosphere. Isoprene is used as monomer in various industrial applications for the production of elastomers e.g., polyisoprene, styrene thermoplastic and butyl rubber (OECD, 2005). Ethylene production, automobile exhausts, petrochemical industries and burning of carbonaceous materials are the major anthropogenic sources of isoprene. All such units emit significant amount of isoprene in the environment (Lewis, 1997; Reimann et al., 2000) making its global emission in the range of 450–700 Tg C/yr which is comparable to that of methane. Isoprene alters the atmospheric chemistry through photochemical reactions with oxides of nitrogen and thus contributes to the generation of tropospheric ozone (Fehsenfeld et al., 1992). Additionally, it produces aerosols, carbon monoxide and indirectly

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contributes to the global warming by lengthening the residence time of greenhouse gases. The exposure to isoprene through air can be deleterious to human health (Purves et al., 2004). It is associated with catarrhal inflammation, sub-trophic and atrophic processes in the upper respiratory tract, etc. (OECD, 2005). Deterioration of olfactory organs has been noticed in isoprene rubber plant workers (IARC, 1994; Doyle et al., 2004). It is classified as Group 2B carcinogen and is proven to cause cancer even at very low exposure level (Yoon and Park, 2002). Hence, its removal from the contaminated environment is imperative.

Chemical and physical methods of transformation of isoprene to benign forms are inefficient and generate secondary pollutants (Elkanzi and Kheng, 2000; Rudzinski, 2004). Therefore, biodegradation has attracted the attention of researchers for its efficient abatement. The pioneer work on biodegradation of isoprene was done by Hou et al. (1981). They reported that *Methanotrophs* and *Xanthobacter* were able to oxidize isoprene. This was followed by a series of work that led to the identification of several isoprene degrading bacterial species such as *Nocardia* (van Ginkel et al., 1987), *Alcaligenes* and *Rhodococcus* (Ewers et al., 1990; Vlieg et al., 1998) with the potential to utilize isoprene as carbon and energy source. The work of Cleveland and Yavitt (1997, 1998) indicated that soil acts as sink for isoprene and its biodegradation is carried out by *Arthrobacter* residing in soil. Alvarez et al. (2009) studied consumption of isoprene from marine and coastal environment by isoprene degrading bacteria such as *Actinobacteria*, *Alphaproteobacteria*, *Bacteroidetes* and *Alcanivorax*.

Most of the earlier reported studies on biodegradation of isoprene are conducted in batch mode. The published information on the biodegradation of isoprene in continuous mode is scanty (Yoon and Park, 2002). Further only few studies on the kinetics of biodegradation of isoprene, which is essential for design of continuous bioreactors have been reported (Vlieg et al., 1998, 1999). In view of this the present study was planned to isolate, characterize and assess the potential of bacterial isolates for isoprene degradation in batch mode. The most efficient bacterial isolate, as revealed by the batch studies, was used as inoculum in a specifically design suspended cell bioscrubber and polyurethane foam packed biofilter units connected in series. The behavior of the two units of bioreactor was analyzed separately as well as in combination. The performance of the bioreactor was assessed at different flow rates and concentrations of isoprene. The IR spectroscopic analysis of the leachate was also carried out to know the molecular nature of degradation products.

2. Methods

2.1. Enrichment, isolation and biochemical tests of isoprene degrading bacterial isolates

Cleveland and Yavitt (1997, 1998) showed the existence of isoprene degrading bacteria in soil. Hence, it is assumed that the isoprene contaminated site is likely to harbor the isoprene catabolizing bacteria. Considering this, the soil samples used in this study were collected from Chowka Ghat, Varanasi, U.P., India (25°19′59.885″N; 82°59′47.381″E; 129 m above mean sea level) an area having automobile garages and spare parts shops. Waste rubber tires and tubes, etc., are being regularly dumped at the sampling site since long. Soil cores were collected from 0 to 15 cm depth. The soil samples were air dried in laboratory at room temperature then mixed thoroughly and sieved using a 2 mm mesh screen to remove debris. The sieved soil samples were kept at 4 °C for microbial analysis. Samples were collected in triplicate to maintain the heterogeneity.

Enrichment was done by mixing 50 g of soil with 10 μL of pure isoprene (Merck Company, Germany) and incubating at 28 °C in dark for three weeks with continuous mixing of soil. Isoprene degrading bacteria were isolated using the mineral salts medium (MSM) (g/L) $Na_2HPO_4{\cdot}12H_2O$ (9.0), KH_2PO_4 (1.5), NH_4NO_3 (1.0), $MgSO_4{\cdot}7H_2O$ (0.2), $CaCl_2{\cdot}2H_2O$ (0.02), Fe(III)[NH_4] citrate (0.0012), having 0.1 mL of the trace element ($FeSO_4{\cdot}7H_2O$, 50 mg; $ZnSO_4{\cdot}7H_2O$, 1 mg; $MnCl_2{\cdot}4H_2O$, 0.3 mg; H_3BO_3, 3 mg; $CoCl_2{\cdot}6H_2O$, 2 mg; $CuCl_2{\cdot}2H_2O$, 0.1 mg; $NiCl_2{\cdot}6H_2O$, 0.2 mg; $Na_2MoO_4{\cdot}H_2O$, 0.3 mg in 100 mL) in 100 mL of media adjusted to pH 7.0. Pure culture of isolates was obtained by repeated plating over isoprene coated MSM agar medium. The cell growth was determined by measuring OD_{600} against control. Protein estimation was done as described by Shukla et al. (2010). The control was prepared in MSM medium without isoprene. Biochemical tests for all the bacterial isolates were performed, following the protocol given in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

2.2. Molecular characterization of bacterial isolates

Genomic DNA extraction and quantification was performed for bacterial isolates as described by Maya et al. (2011). Eubacterial 16S rRNA gene fragments were amplified from isolated DNA using 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) forward primer and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) reverse primer sets. The amplification mixtures having a total volume of 25 μL contained 100 ng template DNA, 1× buffer (Fermentas, UK), 0.5 mM $MgCl_2$, 100 μM each of dNTPs (Fermentas, UK), 0.5 μM of each primer (Sigma Aldrich, USA) and 1U *Taq* DNA polymerase (New England Biolabs, Beverly, MA). The PCR was carried out in a thermal cycler (My Cycler™ Thermal Cycler, Bio Rad Laboratories, Inc., Australia). Thermal cycling conditions were set as: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 1.30 min. The final extension temperature was set at 72 °C for 5 min. Amplicon size was checked by the electrophoresis of PCR product on 1% agarose gel. Amplification reaction was performed in triplicate and the pooled PCR products were purified using QIAquick PCR Purification Kit (Qiagen GmbH D 40724, Hilden). The purified PCR product was sequenced by Chromous Biotech., Bangalore, India. The sequence analyses were performed as described previously (Maya et al., 2011). The nucleotide sequences of the 16S rRNA gene of the bacterial isolates have been deposited in the GenBank nucleotide sequence databases under the accession numbers KM226325–KM226329.

2.3. Isoprene degradation in batch mode

The growth of bacterial isolates at five isoprene concentrations: 100, 500, 1000, 1500 and 2000 ppm were monitored. No growth was observed above 1000 ppm. Hence, the isoprene samples with concentration ranging from 100 to 1000 ppm were prepared in serum bottles containing 10 ml MSM and 100 μL bacterial inoculum. All the vials were crimped by butyl rubber stopper and aluminum caps. The bottles were then incubated at 28 °C in dark at 100 rpm. Residual isoprene concentration in the headspace and liquid media was analyzed after every 24 h using gas chromatograph (Model 3800 GC, Varian, BV, the Netherlands) equipped with flame ionization detector (FID) and a fused silica capillary column (CP SIL 5CB; 15 m × 0.25 mm). For liquid media, isoprene from samples was first extracted in pentane which was then injected into GC. The carrier gas was N_2 at a flow rate of 300 mL/min and the injector and detector temperatures were set at 230 and 250 °C, respectively. The oven temperature was set at 40 °C for 5 min and then ramped to 90 °C at the rate of 5 °C/min for 2 min. The retention time of isoprene was 4.7 min. Isoprene concentration