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Biodegradation of toluene vapor by evaporative cooler model based biofilter

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Abstract The biodegradation of toluene vapor was investigated using a new type of biofilter equipped with a laboratory-scale evaporative cooler model packed with wood wool fibers (area: 360 cm^2). For the purpose of this study, the biofilter system was inoculated with Pseudomonas sp. RSST (MG 279053). The performance of this biofilter, assessed in terms of toluene removal efficiency (and elimination capacity), was as high as 99 % at a loading rate of 6 $g/h \cdot m^2$. The toluene removal efficiency decreased in an exponential manner with the increase in the loading rate. The cooler model-based biofilter was able to remove more than 99 % of toluene using Pseudomonas sp. RSST (MG 279053) as an effective inoculum. This biofilter is designed to operate under batch conditions for the removal of toluene in confined environments (e.g., automotive plants, boiler rooms in manufacturing facilities, and offshore drilling platforms).

Key words: Biofiltration, Removal efficiency, Toluene vapor, Cooler model based Biofilter, *Pseudomonas sp. RSST (MG 279053)*

1. Introduction

A list of 188 pollutants was promulgated as hazardous air pollutants (HAPs) based on their inherent toxicity by the 1990 Clean Air Act Amendments (CAAA) of the United States Environmental Protection Agency.[1] As a member of the HAP list, toluene is used widely in various industrial operations as a raw material for the synthesis of compounds such as tri-nitro toluene (TNT), chloramine-T, saccharin, and many dyestuffs.[1] Toluene has been reported to be carcinogenic to cause damage to the liver and kidney; it can paralyze the central nervous system at a concentration of 200 ppm.[1,2] As toluene is used widely in various industrial operations, it is of prior significance to run proper treatment system for the regulation of its emission into the natural environment. To this end, the removal of toluene should be secured in light of its high vapor pressure (28.6 mmHg at 25 °C) and high polarity (as characterized by its solubility in water: 0.53 g l^{-1} at

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25 °C).

A variety of industrial sources are identified to emit volatile organic compounds (VOCs). Because of the involvement in the greenhouse effect and photochemical smog, VOCs have been subjected to increasingly stringent environmental regulations over the past decades.[3,4] In recent years, bioreactor-based treatments of waste stream gasses containing VOCs (including very odorous compounds) has gained much attention. Such treatment systems are thus projected to offer a more cost effective and more environmentally friendly alternative to conventional air pollution control technologies. Biofiltration of off-gas containing VOCs is a relatively new field of bioprocess engineering that can be adopted for the air quality management under mild pollution conditions.[5]

Due to the low level (sub-ppm) emission of toluene (and other VOCs) from various sources, the use of conventional methods (e.g., adsorption, incineration, and condensation) is often unfeasible for its treatments in an economic sense.[6,7] Hence, the use of bio-treatment methods based on aerobic biodegradation in biofilters appears to be an attractive option to consider. The continued reduction in allowable levels of VOC emissions, as embodied in the Clean Air Act, has forced operators of paint shops to search for new methods to limit VOC emissions from their finishing processes. Several choices have been proposed such as electro coating or powder coating. Moreover, the use of very high solids and even water-based sprayable coatings was also suggested; however, for certain applications such as an automobile or plastic surface coating, solvent-based coatings are still the norm due to the need for very high-quality finishes at high transfer efficiencies. For the advanced paintings (spray paint) of steel and plastic products, the surface coaters are forced to use solvent based coatings. The installation of add-on control equipment to clean the booth exhausthas been suggested as the most effective option to reduce VOC emissions from spray booths. The effectiveness of some add-on abatement technologies such as incineration or carbon adsorption has been technically provento a certain degree. However, for the purpose of VOC control, the employment of such techniques in spray booth can be prohibitively expensive.

Biofiltration is based on biological oxidation of VOCs using aerobic microorganisms immobilized on solid particles of bed media such as peat, compost, wood chips, polyurethane foam, and their mixtures packed in a column.[8-10] The biofilter is essentially a fixed-film bio-reactor that provides a large contact area between the gas stream and the microorganisms supported on a porous granular solid surface. These microorganisms exist both in the medium and the bio-film.[7,11,12] As the polluted gas stream passes through the medium, VOCs are partitioned into the bio-film through which biological oxidation proceeds under aerobic conditions.[13] The main advantage of biofiltration is that the pollutants are converted into harmless end-products instead of being transferred from one phase to another.[3,14] Moreover, such approach is also advantageous in that excellent operational stability can be gained at relatively low cost.[6,11]

Most high production paint spray booths are either multi-zone booths with dedicated zones for manual and automatic paint application or single-zone, fully automatic booths. The highest concentrations of VOCs are found in the exhaust from the automatic zones. The general tendency towards the automation of painting process demands the development of a novel abatement technique. The aim of this study was hence set to demonstrate the feasibility of a novel evaporative cooler model based biofilter which could be used to achieve high removal efficiencies of harmful VOCs in a confined environment such as paint spray booth. In line with general expectation, the high removal efficiency of this particular biofilter should be mainly ascribable to its design. The fan of the cooler keeps circulating the air while sucking the air loaded with the toluene vapor through the cooler pads. This process was repeated for multiple times (e.g., at least as triplicates). As a result, very high degradation of toluene took place, reaching almost 100% (*Table* 1; *Fig.* 2). Moreover, biodegradation of toluene also took place in the leachate itself. As toluene vapor was absorbed in the leachate, it was degraded by the bacteria already present in the immobilized form of leachate. As such, a positive concentration gradient

Table 1. Dimensional details, schematics and performance of evaporative cooler model based biofilter at optimal operating conditions for treatment of waste gas containing toluene

Parameters	Values
Dimension (cm)	31 × 31 × 52
Voltage of motor (V)	220
RPM of motor of cooler	1400-2800
Velocity of out coming air (m/s)	2
No. of biofilter media pads	3
Area of each pad (cm^2)	360
Total pad area (cm^2)	1080
Biofilter media	Wood wool
Flow rate of nutrient solution on the pads (mL/min)	110
Inlet Concentration (mg/L)	4.13-44.2
Outlet Concentration (mg/L)	0.005-3.91
Removal Efficiencies (%)	91-99
Elimination Capacity (g m^{-2} hr^{-1})	4-57.2
Inlet toluene loading rate (g m^{-2} hr^{-1})	6-60
Bacterial Details	Pseudomonas sp. RSST (MG 279053)

was observed for the absorption of toluene.

2. Materials and Methods

2.1. Toluene-degrading potential microorganisms, chemicals, and basal media

To induce the degradation of toluene using a specific microorganism, *Pseudomonas putida* (MTCC 102) strain was procured from the 'Microbial Type Culture Collection and Gene Bank' (Chandigarh, India). The strain was revived in a 250 mL shake flask containing 100 mL of nutrient broth composed of yeast extract (0.2 g), beef extract (0.1 g), peptone (0.5 g), and NaCl (0.5 g). The flask was then incubated for 16 hrs in an incubator shaker at 26°C and 140 rpm. This incubated suspension of *Pseudomonas putida*was used as inoculum for the cooler model biofilter. The packing of the biofilter was routinely supplied with a nutrient solution to maintain the growth of microorganisms in the biofilter media.

The composition of mineral salts medium (g l^{-1}) used in this study was as follows: MgO, 0.20; NH_4Cl, 5.11; NaCl, 10.0; $FeCl_3$ (anhy.), 0.05; $CaCl_2$ (anhy.), 0.02; NH_2CONH_2, 1.00; KH_2PO_4, 0.85; K_2HPO_4, 0.22; and $NaNO_3$, 0.12. All these chemicals were purchased from Hi Media, India. Toluene (99.8 % pure analytical grade) was purchased from M/s Sigma Aldrich Co. Germany.

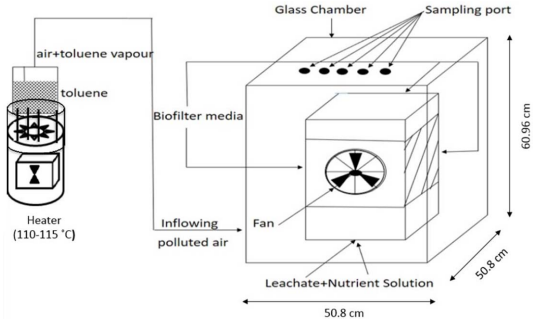

Fig. 1. Schematic diagram of cooler model based biofilter and experimental setup for the treatment of waste gas containing toluene.

2.2. Molecular characterization, sequencing and phylogenetic analysis

The total genomic DNA from the pure culture of strain RSST was extracted by following the method described by.[15] The washed DNA pellet was incubated at 37°C for 30 min for removal of ethanol, and then dissolved in 50 µL of TE buffer. The PCR reactions was carried out in BioRad thermal cycler and amplification was performed by using universal 16S rDNA primers pA (5'-AGTTTGATCCTGGCTAG-3') and pH (5'-AGGAGGTGATCCAGCCGCA-3').[15] The amplification was carried out in a 100 µL volume by mixing 50-100 ng template DNA with the polymerase reaction buffer (10X); 100 µM (each) dATP, dCTP, dTTP and dGTP; primers pA and pH (100 ng each) and 1.0 U Taq polymerase. The amplification conditions were as follows: initial denaturationof 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 45 s at 50 °C and 1.5 min at 72 °C, and a final extension period of 8 min at 72 °C. After amplification the PCR products was resolved by electrophoresis in 1.5 % agarose gel in 1X TAE buffer. Gels were stained in ethidium bromide (10 mg/mL) and gel images was visualized on a gel documentation system. The amplified 16S rRNA genes PCR product was purified with a Nucleo-pore purification kit

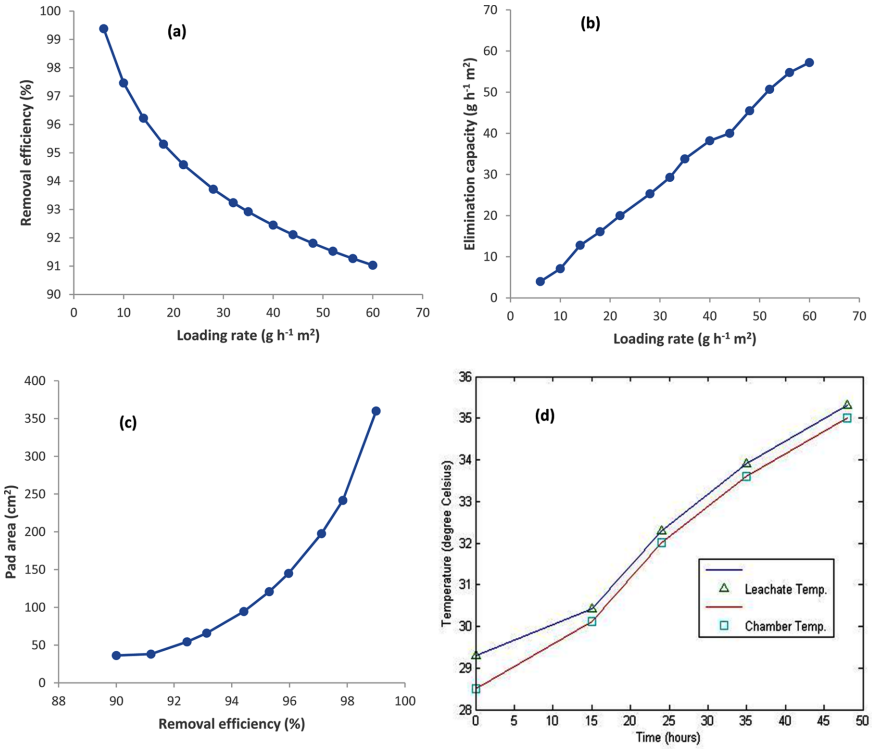

Fig. 2. Bench scale cooler model based biofilter performance during treatment of toluene in laboratory, (a) Influence of the inlet loading on the removal efficiency of the biofilter, (b) Influence of the inlet loading on the elimination capacity of the biofilter, (c) Pad area requirement for different toluene removal efficiencies; and (d) Leachate and chamber temperature variations.

(Genetix Biotech Asia Pvt. Ltd.). The nucleotide sequence was di-deoxy cycle sequenced with fluorescent terminators (Big Dye, Applied Biosystems) and run in 3130xl Applied Biosystems ABI prism automated DNA sequencer. 16S rRNA gene amplicons was sequenced from both ends by using primers pA and pH and consensus sequence was generated. The partial 16S rRNA gene sequences of the strain RSST was compared with those available in the databases (http://www.ncbi.nlm.nih.gov/BLAST/) and identification was determined on the basis of sequence similarity of > 97 % with the closest relative in the NCBI GenBank. The phylogenetic tree was constructed on the aligned datasets using the neighbour-joining method implemented in the program MEGA 6.0.[16] Bootstrap analysis was performed on 1,000 random samples taken from the multiple alignments. The sequences of the strain RSST was submitted to NCBI GenBank and accession numbers was assigned as MG279053.

2.3. Analytical tools (GC Analysis): terms and measurement protocols

Gas samples were collected from the sampling ports at the top of the glass chamber by Hamilton syringe (1 mL capacity). Samples were analyzed using a gas chromatograph (Model No.: GC 7610 and Trace 1110, Gas Chromatograph, Thermo Scientific, Japan) equipped with flame-ionization detector (FID) and a Porapack column (1/8" ID, liquid – 10 % FFAP, solid – Ch-WIHP, 80/100 mesh). Nitrogen was used as a carrier gas. Temperatures of the injector, oven, and detector were 170, 130, and 240 °C, respectively. The gas chromatograph was standardized weekly. The detection limit was determined to be 0.18 ppm at a confidence level of 99 % under repeatable conditions (e.g., same instrument, same operator, identical target pollutant, and constant operating conditions). The removal experiments of toluene were conducted as duplicate run, and the further evaluation of data was

done using the average values of those replicate runs. Operation and performance of a biofilter are generally reported in terms of removal efficiency (or pollutant elimination capacity) as a function of the loaded amount of target gas. These terms are defined as[8]:

$$\text{Removal Efficiency (RE)} = ((C_{in} - C_{out}) / C_{in}) \times 100\ (\%) \quad (1)$$

$$\text{Elimination Capacity (EC)} = ((C_{in} - C_{out}) / A) \times Q\ (g\ m^{-2}\ h^{-1}) \quad (2)$$

$$\text{Pollutant Loading (L)} = (C_{in} / A) \times Q\ (g\ m^{-2}\ h^{-1}) \quad (3)$$

Where C_{in} and C_{out} are pollutant concentrations at the inlet and outlet, respectively, A is the total biofilter media pad area (m^2), and Q is the airflow rate ($m^3\ h^{-1}$). Experimental data have been used to calculate these parameters. The efficient functioning of any biofilter unit strongly depended on the loading amount of pollutant at the inlet.

2.4. Biofilter specification and performance evaluation

A schematic diagram of a cooler based biofilter model and experimental setup is shown in *Fig.* 1. The lab scale biofilter consisted of a customized glass chamber (50.8 × 50.8 × 60.96 cm) fitted with GC (Gas Chromatography) septa at the top for taking out gas samples (1 mL sample per injection) for GC analysis. The glass chamber simulated a confined environment. Inside this chamber, a simple evaporative cooler with a total pad area of three-layered filter (360 × 3 = 1080 cm^2) was placed. Wood wool was used as the packing media in the pads. Due to its high volume and large surface area, wood wool is effective for the retention of water or moisture. The width of wood wool fibers varied from 1.5 to 20 mm, while their length was usually around 500 mm. *Pseudomonas putida* was immobilized within nutrient solution inside the cooler. This solution was circulated (110 mL/min per pad) to the cooler pads with the aid of a peristaltic pump (Model – TMA 200, TMA International, New Delhi, India; Power – 6 W, maximum flow – 400 L/h, maximum head – 0.7 m), leading to the immobilization of bacteria on the pads. Drippings of the toluene solution from the cooler pads were collected at the bottom in the nutrient solution, which results in the formation of a leachate. The pump was run for 24 hours for proper immobilization of bacteria on the cooler pads. A temperature controlled heating mantle was used to maintain temperature ranges near the boiling point of toluene (110-115 °C) to evaporate it into vapour-phase in the range of 50-200 ppm through a 250 mL bubbler containing toluene (purity (99.8 %); relative density (0.865 g mL^{-1} at 25 °C); and water solubility (0.5 g l^{-1}: at 15 °C). Seven to eight liters of nutrient solution with known composition were kept inside the cooler for the experiment. The biofilter setup for toluene removal was run for 48 hours. As almost complete removal of toluene was achieved within 48 hours of operation, its operation was ceased at that point. Moreover, the acclimatization period of microbes in this experiment was very low (owing to the pure nature of the microbial strain) which further shortened the operation time. The biofilter was operated at an ambient temperature in the summer where the temperature normally varies in the range of 3 5± 5 °C.

3. Results and Discussion

3.1. Continuous biodegradation experiments and performance of cooler model based biofilter

Biofilter was operated at an inlet concentration of toluene in the range of 4.13-44.3 mgL^{-1} (1,096 to 11,760 ppm) to observe its outlet concentration typically in the range of 0.0045-3.91 mgL^{-1} (1.19 to 1,038 ppm). In Fig. 2(a), the removal efficiency of toluene is plotted as a function of its loading concentrations throughout the experimental period (i.e., 60 hours). Removal efficiency of toluene has been observed to decrease from 99.4% (0 hr) to 91.0% (60 hr) when loading increased from 10 to 60 $g.m^{-2}h^{-1}$. This could be due to insufficient contact time between gas and biofilms.[7] Substrate inhibition at a high concentration of toluene may also be responsible for poor removal.[1,5] The removal efficiency of toluene followed an exponential pattern with the inlet loading as approximated by the equation RE = a

× (L^b), where 'a' and 'b' are constants whose values were found to be '106.4' and '-0.03811' respectively, with 95% confidence bounds using curve fitting.

The elimination capacity is defined as the amount of pollutant degraded per unit time, normalized to the area of packed bed and is used to reflect the capacity of the biofilter to remove the pollutants. It is also plotted as a function of its loading concentration in *Fig.* 2(b). At lower inlet loadings, it was evident to see the low values of removal efficiency (and elimination capacity); *Figs.* 2(a) and 2(b) was drawn to represent either acclimation period or nutrient deficient period. As such, the maximum elimination capacity of the biofilter was 57.2 g m^{-2} hr^{-1} at inlet load of 60 g m^{-2} hr^{-1}.

Effect of the surface area of cooler pad has also been assessedin terms of removal efficiencies (*Fig.* 2(c)). The removal efficiencies were observed to increase (90-99 %) with increasing pad area (36.3-360 cm^2); hence, the results confirmed that larger surface are afacilitated the growth of microorganisms responsible for the biodegradation of toluene. This gives us an indication of how much area of biofilter media is required to attain a particular removal efficiency of the biofilter. Accordingly, the scale up of the bio filtering system can be done for a chamber with a large volume.

3.2. Temperature variations

Biofiltration is a system to induce bio-oxidation of contaminated air using a fixed bed of organic (or inorganic) media containing microorganisms with the potential to minimize pollutants aerobically. Biotransformation process of VOCs can be expressed as:

Microorganism

$$VOCs + O_2 \rightarrow Biomass + H_2O + CO_2 + Heat\ (Energy)$$

The more biodegradation takes place; the more heat is generated in the system. Temperature control is very important in biofiltration as the biological activity is strongly affected by changes in temperature. A proper range of temperature is 14 °C to 40 °C for the operation of biofiltration, which is suitable for the growth of mesophilic microorganisms.[17] As elucidated by many studies, such effect can be described by the Arrhenius equation in the sub-optimum range (15-35 °C). Although the operation of biofilter under warmer conditions generally supports more active organisms, the efficiency of toluene removal is not necessarily favorable due to other variables (e.g., the physio-chemical effects of higher temperature). For instance, the transfer of the contaminant from the air to the microorganisms will be less effective, as the extent of dissolution decreases with the rise in temperature.

In the present study, the temperature changes in both the leachate and the chamber were monitored throughout the course of the experiment; as per expectation, both temperatures were found to keep rising with their peak values of 35.3 and 35 °C, respectively (*Fig.* 2(d)).

3.3. Discussion on the biofilter design

Dixit *et al*. described bio filtration of a mixture of n-propanol (as a model hydrophilic VOC)) and toluene (as a model hydrophobic VOC) in a biofilter packed with compost–woodchip mixture (toluene vapors at loadings up to 175 g m^{-3} h^{-1}).[1] They observed removal efficiencies of 70 % – 99 %. The performance of this particular biofilter, if evaluated in terms of removal efficiency, was far superior over other traditional biofilters. Other researchers reported removal efficiencies in the 90 % + region which is very difficult to maintain.[8,18] Removal of toluene using an agro-waste based biofilter was studied utilizing activated sludge as the inoculum.[8] The reported removal efficiency was 59.8 % with an elimination capacity of 174.6 mg l^{-1} hr^{-1} and an effective bed retention time (EBRT) of 154 seconds. [19,20] reported 69.6 % removal of toluene using small stones of packing media immobilized with bacteria and 180 seconds EBRT with elimination capacity of 40.3 mg/L/hr. Removal efficiencies of 78.4 % have been reported using activated sludge at 74.2 seconds of EBRT with 36.0 mg/l/hr elimination capacity.[21] The fate of toluene during biofiltration can lead to the formation of various intermediates, such as hydroxylated toluene, benzyl alcohol, benzaldehyde, benzoate, catechol and final

mineralization products (CO_2 and H_2O).[1,7]

3.4. Bacteriological status of the biofilter during treatment

Characterization of specific microorganisms growing on a toluene plate had shown one morphological property (colony). The colony of this culture was picked up and streaked on three different slants containing toluene. This culture growing on the biofilter was designated as RSS-1. It was seen to have the potential to degrade toluene when tested separately as well as in mixed biomass. Further, this culture was also identified by fatty acid methyl esterification (FAME) analysis as a *Pseudomonas putida*. The FAME profile of bacterium was matched with a fatty acid profile data base library RTSBA6600. The toluene-degrading isolate was identified as *Pseudomonas putida* based on sim index. The result of this analysis indicates that the microbial profile responsible for treating toluene in the biofilter had changed, although it was initially seeded with *Pseudomonas putida* during the start up. As such, it is not easy to maintain a single pure culture system for the biofilter method. However, it is always desirable to have a mixed population of microorganisms for the biotechnological processes in an open system. This is because the mixed biomass can degrade and stabilize the constituents more effectively. Molecular characterization based on 16S rRNA gene homology of 1428-bp partial sequence confirmed that potential toluene degrading bacterial strain RSST belongs to *Pseudomonas* sp..[22] In the phylogenetic tree, strain RSST and other related *Pseudomonas* species were grouped together (*Fig.* 3).

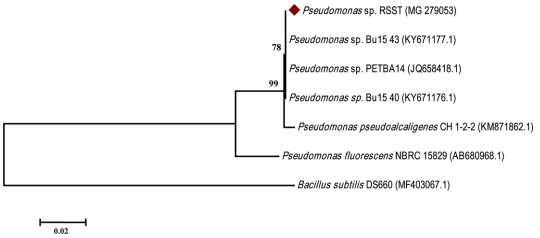

Fig. 3. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing relationships between strain RSST and its related bacterial species. *Bacillus subtilis* DS660 (MF403067.1) was taken as an outgroup. The numbers represent the confidence levels from 1000 replicate bootstrap sampling.

4. Conclusions

This study demonstrates the effective removal of toluene vapor from the air in a confined chamber using an evaporative cooler model based biofilter inoculated with *Pseudomonas sp. RSST (MG 279053)*. The purpose of this study was to show that a novel biofiltration method could be used to reduce the concentration of harmful VOCs in confined chambers such as a paint spray booth. Toluene removal efficiencies were achieved in the 90 % + level at the inlet toluene loading rates in the range of 6-60 g/h-m^2. Biofilter was capable of achieving as much as 99 % toluene removals at a loading rate of 6 g/h-m^2. The maximum elimination capacity of the biofilter was 57.2 g m^{-2} hr^{-1} at inlet load of 60 g m^{-2} hr^{-1}.

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References

1. R. M. Dixit, S. C. Deshmukh, A. A. Gadhe, G. S. Kannade, S. K. Lokhande, R. A. Pandey, A. N. Vaidya, S. N.

Mudliar, and M. A. Deshusses, *Environ. Technol.*, **33**, 751-760 (2012).
2. M. Murata, M. Tsujikawa, and S. Kawanishi, *Biochem. Biophys. Res. Commun.*, **261**, 478-483 (1999).
3. S. Mudliar, B. Giri, K. Padoley, D. Satpute, R. Dixit, P. Bhatt, R. Pandey, A. Juwarkar, and A. Vaidya, *J. Environ. Manage.*, **91**, 1039-1054 (2010).
4. B. S. Giri, K. H. Kim, R. A. Pandey, J. Cho, H. Song, and Y. S. Kim, *Process Biochem.*, **49**, 1543-1554 (2014).
5. B. S. Giri, and R. A. Pandey, *Bioresour. Technol.*, **142**, 420-427 (2013).
6. G. Leson, and A. M. Winer, *J. Air. Waste. Manage. Assoc.*, **41**, 1045-1054 (1991).
7. R. S. Singh, B. N. Rai, and S. N. Upadhyay, *Process Saf. Environ. Prot.*, **88**, 366-371 (2010).
8. R. S. Singh, B. N. Rai, and S. N. Upadhyay, *Environ. Technol.*, **27**, 349-357 (2006).
9. S. J. Ergas, K. Kinney, M. E. Fuller, and K. M. Scow, *Biotechnol. Bioeng*, **44**, 1048-1054 (1994).
10. A. Mallakin, and O. P. Ward, *J. Ind. Microbiol.*, **16**, 309-318 (1996).
11. A. K. Shukla, R. S. Singh, S. N. Upadhyay, and S. K. Dubey, *Bioresour. Technol.*, **101**, 8119-8126 (2010).
12. J. Huang, and K. L. Pinder, *Biotechnol. Bioeng.*, **45**, 212-218 (1995).
13. C. Alonso, M. T. Suidan, G. A. Sorial, F. L. Smith, P. Biswas, P. J. Smith, and R. C. Brenner, *Biotechnol. Bioeng.*, **54**, 583-594 (1997).
14. B. S. Giri, S. N. Mudliar, S. C. Deshmukh, S. Banerjee, and R. A. Pandey, *Bioresour. Technol.*, **101**, 2185-2190 (2010).
15. A. Sharma, P. Singh, S. Kumar, P. L. Kashyap, A. K. Srivastava, H. Chakdar, R. N. Singh, R. Kaushik, A. K. Saxena, and A.K. Sharma, *Geomicrobiol. J.*, **32**, 170-180 (2015).
16. K. Tamura, J. Dudley, M. Nei, and S. Kumar, *Mol. Biol. Evol.*, **24**, 1596-1599 (2007).
17. C. Lu, M. R. Lin, and C. Chu, *J. Environ. Eng.*, **125**, 775-779 (1999).
18. D. W. Park, S. S. Kim, S. Haam, I. S. Ahn, E. B. Kim, and W. S. Kim, *Environ. Technol.*, **23**, 309-318 (2002).
19. G. Gallastegui, A. Avalos Ramirez, A. Elias, J. P. Jones, and M. Heitz, *Bioresour. Technol.*, **102**, 7657-7665 (2011).
20. G. Gallastegui, A. Barona, N. Rojo, L. Gurtubay, and A. Elías, *Process Saf. Environ. Prot.*, **91**, 112-122 (2013).
21. Y. Zhu, S. Li, Y. Luo, H. Ma, and Y. Wang, *PeerJ*, **4**, e2045 (2016).
22. T. M. Chong, W. F. Yin, J. W. Chen, S. Mondy, C. Grandclement, D. Faure, Y. Dessaux, and K. G. Chan, *AMB Express*, **6**, 95 (2016).