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Benzene Biodegradation and its Kinetics Study by Sphingomonas sp. and P.putida

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Abstract—*Benzene degradation potential of five bacterial communities namely Sphingomonas sp. (MTCC 8992), P. puidta (MTCC 1192), Rhodococcus rhodochrous (MTCC 1767), Bacillus sphaericus (MTCC 8103) and Alcaligenes xylosoxidans (MTCC 1685) were investigated in a batch experiments in concentration range of 50-500 mg l^{-1}. The Sphingomonas sp. degraded a maximum of 65 % of benzene followed by P. putida (64.2 %), Rhodococcus rhodochrous (56 %), Bacillus sphaericus (52 %) and Alcaligenes xylosoxidans (47 %). Sphingomonas sp.8992 and P. putida 1192 have shown approximately equal and maximum degradation potential for benzene at concentration of 250 mg l^{-1}. The temperature and pH for maximum degradation were optimized and found to be 30 ^oC and 7, respectively. Kinetic parameter non-inhibitory (Monod) model was fitted to the experimental data. The kinetic parameters such as maximum specific growth rate μ_{max}, half saturation constant K_s and ratio (μ_{max} / K_s) were also calculated and found to be approximately same for Sphingomonas sp. and P.putida.*

1. INTRODUCTION

The petrochemical industry has been classified as hazardous group of industries under the factories (Arafa 2003). The waste generated in the petrochemical industries mainly contains the complex hazardous compounds such as benzene, toluene, xylene and phenol, etc. In these compounds, benzene contamination is of great public concern throughout the world due to its solubility in water, toxicity, and volatility (Farhadian et al. 2009). It is widely used as industrial solvents for organic synthesis and equipment cleansing and consequently often release in the environment due to accidental leakage in underground storage tanks and pipelines, improper waste disposal practices, and leaching from landfills (Shim et al. 2005).

Due to its toxicity and carcinogenicity, benzene represents a significant threat to human health and is currently recognized as one of the most important contaminants in the environment.

The conventional remediation techniques, such as thermal, extraction, steam stripping, chemical oxidation, etc. are used to reduce high concentrations of pollutants (Takahata 2006). However, these processes have several limitations such as high cost and generation of additional toxic products. Hence, there is a scope for more reliable, economical and eco-friendly technique for the removal of pollutants from the contaminated environment. Bioremediation is a technique which satisfies the above requirements because it involves the manipulation of environmental parameters to allow the microbial growth and enhanced degradation of pollutants without any damage to the environment (Fulekar 2005).

2. MATERIAL AND METHOD

Microorganism and culture media

Organisms

The bacterium *P. pudita* (MTCC 1192) *Sphingomonas* sp. (MTCC 8992), *Rhodococcus rhodochrous (*MTCC 1767), *Bacillus sphaericus* (MTCC 8103) and *Alcaligenes xylosoxidans*.(MTCC 1685) were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India, in dry culture form. The cultures were revived in the nutrient broth liquid and further plating was performed on solid agar Petri dish. Stock cultures were then obtained by standard spread plate microbial techniques.

Chemicals and growth of inoculum

All the chemicals were purchased (AR grade) with more than 99.9 % purity. The chemicals were from Merck, India. The medium was sterilized in two parts to avoid precipitation of ferrous salts during autoclaving. The bacterial culture were grown in a defined mineral salts medium (MSM). Benzene was added as carbon source for cell growth, was aseptically added to MSM in laminar flow using a micro-syringe. The pH of the medium was adjusted to 7 by adding HCl. In a separate set of experiments, growth of five bacterial species was studied in presence of 50-500mg l^{-1} of benzene, respectively. The bacterial growth was regularly monitored by Elico UV-VIS spectrophotometer Model no SL-159 at 600 nm for 10 days.

Biodegradation of benzene

Biodegradation studies were carried out using five bacterial sp. at initial benzene concentrations of 50-500 mg l^{-1} Beyond the concentration of 250 mg l^{-1} inhibition was observed. For the biodegradation studies, all the bacterial species were initially pre-cultured in nutrient broth medium to increase the bacterial density. Cell suspensions of each bacterium with the volume of 1ml were inoculated in 100 ml MSM medium in separate serum bottles and sealed with butyl rubber stoppers and aluminum crimp caps containing each 50-500 mg l^{-1} of benzene. The incubation was done at 30 ^{0}C on a shaker at 150 rpm for 10 days. Residual benzene was analyzed by GC-FID, respectively, after 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 h of incubation periods. Benzene concentrations were analyzed using a thermo-fisher 7610 gas chromatograph (GC). Details are given in elsewhere (Kureel et al., 2015).

SEM observation and FTIR analysis

The bacterium *Sphingomonas* sp. and *P.putida* morphology was analyzed using scanning electron microscopy; a strip of the filter paper size (0.22 µm) was used to filter the bacterial sample. Further, the filter paper dried overnight at 40 ^{0}C in the oven. The morphology of the bacteria was examined using a low-vacuum in order to minimize the harm to the bacterium sample.

FT-IR analysis was carried out for bacterial liquid culture taken after 10 days. The spectrum of the functional group and the bands were recorded in the region from 4000 to 400 cm^{-1}.

3. RESULTS AND DISCUSSION

Results suggest that there is no substrate inhibition up to the respective maximum concentrations. Considering the response of bacterial species for maximum degradation, concentrations of 250, 200, 150, 100 and 50 mg l^{-1} was selected for biodegradation studies. The present study suggested that *Sphingomonas* sp. and *P. putida* have shown higher degradation potential for benzene followed by *Rhodococcus rhodochrous*, *Bacillus sphaericus* and *Alcaligenes xylosoxidans*. The growth of bacterial species in the presence of benzene at concentrations, 50-250 mg l^{-1} is shown in Fig. 1. The growth curves for different bacterial species at optimize concentration of benzene. Sigmoid growth curves were observed for all bacterial species in the presence of benzene. The variation of benzene concentrations with respect to time is shown in Fig. 2. The initial benzene concentration for five bacterial sp. was found to decrease with respect to time. It indicates that benzene is utilized as a carbon and energy source (Devinny et al. 1999).

Kinetics of biodegradation

In order to predict on evaluate the biodegradation of organic pollutants, researchers have developed mathematical models that includes substrate utilization, bacterial growth and decay and utilization of electron accepters. The kinetics of microbial growth and utilization of substrate is described using Monod kinetic model (Monod 1949). The Monod kinetic parameters were estimated for only substrate degradation curve with microbial yield coefficient obtain from two measured data points of microbial growth at initial and after complete degradation. The equation is as follows:

$$\mu = \frac{1}{x}\frac{dx}{dt} = \frac{\mu_{max} S}{K_s + S}$$

where µ is specific growth rate (h^{-1}), μ_{max} is maximum specific growth rate (h^{-1}), K_S is half-saturation constant (mg l^{-1}), X, S, and t are microbial cell, initial substrate concentrations

(mg l^{-1}), and time, respectively.

The estimated gowth kinetic parameters (μ_{max} and K_s) obtained from Monod model for *Sphingomonas* sp. and *P.putida* were 0.185 h^{-1}, 31.12mg l^{-1} and 0.134h^{-1}, 28.4 mg l^{-1}, respectivly**).** The values of μ_{max} and K_s in the present study were found to be higher than earlier reported values for *P.aeruginosa* (μ_{max} = .14 h^{-1} and K_s= 4.5 mg l^{-1}) (Kim et al. 2005). Higher values of μ_{max} and K_s in *Sphingomonas* sp. due to high substrate concentration. This is suggesting that these two parameters corelated to each other.

Scanning electron microscopy

Scanning electron micrographs corresponding to a free-biofilm and a ten-day biofilm development in presence of benzene as carbon source is shown in Fig. 4(a-d). The result shows that there was no change observed in the morphology of *Sphingomonas* sp. and *P.putida.* It could be conformed that bacterium have potential to withstand the pollutant load.

FT-IR analysis

The result indicates that benzene was degraded in liquid culture.

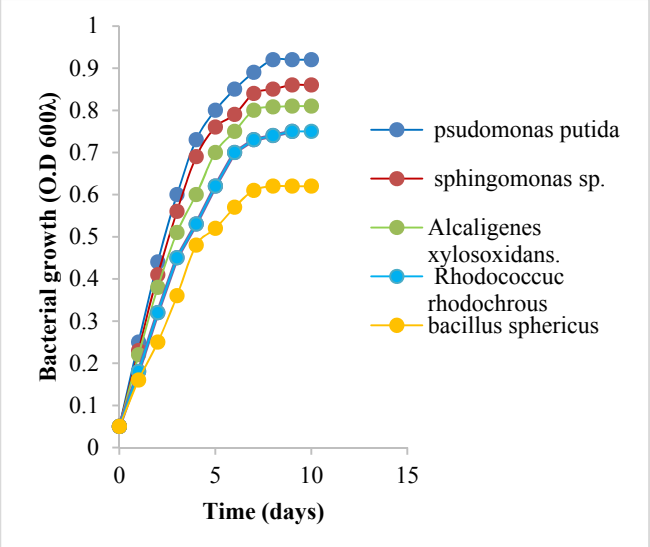

Fig. 1: Growth of different bacterial sp at same concentration of benzene at 50-250 mg/l.

The peak at band position 1636.3 cm^{-1} corresponding to the carbonyl groups (C=O) indicates that benzene was degraded by the *Sphingomonas sp and P.putida* during the experiment. In addition to above phenol and catechol was also detected. In the control sample the band spectrum of benzene was found in the range from 3196.9-3238.4 cm^{-1} is shown in Fig. 3b.

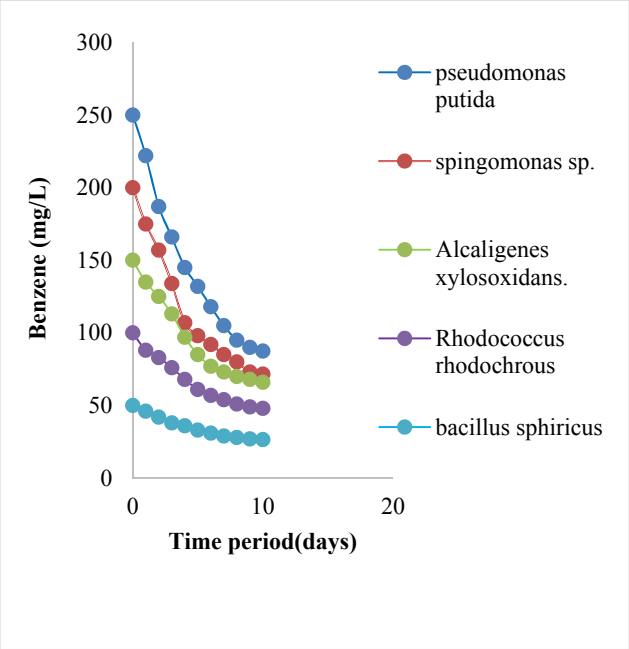

Fig. 2: Batch biodegradation of benzene at concentrations of 250 (*Sphingomonas* sp. and *P. putida*), 200 (*R. rhodochrous*), 150 (*Bacillus sphaericus*), 100 mg/l (*Alcaligenes xylosoxidans).*

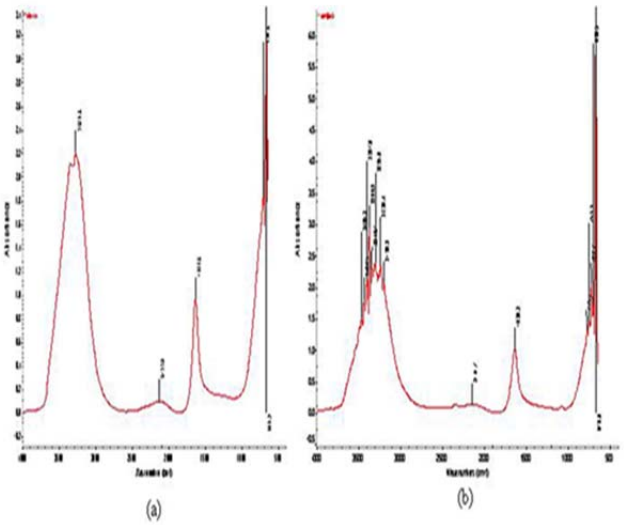

Fig. 3

Fig.3 (a) Band position of control sample (benzene), (b) inoculated sample

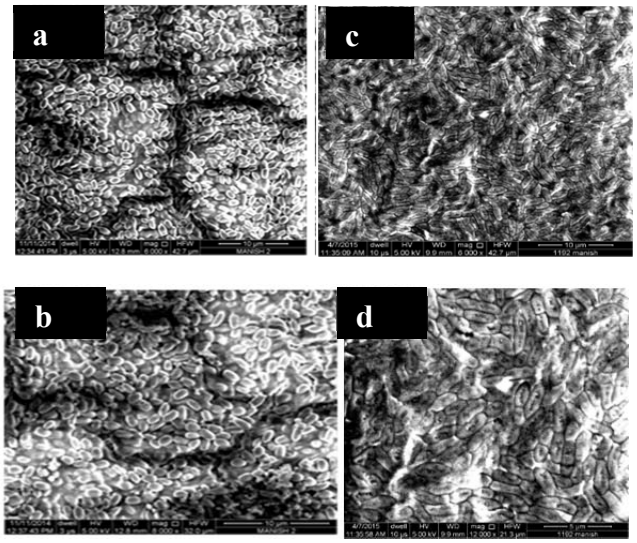

Fig. 4: Scanning electron micrographs of *Sphingomonas* sp.(a,b) and *Psuedomonas putida* (c,d) morphology (a.c) before and (b,d) after the experiment was observed no change in bacterial morphology.

4. CONCLUSION

In batch biodegradation experiments all five bacterial sp. exhibited the potential for benzene degradation. The kinetic model fitted well for the biodegradation of benzene. This is the first report of benzene utilization by *Sphingomonas* sp. Further, the efficacy of this bacterial species would be utilized for the removal of benzene in continuous bioreactor system. As indicated by the kinetic constants, this study would be helpful in the practical application of this bacterial sp. for removal of benzene from the contaminated environment.

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