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Bioremediation of Volatile Organic Compound Benzene by *P.putida(1192)*

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Abstract—*Benzene is volatile organic compounds commonly found in petroleum derivatives. Biodegradation appears to be an economical, energy efficient and environmentally sound approach for treating benzene contamination under aerobic conditions. Application of a sensitive, rapid, inexpensive, and reliable ecotoxicity test is vitally important in mixture toxicity studies. UV-VIS Spectrophotometry is employed as a general analytical tool for the characterization of the optical density (OD) microorganisum. Degradation of aromatic pollutants is essential in detoxification of wastewater and contaminated soils. The experiment was carried out for degradation of volatile organic compound benzene by bacteria P.putida(1192). at different concentration of benzene vary from 50 mg /L to 400 mg/L at different temperature and pH. The degradation and bacterial growth was studied. The maximum degradation was observed at 250 mg/L. at 30 ^{0}C and 7±0.1 pH.*

1. INTRODUCTION:

Petrochemical industry is the fastest growing industry, manufacturing variety of chemicals beneficial for the other chemical processes and operations. However the waste generated during the manufacturing of basic raw material and intermediates are of the prime concern to the environmentalist. The petrochemical industry has been classified as hazardous group of industries under the Factories Act, 1948 Section 2(cb) and requires the compliance within the prescribed standards (M.A. Arafa, 2003). Benzene can additionally cause hematological effects which may ultimately lead to aplastic anemia and development of acutemyelogenous leukemia (ATSDR, 2004). Due to its toxicity and relatively high solubility, benzene represents a significant human health threat and is currently recognized as one of the most important contaminants in the United States. The USEPA has set a maximum permissible level of benzene in drinking water at 5 µg/L and set a goal of 0 µg/L for benzene in drinking and surface water. Consequently, benzene is often a single compound that drives the need for corrective action at sites contaminated with petroleum product releases (Da Silva and Alvarez, 2007).

The wastes generated are commonly found to contain the complex hazardous compounds like benzene, toluene, xylene and phenol, etc. Benzene is one of the pollutants of petrochemical waste which is highly toxic and carcinogenic. Human exposure to benzene is a global environmental problem. After inhalation or absorption, benzene targets organs viz. liver, kidney, lung, heart and brain, etc. Benzene causes haematotoxicity through its phenolic metabolites that act in concert to produce DNA strand breaks and chromosomal damage (S.V. Rana, Y. Verma, 2005); hence benzene requires advance treatment to bring treated waste according to the prescribed level (K.J. Oh *et al*., 2002). In spite of the present physicochemical and biological treatment of waste the hazardous contaminants are still found persisting in the environment resulting into an increased *level* of pollution and causes environmental impact (S. Vigneswaran *et al*., 2001; M.S. Wei, K.H. Huang,2001). The ecologically acceptable treatment method for benzene and other waste hydrocarbons is amajor challenge confronting to the petrochemical industries as well as other chemical industries. Thus the recent advances in bioremediation techniques for the treatment of toxic waste will be of high significance (M.H. Fulekar, 2005). Bioremediation techniques are typically more economical than traditional methods of waste treatment such as incineration, absorbent/adsorbent techniques, catalytic destruction, etc. Bioremediation technologies are improving as greater knowledge and experience are being gained in the field. Bioremediation application can be more effective where environmental conditions permit microbial growth and activity; its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate (M.H. Fulekar, 2005). Objective of the research is to determine the potential of bacterial community *pseudomonas putida, Rhodococcus rhodochrous. and bacillus sphaericus for* bioremediation of benzene in laboratory scale batch bioreactor and to optimize the performance parameters (of pH, temperature, and concentration).

2. MATERIAL AND METHOD

2.1 Microorganism and culture media

This VOC,S degrader was purchased from Microbial Type Culture Collection and Gene Bank Institute of Microbial Technology Sector 39-A, Chandigarh–160036 (India). Cells were grown in a minimum medium contained different amount of benzene (50–250mg/L) as a sole source of carbon. All chemicals and benzene are purchased from gyan scientific merk product purity of 99.9%. and composition of mineral salt medium was K_2HPO_4, 4.27 g/L; KH2PO4, 3.48 g/L; $(NH_4)_2SO_4$, 0.34 g/L; $MgSO_4.7H_2O$, 0.46 g/L; $FeSO_4$, 0.001 g/L; $CaCl_2.2H_2O$, 0.018 g/L; $CuCl_2.2H_2O$, 0.01 mg/L; $CoCl_2.6H_2O$, 0.2 mg/L; $ZnSO_4.7H_2O$, 0.1 mg/L; $MnCl_2.4H_2O$, 0.03 mg/L; $Na_2MoO_4.2H_2O$, 0.03 mg/L; and $NiCl_2.6H_2O$, 0.02 mg/L.

2.2 Batch experiments

Batch experiment was studied in laboratory *P.putida*. Growing in nutrient (MSM) mineral salt medium containing benzene as sole carbon and energy source was harvested in the late exponential phase. The experiments were conducted in 100-mL serum glass bottles containing 80 mL of the mineral salt medium and diffent amounts of benzene concentration. Media and materials were all sterilized and control were included in all experiments. The bottles were incubated in at 30^0C shaken at 150 rpm. The variation of biomass and substrate concentrations in each bottle was then monitored periodically.

2.3 Analytical methods

The suspended cells concentration was determined by measuring the optical density at 600 nm with a UV/VIS The suspended cells concentration was determined by measuring the optical density at 600 nm with a UV/VIS spectrophotometer (Elico-6000, Hanson Tech. Co. Ltd., Korea) based on the established standard curve of optical density versus time. Benzene degradation was analysed by GC (gas chromatograph equipped with flame ionization detector FID) capillary column BP-5. Injector , detector and column temperature was hold at 150, 150 and 100 0C, respectively, Nitrogen gas surved as carrier gas and oxygen and hydrogen as fuel gas for the FID.

3. RESULT AND DISSCUSSION:

The fig.1 shows growth curve of deffernt bacterial species and maximum growth was observed for bacterial sp *P.putida.* and the fig.2 shows variation of benzene with respect to time and maximum degradation was observed at 250mg/l of benzene. following batch experiment was performing in laboratory .

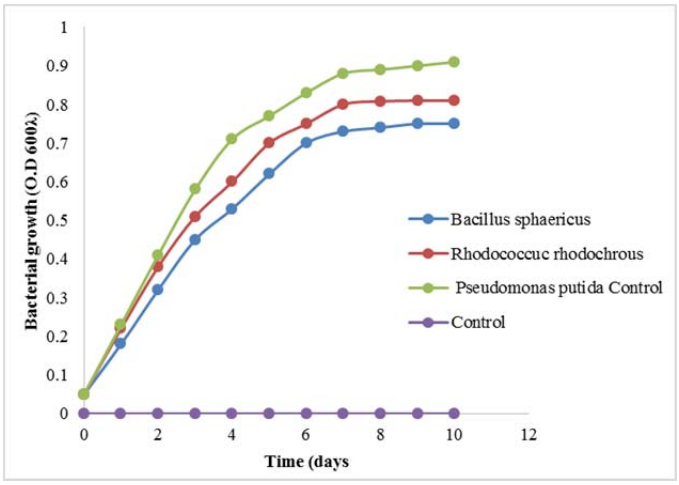

Fig. 1: Show bacterial growth vs time at same concentration of benzene at concentration of 250mg/l.

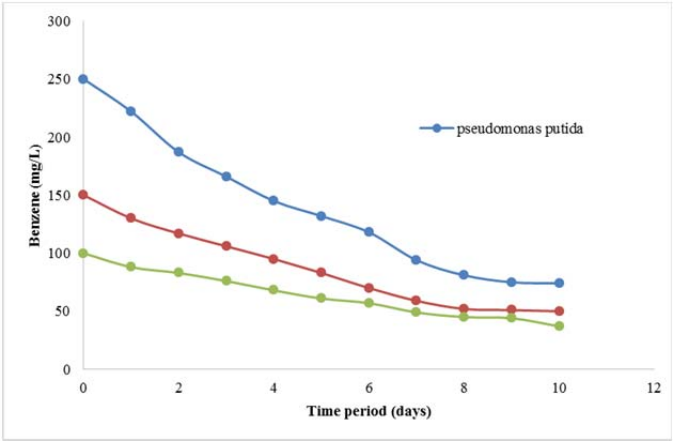

Fig. 2: Show degradation of benzene with respect to time.

4. CONCLUSION

This study show that the use of *P.pudida* for benzene degradation is more efficient bacterial specie then then other two sp. Maximum growth and degradation was observed for this sp.The results show that *P.putida* exhibit the greatest efficacy in benzene degradation. Therefore the present research study on bioremediation of hazardous compounds using *P. putida* 1192 as potential organism in Batch experiment has provided an innovative research in the area the of bioremediation.

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