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Biodegradation of benzene in a batch reactor using Indigenous mixed microbial culture

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Abstract: Benzene is a volatile organic compound, which is commonly used as process solvent in Industry. It has severe effect on atmospheric environment and human health. The removal of benzene is carried out by biological process. A series of batch experiments were performed using mixed culture to examine the biodegradation of benzene. Batch degradation of benzene was carried out at initial concentrations varying from 50 to 400 mg/l to obtain the kinetic parameters of microbial growth. The results revealed the feasibility of biodegradation of these compounds with maximum specific growth rate of 0.047 hr^{-1}. The maximum removal efficiency of benzene was obtained at 50 mg/l (95%).The degradation rate are calculated. From the biodegradation experiment the substrate inhibition was found to be at 200 mg/l.
Key words: biodegradation, Benzene, microbial growth, degradation.

1.Introduction

Air pollution has become a topic of increasing environmental concern in many cities all over the world. Of particular importance in recent years, are the volatile organic compounds (VOCs), which arise from many industrial operations, particularly involving organic solvents. Benzene is a VOC used primarily as a raw material in the synthesis of styrene, nylon, aniline, polyester resins, detergents, and other products used in the production of drugs, dyes, dry cleaning process, insecticides, and plastics. New coking, liquefaction, and gasification processes for coal are all potential sources of benzene. Benzene is used as an additive in gasoline, but it also is present naturally in gasoline, because it occurs naturally in crude oil and is a by-product of oil refining processes. The percentage of benzene in unleaded gasoline is approximately 1 to 2 percent by volume.

Benzene is used as a constituent in motor fuels**;** as a solvent for fats, waxes, resins, oils, inks, paints, plastics, and rubber; in the extraction of oils from seeds and nuts; and in photogravure printing. Benzene is used as a chemical intermediate. Benzene is also used in the manufacture of detergents, explosives, pharmaceuticals, artificial leather, linoleum, oil cloth, varnishes, lacquers, and dyestuffs. [1, 2]

The health effects of acute (short-term) exposure to benzene through inhalation consist mainly of nervous system effects including decreased visual, auditory, and motor functions but these effects are reversible once exposure ceases. Long-term exposure to benzene has the potential to cause chronic health effects including central nervous system (CNS) damages, cardiac effects, and lung cancers. [3, 4].There are several physico-chemical technologies for benzene removal from waste gas streams and liquid streams, but these methods require high capital investment and running costs especially when dealing with high flow rates containing low pollutant concentrations. Biological processes as cheap, environmental friendly, simple and reliable technologies are promising alternatives to control benzene pollution in the waste water. [5]

Previous research and literature has mentioned the effectiveness of many bacterial strains with regard to benzene degradation, and the most commonly mentioned one is Pseudomonas spp., especially the Pseudomonas

putida [6, 7, 8]. It has been the popular research strain of Pseudomonas, but other strains of Pseudomonas have also been studied in benzene degradation, such as: P. aeruginosa[9]; Jean et al. [10] used a glass sand tank in the laboratory to study the effect of inorganic nutrients (sulfate, phosphate, and ammonium chloride) on the aerobic biodegradation of benzene by Pseudomonas spp., revealing that the increase in nutrient levels resulted in enhanced bacterial growth and benzene degradation.

A great deal of research has been conducted on the use of different bacteria for benzene remediation and many Studies use pure strain in petroleum carbon-chemical remediation, but there are only few reports that specifically mention the use of mixed culture for benzene biodegradation. In this study the mixed culture was used for pollutant biodegradation (benzene) and evaluates their biodegradation efficiency.

3. Materials and Methods

3.1 Microorganisms and Culture Media:

All chemicals used in this study were of laboratory and HPLC grade. Toluene (both laboratory and HPLC grade) were purchased from Merck Limited, India. The microbial mixed culture was obtained from Cow dung's Compost. The culture was initially grown in 250 ml Erlenmeyer flask containing 100 ml of mineral salt medium (MSM) containing the following composition (g/L): Na_2HPO_4 – 5, K_2HPO_4 – 4.0, KH_2PO_4 – 4.0, $(NH_4)_2$ PO_4 – 1.0, $MgSO_4.7H_2O$ – 0.25, $CaSO_4$ – 0.25, $FeSO_4.H_2O$ – 0.08, Dextrose – 2.0 in distilled water. The pH of the mineral salt media was adjusted to 6.85 and the cultures were grown under ambient condition.

3.2 Batch Degradation Studies:

The mixed microbial culture was pre-cultured in 100 mL of the MSM containing 25 mg/L of benzene as the carbon source for about 48 hrs. The batch culture biodegradation of benzene was carried out over a concentration range of 50-400 mg/L of benzene individually in 250 mL Erlenmeyer flasks. Flasks were closed with cork and sealed with aluminum foil to minimize the loss of benzene by evaporation. Samples collected at regular intervals were analyzed for biomass and residual benzene concentration. The samples were analyzed using HPLC equipped with UV-detector.

3.3 Biomass concentration:

The biomass concentration was estimated using wet weight method. The 1ml of sample was taken in eppendorf tubes. Then it was centrifuged at 2000 rpm for 20 minutes. The supernatant was separated from biomass. The eppendorf tube containing biomass was weighed. The difference between the weight of eppendorf tube with biomass and the empty eppendorf tube was used to the biomass concentration.

3.4 Benzene concentration (liquid phase):

Benzene concentration was determined by using High Performance Liquid Chromatography (HPLC) equipped with UV detector using LC solution software.The analytical conditions of HPLC for toluene were maintained as mentioned as follows: Column: C18, Mobile phase: Methanol/H_2O (70:30), Flow rate: 1ml/min, UV wavelength: 254nm, Pump A: 135 Kg F/cm^2 , Pump B: 135 Kg F/cm^2 ,Injection volume: 20µl

4. Results and Discussion

Batch Degradation Studies (Benzene)

The effect of benzene on the growth of mixed cultures, the growth profile was determined at various concentrations of benzene in the range 50 - 400 mg/L in the mineral salt medium. The biomass growth profiles are shown in Figure 1. It was observed that the biomass growth profile, particularly at low benzene concentrations was typical of a conventional biodegradation process with lag, log and stationary phases. However, at the highest concentration at 200 mg/L the growth rate was significantly reduced. Figure 2 shows the specific growth rate for different initial concentrations of benzene. Complete removal of benzene was not achieved in the range of substrate concentrations in this study. However at low concentrations (50 mg/L) nearly 95 % of benzene was removed by the mixed cultures. The maximum specific growth rate is 0.047 hr^{-1}.

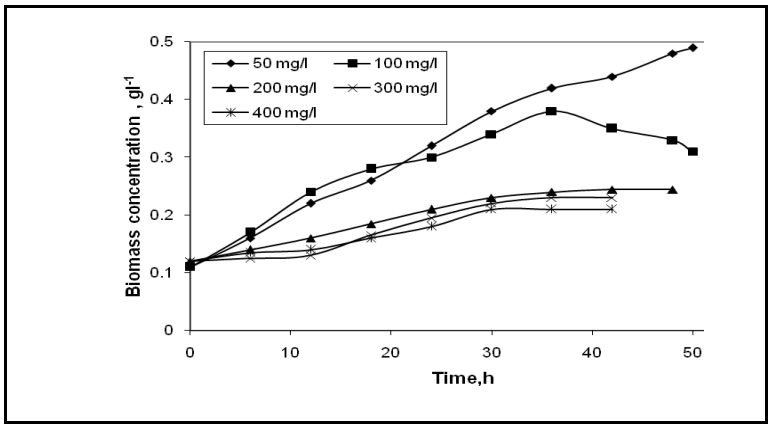

Fig. 1. Biomass growth profile during benzene biodegradation

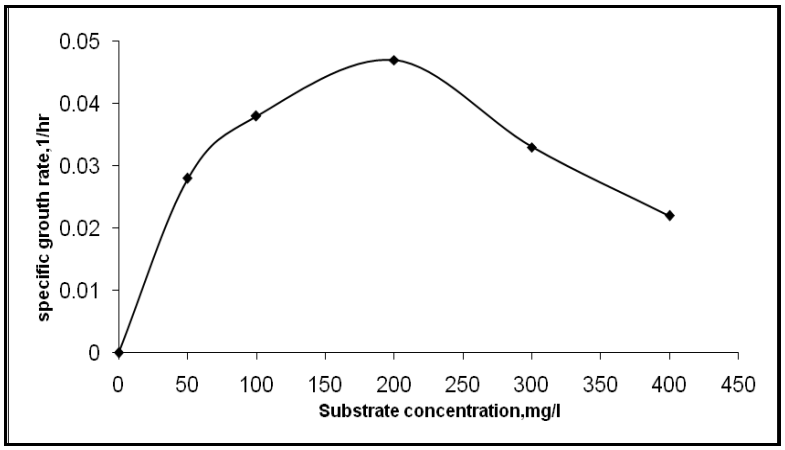

Fig .2. Specific growth profiles at different initial concentrations of benzene

The degradation rates were calculated on the basis of the equilibrium concentration reached at each concentration during the biodegradation study and plotted against benzene concentration in Figure 3.

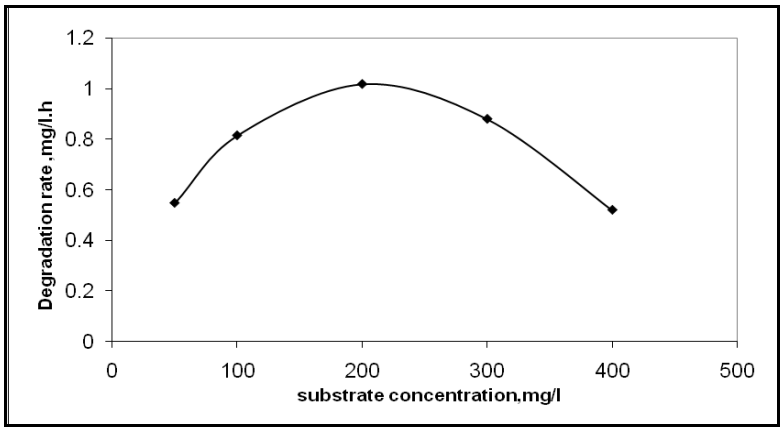

Fig .3. Degradation rate profile of mixed cultures at different initial concentrations of benzene

The degradation rate first increase with benzene concentration and then decreased with increasing concentrations of benzene in the liquid culture. Maximum degradation rate is found to be 1.08 mg l^{-1} h^{-1}.These profiles indicate that the microbial metabolism was inhibited at higher concentration of benzene. The inhibition of benzene degradation may be due to the effect on cell metabolisms as a result of production of acidic intermediates. It may also be due to the inhibition of cell growth resulting from changes at cellular and genetic levels. The presence of high concentrations of toxic compounds like benzene is reported to have an effect on the growth of cells utilizing them as the sole carbon and energy source, which is attributed to changes occurring at the cellular and molecular levels. The formation of hydroxyl radical and acid metabolites during high benzene

concentration also affects the degradation pattern. Similar inhibition effect was observed in benzene biodegradation using *Pseudomonas putida* [11].

4. Conclusions

A mixed culture from waste water treatment plant was acclimatized to benzene. The biodegradation potential of acclimatized mixed culture to utilize benzene was tested in batch experiments at initial concentrations of waste water ranging from ~50-400 mg/L. The culture was able to grow well with benzene as the sole carbon source at the concentration range studied, despite the prevalence of inhibition at high concentrations of substrate with a specific growth rate maximum of 0.047 h^{-1}. The mixed cultures exhibited substrate inhibition concentration higher than 200 mg/L of benzene. The maximum degradation rate is found to be 1.08 mg l^{-1} h^{-1}.

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