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Effect of pH and Inoculums Size on Benzene Biodegradation using Mixed Culture

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ABSTRACT

Benzene is a toxic compound which is widely used as raw material in synthesis of styrene, nylon and in production of drugs, dyes, plastics etc. Because of toxicity, there is need to decontaminate the benzene. Benzene in waste water and biodegradation is very useful alternation to conventional clean – up methods. The success of this depends on mixed culture able to degrade in a changeable environment. The aim of work is to study the influence of pH of the medium and effect of inoculum size on benzene biodegradation by mixed culture. The effect of pH and inoculums size on biodegradation of benzene at initial concentration of 100 mg/L. pH is varied from 3.0 to 9.0. Benzene degradation was rapid at neutral pH (7.0) and decrease further increase or decrease pH. Benzene was degraded at different inoculum sizes. The degradation increases with inoculum size, but culture inoculated at 2.5 x 107 CFU/mL shows maximum degradation rate. This result is useful to understand the physiological and biochemical characters of mixed culture at changeable environmental condition.

1. Introduction

Benzene is used primarily as a raw material in the synthesis of styrene (polystyrene plastics and synthetic rubber), phenol (phenolic resins), nylon, aniline (a colorless/odorless benzene derivative), 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 for printing and lithography, paint, rubber, dry cleaning, adhesives and coatings, detergents, extraction and rectification, preparation and use of inks in the graphic arts industries; as a thinner for paints and as a degreasing agent. Benzene is used as an additive in gasoline. Benzene is used as a constituent in motor fuels [1].

Benzene is volatile xenobiotic compounds that are prevalent in industrial wastewaters and gasoline or petroleum contaminated ground waters. Benzene is regarded as the most hazardous compound of the BTX group. The International Agency for Research Cancer (IARC) and United State Environmental Protection Agency (USEPA) have classified benzene to be a Group A and Class 1 human carcinogen respectively [2]. Short term human exposures to relatively high concentrations of benzene can give rise to various adverse health effects such as headaches, dizziness, inability to concentrate, impaired short term memory and tremors. While long term exposures can give rise to more complex health effects that include haematotoxicity, genotoxicity, immunological and reproductive effects as well as various cancers. Various studies have shown chronic exposures to benzene with service station attendants may result in the occurrence of cancer and other adverse health effects [3].

Various physical-chemical methods were reported in the literature for the treatment of benzene. However, biological treatment is an attractive approach for removing benzene from contaminated water. Biological treatment appears to be an economical, energy efficient and environmentally sound approach for treating benzene contaminated water. Microorganisms are able to degrade benzene under aerobic, as well as anaerobic conditions [4].

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* sp. [5].

The success of bioremediation may depend on the availability of microbial strains that can mineralize high levels of benzene and withstand adverse conditions to compete under *in situ* conditions. Effective bacterial inoculums should be able to tolerate high levels of benzene while maintaining a level of activity to provide efficient mineralization. Understanding the physiological and biochemical properties of benzene-degrading bacteria is required before optimum use of bacteria in environmental applications [6, 7].In order to find a strain able to degrade benzene in a changeable environment; we studied the effect of inoculums size and the influence of the pH of the medium on benzene degradation by a mixed culture. The objective of this research is to study the effect of inoculums size and pH on biodegradation of benzene using mixed culture.

2. Experimental Methods

2.1 Microorganisms and Culture Media

The microbial mixed culture was obtained from cow dung 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 – 5.0, 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.

2.2 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 at concentration of 50 mg/L of benzene at different pH and inoculums size. 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.

2.3 Analytical method

An HPLC chromatograph equipped with a UV detector (Model UV-1700 Shimadzu) was used for analysis. The UV detector was set at 254 nm. The high performance liquid chromatography column was a C18 bond pack 3µm (25 cm × 4.6 mm) analytical column. Chromatography was isocratic in a mobile phase consisting of water-methanol (30:70). The flow rate was set at 1 mL/min. All chemicals and water used were HPLC grade.

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3. Results and Discussion

3.1 Enrichment of mixed culture

Benzene degrading mixed microbial culture was isolated and enriched form a cow dung compost. The culture was initially grown in 250 mL Erlenmeyer flask containing 100 mL of mineral salt medium (MSM) .The pH of the mineral salt media was adjusted to 7.0 under agitation condition (200 rpm). The culture was acclimatized for a period of 3 weeks to grow in MSM containing benzene as the sole carbon source. This enriched mixed culture is used for biodegradation experiment.

3.2 Effect of pH on benzene degradation

Fig. 1 shows the removal performance of benzene with respect to various pH values. When the pH increased from 3.0 to 9.0, the initial benzene concentration decreased from 100 mg/L to 27 mg/L (removal efficiency of 73 %) after 26 hours. Further increase of pH values beyond 7.0 resulted in a gradual increase of residual benzene concentrations indicating lower benzene degradation efficiency. Hamed *et al*. studied the effect of pH on benzene degradation and showed the degradation of benzene was higher at pH 6.7 [8]. Similar study was carried out by Li *et al* [9] on benzene and it derivatives using *planococcus* sp. *ZD22.* Its optimal conditions for biodegradation of benzene were 20 °C at pH 9.5.

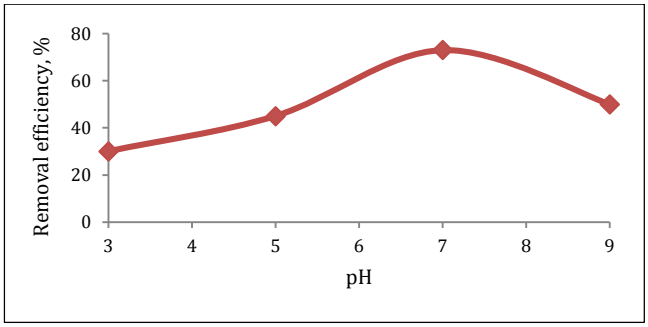

Fig. 1 Effect of pH on benzene degradation

3.3 Effect of inoculum size on benzene degradation

Fig. 2 compares the removal performance of benzene with respect to inoculums size. When the inoculums size increased from 2 to 6 mL, the initial benzene concentration decreased from 100 mg/L to 30 mg/L (removal efficiency of 70 %) after 24 hours. Further increase of inoculums beyond 4 mL resulted in a gradual increase of residual benzene concentrations indicating lower benzene degradation efficiency. When the inoculums size was varied to 6 mL, the residual benzene concentration was as high as 60 mg/L. You *et al* [10] studied the effect of inoculums size on benzene degradation and showed the degradation of benzene was higher at inoculums size of 5 mL. Reardon *et al* studied the effect of the inoculum size, expressed as an initial substrate mass to initial biomass ratio (S_0/X_0), was evaluated in batch cultivations with benzene. The optimum value found to be 30g/g [11].

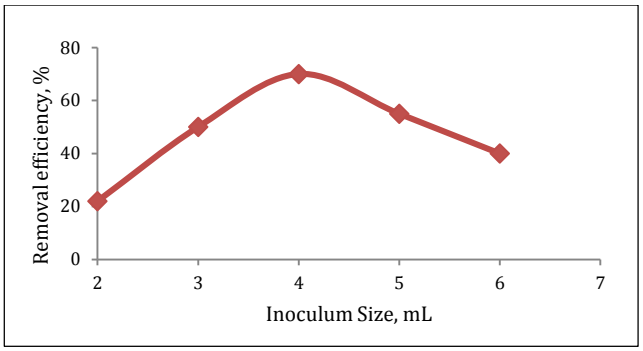

Fig. 2 Effect of inoculums size on benzene degradation

4. Conclusion

Our study showed the change in pH values of the culture medium and change in inoculums size affected the specific rate of benzene biodegradation by mixed culture. At pH of 7.0 and inoculums size of 4 mL ($2.5x10^7$ CFU/mL) were optimal conditions to obtain the maximal degradation of benzene in batch experiment systems. The information provided here can be used to optimize degradation condition in the field, by adjusting the pH to provide the inoculums with competitive advantages over the natural flora.

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