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Benzene biodegradation by indigenous mixed microbial culture: Kinetic modeling and process optimization

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ABSTRACT

Benzene is one of hazardous pollutants generated from paint, chemical, and petrochemical industries have a harmful impact on human health and the atmosphere. This study reports benzene biodegradation by indigenous mixed microbial culture in shake flasks over a concentration ranging from 25 to 600 mg/l, and the kinetics involved in the process has been modeled. Experimental data obtained were fitted to both inhibition and noninhibition models to determine the biokinetic constants. Haldane model was best to predict the experimental data. The central composite design was further used for optimization of pH and benzene concentration to enhance the benzene biodegradation. At an optimum pH of 7.05 and benzene concentration of 332.82 mg/l, the maximum estimated specific growth rate and degradation rate were 0.05 1/h and 6.01 mg/l h, respectively. The LC-MS analysis of sample indicate the presence of catechol, cis-1,2-dihydrobenzene-1,2-diol, and 2-hydroxymuconate semialdehyde as intermediates, which justifies the developed pathway of benzene biodegradation. The predominant microorganism in the mixed culture responsible for benzene degradation was later identified to be *Enterobacter cloacae* SG208. The results provide insight into the process of benzene biodegradation and prove the potential of indigenous mixed culture for treatment of benzene.

1. Introduction

Benzene is one of major VOCs produced from natural and anthropogenic activities. Benzene has vast applications in industries and also causes environmental pollution (Liu et al., 2010). It is the main component in petrochemical effluents and its exposure is a global environmental problem (Singh and Fulekar, 2010). Both liquid and gaseous benzene at higher concentrations can cause a significant threat owing to their toxic and carcinogenic properties (Rene et al., 2015), and cause both short-term as well as long-term health effects (MDH, 2010). Physico-chemical methods have many drawbacks - generate a huge amount of secondary wastes, high energy cost, and serious consequences on the environment (Padhi and Gokhale, 2014). Therefore, the biodegradation method will be of high significance and eco-friendly for the treatment of benzene.

Microorganisms metabolize aromatic hydrocarbons for their growth and development, which is an effective way of treating hazardous wastes (Ojumu et al., 2005). Several microorganisms are isolated and identified having ability to degrade aromatic hydrocarbons such as benzene and its derivatives, from which *Pseudomonas* is the most efficient strain (Ferhan et al., 2002). Benzene biodegradation is investigated by many researchers using pure culture (Reardon et al., 2000; Zhang et al., 2013), coculture (Shim and Yang, 1999), mixed microbial culture (Maliyekkal et al., 2004), and activated sludge (Lodaya et al., 1991). The effect of benzene, toluene, and xylene concentration on the specific growth rate and degradation rate of mixed microbial culture was studied by Maliyekkal et al. (2004). Monod and Haldane kinetic models can accurately predict the biodegradation of toxic compounds like benzene and toluene (Priya and Philip, 2013). Kim et al. (2005) investigated the kinetics of benzene biodegradation and estimated the biokinetic constants like maximum specific growth rate (μ_{max}), half saturation constant (K_s), and inhibition constant (K_i) using *Pseudomonas* species.

The microbial cell growth varies for different external source of carbon unless they are acclimatized to that specific substrate. However, previous studies showed that even after acclimatization microorganism exhibit inhibition due to the high toxicity of substrate (D'adamo et al., 1984). High substrate concentration inhibits

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the growth of microorganism whereas low substrate concentration leads to starvation (Singh and Fulekar, 2010). Therefore, determination of optimum pollutant concentration for microorganism in biodegradation is extremely important. Previous studies have applied one-factor-at-a-time (OFAT) method for process optimization to improve biodegradation. It, however, takes more time and is inefficient in determining the interactions among variables and prediction of optimum environment (Zu et al., 2013). Therefore, using an alternative optimization method based on response surface methodology (RSM), such as central composite design (CCD), overcomes the above mentioned drawbacks (Yao et al., 2009). The same method was used by Sahoo et al. (2010) to optimize the media components to enhance 4-chlorophenol biodegradation and the specific growth rate of the culture. Several studies have demonstrated degradation of benzene along with other pollutants, but studies on degradation of benzene as a single pollutant and its kinetic modeling is scarce in the literature. Moreover, no work has been reported on process optimization of benzene biodegradation. In this study, the optimum environment for benzene biodegradation was determined, which may be applied in a large-scale biological system to enhance the degradation of benzene from industrial waste streams.

In this study, batch kinetics has been performed by varying the benzene concentrations from 25 to 600 mg/l to determine the specific growth rate and degradation rate using mixed microbial culture. The experimental data has been used to determine the biokinetic constants such as μ_{max}, K_s, and K_i using Haldane, Monod inhibition (hyperbolic inhibition) and Aiba (exponential inhibition) substrate inhibition models, and noninhibition model of Monod. Further, CCD followed by RSM has been employed to optimize the different process variables to enhance the specific growth rate and degradation rate of the culture. At the optimum combination, the metabolic intermediates produced during benzene biodegradation have also been analyzed. An attempt has been made to isolate the predominant benzene degrading microorganism in mixed culture and identified using morphological, biochemical, physiological, and 16S rDNA techniques.

2. Materials and methods

2.1. Nutrient media

The composition of mineral salt medium (MSM) reported by Vrionis et al. (2002) used in this study with slight changes in grams per liter included: $(NH_4)_2SO_4$ (2), K_2HPO_4 (1.6), KH_2PO_4 (0.8), $MgSO_4 \cdot 7H_2O$ (1.0), $CaCl_2$ (0.125), $ZnSO_4 \cdot 7H_2O$ (0.06), $MnSO_4$ (0.0375), H_3BO_3 (0.0225), $NiCl_2$ (0.018), $COCl_2$ (0.014), $(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$ (0.0134), $FeCl_3$ (0.003), and $CuSO_4 \cdot 5H_2O$ (0.0025). The pH was 6.7 ± 0.05, after supplementing all the nutrients in the MSM. Before enrichment of mixed microbial culture, MSM was sterilized by autoclave at 121 °C for 15 min.

2.2. Enrichment of mixed microbial culture

The activated sludge was collected from a wastewater treatment plant of petrochemical industry located in Guwahati, India. The sludge was initially allowed to settle for a few hours, and then 10 ml of settled sludge was mixed with 100 ml of saline water (1%). After shaking for 30 min the sludge was again allowed to settle for 1 min to remove the inorganic solids. Further, supernatant was centrifuged (Remi, India) at 10,000 rpm for 3 min to obtain the microbial cells. This technique was used by Datta and Philip (2012) to isolate the mixed microbial culture from activated sludge. The microbial culture was then enriched in MSM containing 1 g/l of glucose by varying benzene concentration in the range of 25–600 mg/l under the agitation of 150 rpm at 30 °C for a period of 16 days.

2.3. Analytical methods

2.3.1. Cell density

The cell density or cell growth was determined by measuring its absorbance at 600 nm wavelength (OD_{600}) using a UV-visible spectrophotometer (Cary 50, Varian, United States). OD_{600} was then expressed as dry cell weight by a calibration curve plotted between OD_{600} versus dry weight of biomass (Sahoo et al., 2014). One unit of OD_{600} was equal to 525 mg/l of dry cell weight.

2.3.2. Benzene analysis

Benzene concentration in samples was estimated by gas chromatography (GC, Model: Dhruva, CIC, Vadodara, India) equipped with a flame ionization detector is briefly mentioned in our previous paper (Padhi and Gokhale, 2016). As per the standard method known amount of benzene was injected in n-hexane into a closed bottle equipped with Teflon septum and a calibration curve was prepared (Datta and Philip, 2012). Samples were centrifuged at 10,000 rpm for 10 min to separate out the cell mass from the supernatant, and then residual benzene was extracted with n-hexane for analysis in GC. The benzene concentration was determined from the calibration curve. In order to analyze metabolites during benzene biodegradation liquid chromatography mass spectrometer (LC-MS, Model: Q-Tof Premier, United States) was used.

2.4. Batch biodegradation studies

Before optimization, batch studies were carried out to understand the biodegradation kinetics of benzene at a medium pH of 6.7 ± 0.05 by varying the benzene concentration from 25 to 600 mg/l. Erlenmeyer flasks of 250 ml capacity contained 100 ml of MSM with Teflon liner screw cap and a sampling port on the bottom were used for the batch biodegradation studies. The enriched mixed culture was grown overnight and inoculated in the flasks so that to obtain an initial OD_{600} varied from 0.11 to 0.15. The flasks were kept on shaking incubator (LSI-1005R, India) at 30 °C with 150 rpm. For each concentration, batch experiments were performed in duplicate along with abiotic control. The flasks were removed at an interval of 6 h time span up to 12 h, followed by at every 12 h over a period of 72 h. Sample volume of 2 ml were collected from the sampling port of flasks using the gas tight syringe to estimate cell growth and their corresponding benzene biodegradation. In control flasks, benzene concentrations were decreased by 10–16% at the end of each batch study, and the degradation rates were estimated after correcting with the control experiments.

2.5. Experimental design for optimization

CCD based on RSM was employed for determining optimum pH and benzene concentration to enhance the specific growth rate and degradation rate of the culture. Two factors ($k = 2$) such as pH and benzene concentration were chosen as the variables. The total numbers of experimental runs were formulated as

$$2^k + 2k + n_0 \cdots \quad (1)$$

where, k is the number of factors, and n_0 is the number of replicates at the center point (Sahoo et al., 2010). Thus, a total of 13 experiments were carried in the present optimization study. For optimization, batch studies were conducted and samples were collected at regular intervals from each experimental run to analyze cell growth and benzene concentrations as described in detailed previously.