Microbial Degradation of Benzene by Mixed Cultures

Divya Baskaran and Ravi Rajamanickam

***Abstract*—** Benzene constitutes major volatile organic compounds (VOC's) produced from naturally and industrially due to heavy demand. It is mostly emitted from paint, chemical and petrochemical industries and released to atmosphere leading a severe environmental pollution. Benzene has a low boiling point or higher vapor pressure at room temperature; they evaporate easily and are hazardous to environment and humans. At higher concentration (>5 µg m-3) of benzene can generate ground level ozone after reacting with oxides of nitrogen which in turn forms smog that is also harmful to humans and vegetation. Benzene is a potent carcinogen and toxic in nature accordingly the health effects are acute and chronic exposure through cardiac effects, lung cancers and central nervous system (CNS) damages. In earlier, physico-chemical technologies are used to remove benzene from polluted air and liquid streams but these methods are requiring high energy and operational cost. Therefore, the biodegradation method will be of high significance and eco-friendly for the treatment of benzene. This study reports benzene biodegradation in a batch system by mixed microbial culture over a concentration ranging from 25 to 400 mg/l at room temperature with initial a pH value of 6.85. The specific growth rate increased with increase in substrate concentration up to maximum value of 0.047 h-1 at 200 mg l-1 and then decreased with increase in concentration. Maximum degradation rate was found to be 1.08 mg l-1 h-1, at high concentration of benzene affects the degradation pattern due to the formation of hydroxyl radicals and acid metabolites. At low fix concentration (50 mg l-1) nearly 95 % of benzene was degraded by the mixed culture. By fitting the specific growth rate with different substrate inhibition models (Haldane, Edwards and Levenspiel), biokinetic constants that are necessary to understand the kinetics of biodegradation process were evaluated. Among the equation Levenspiel biokinetic equation seem to be the best adequate expressions for specific growth rate on benzene with R2 (0.9851). Biochemical test and physical characteristic test were carried out in mixed microbial consortium. The result shows that Pseudomonas sp., Bacillus sp., Escherichia Coli are predominant species found in the mixed culture for the degradation of benzene.

***Keywords*—** Benzene, biodegradation, mixed culture, microbial characteristics, inhibition model

I. Introduction

The volatile organic compounds (VOCs) are emitted into the atmosphere from various industrial and manufacturing operations in large quantities [1,2]. According to The Clean Air Amendments Act (CAAA) of 1990 and modified Clean Air Amendments Act 2001 to regulate GHG (Green House Gases), identified 188 air pollutant out of this list 82 are volatile organic compounds [3]. Among all VOCs, of particular interest is benzene which is used in the production of paint, rubber, dyes and drugs, dry cleaning process especially used as an additive in motor fuels. Benzene is a xenobiotic compound, regarded as most hazardous compound via human carcinogen under the classification of The International Agency for research Cancer (IARC) and United State Environmental Protection Agency [4]. The health effects are actue and chronic exposure to benzene through cardiac effects, lung cancers and central nervous system (CNS) damages [5-7]. In European Union legislation postulates emission of benzene is 79 kt per year into the air and recommends the air quality guidelines with the permissible limit for same is 5 µg m-3 [8].

For air pollution control, the various physico-chemical technologies are used to remove VOCs from polluted air streams which is subjected to many researchers via condensation, absorption, incineration, adsorption, catalytic combustion, chlorination, photo catalytic oxidation, membrane separation etc [9,10]. From then on, these methods are requiring high energy and operational cost when treating of high flow rate of low pollutant concentration [6]. In this connection, biological techniques used for VOC treatment which has become more popular because of they are not only economical efficient and eco-friendly also produce innocuous end-products. Benzenes are free to degrade under anaerobic and aerobic conditions in the existence of microorganism [11,12]. In previous survey, reveals that study the biodegradation of aromatic compounds using pure or mixed microbial culture [13,14]. Bacteria such as pseudomonas and Bacillus were shown as most dominant species. The experimental data obtained from single pollutant was used to estimate the biokinetic parameters. Mixed pollutant studies were conducted to understand the concentration dependent interaction of the compounds in a biosystem. Benzenes are the better growth substrate having higher yield coefficient (1.2) in aerobic biodegradation which showed the faster growth in mixed culture [15]. At higher concentration of substrate inhibited the microorganism growth and starved at low concentration of substrate.

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Modeling the kinetics of microbial growth and its substrate inhibition pattern is an important aspect of any biodegradation study. One of the major problems was the enzyme inhibition in the bacterial degradation due to increasing concentration of degraded product [16]. Through the literature review a number of empirical models such a Haldane model [17], Levensipel model [19] and Edwards model [18] are describing substrate inhibition which are mathematically identical [20].

The main objective of this research was to determine the degradation rate of benzene using acclimated mixed culture in a batch biodegradation and identify the dominant species of microorganism accumulate in the mixed culture.

II. Materials and Methods

A. Culture Media

The mixed microbial culture was obtained from Cow dung Compost collected from nearby farmyard in Chidambaram, India. It contains nutrients such as phosphorus, potassium and nitrogen which are essential for the microbial growth. The composition of mineral salt medium (MSM) in g L-1 of distilled water: Na2HPO4 – 5.0, K2HPO4 – 4.0, KH2PO4 – 4.0, (NH4)2PO4 – 1.0, MgSO4.7H2O – 0.25, CaSO4 – 0.25 and FeSO4.H2O – 0.08. The culture was grown at the ambient condition and the pH of the mineral salt media was adjusted to 6.85.

B. Batch biodegradation

The microbial culture was pre-cultured in 100 ml of MSM containing 25 mg l-1 of benzene as the sole carbon source for about 48 hours. Biodegradation of benzene was carried out individually over a concentration range of 50 – 400 mg l-1 in 250 ml Erlenmeyer flasks. The samples were collected at regular intervals and analyzed for biomass and residual benzene concentration. The experiment were carried out for period of 48 h or until the residual concentration in the flask was found to saturate. The biomass concentration was estimated using wet weight method. Benzene concentration in liquid phase was determined by HPLC equipped with UV detector (Model UV-1700, SHIMADSU).

The analytical conditions of HPLC for benzene were maintained as mentioned as follows:

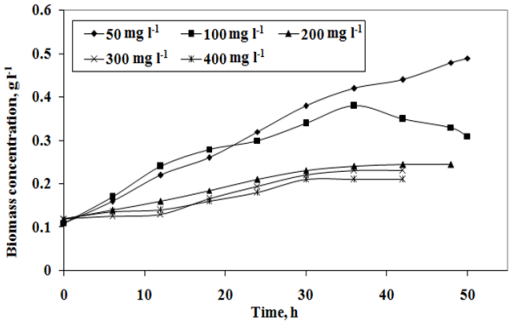
Column:	*C18,*
Mobile phase:	*Methanol/ H_2O (70:30),*
Flow rate:	*1ml min^{-1},*
UV wavelength:	*254 nm,*
Pump A:	*135Kg F cm^{-2}*
Pump B:	*135 Kg F cm^{-2},*
Injection volume:	*20 µl*

Biochemical identification of microorganisms was carried out using standard biochemical tests [21] and microscopic observations. Bergey's Manual of Determinative Bacteriology was used as a reference to identify the isolates [22].

III. Results and Discussion

A. Kinetics of benzene biodegradation

Acclimation of mixed culture isolated from the cow dung compost was carried out grow in the presence of benzene as a sole carbon source. At higher concentration, benzene found to be shown significant inhibitory effect on the cell growth [23]. Initially, glucose was used to adopt the acclimation stage, and later that the culture was grown only in accumulation of benzene. Typical pattern of biomass growth was observed as a function of time is shown in Fig. 1. It was observed that the concentration of benzene between 50 mg l-1 and 200 mg l-1 did not show any substrate inhibitory effect on microbes. From biomass development profile, especially at low benzene fixations was average of an ordinary biodegradation process with slack, log and stationary stages. Therefore, at higher benzene concentration (200 mg l-1) the growth rate was diminished. Fig. 2 shows that the specific growth rate for different initial concentration of benzene. The specific growth rate increased with increase in substrate concentration up to maximum value of 0.047 h-1 at 200 mg



l-1 and then decreased with increase in concentration. It indicates there is affected that the substrate inhibition at high concentration [24,20].

Fig. 1 Biomass growth profile

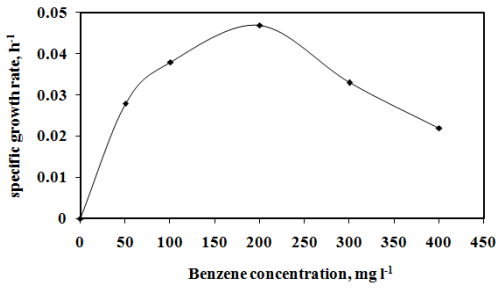


Fig. 2 Specific growth rates at different initial concentrations of benzene

This study was conducted to examine the effect of benzene concentration on the degradation using mixed culture. Fig. 3 shows that the degradation rate profile of mixed culture at different initial concentrations of benzene. From then on, complete removal of benzene was not attained to in the scope of substrate focuses in this study. Maximum degradation rate is found to be 1.08 mg l-1 h-1.This profile indicates that the microbial metabolism was inhibited at higher concentration of benzene. The inhibition of benzene degradation may be due to the effect on cell metabolism 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 concentration of toxic compound like benzene affects the growth of cells. It restricts the cell utilizing them as the sole carbon and energy source. The formation of hydroxyl radicals and acid metabolites due to high benzene concentration which is affects the degradation pattern [25]. Mathur et al. (2010) [26] reported similar inhibitory effect at 200 mg l-1 of benzene using Pseudomonas Putida. Shihabudeen et al. (2004) [27] also reported the substrate inhibition at 150 mg l-1 of benzene using enriched mixed culture.

The method of least squares used to evaluate the parameters μmax, Ks and K1 from the kinetic models. Fig. 4 shows that the experimental and predicted μ values for benzene biodegradation. The kinetic parameters evaluated from the models are given in Table 1, while some typical values observed from literatures on the aerobic degradation of benzene are given in Table 2. From then on, the higher KI value physically means, the culture is less sensitive to substrate inhibition and vice versa. It was observed that the standard deviation is low for the predicted specific growth rate. Levenspiel model indicates a high degree of fit for the models for benzene. However, the model predicted inhibitory concentrations deviated from the experimentally observed inhibitory concentrations in some of the cases. Such variations in model predictions of the inhibitory concentrations are often reported in the literatures [15, 20]. Since empirical nature in all models, it is not viable to use the parameter values as a measure of importance for inhibition.

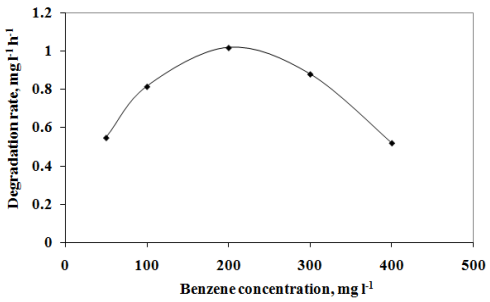


Fig. 3 Degradation rate profile at different Concentrations of benzene

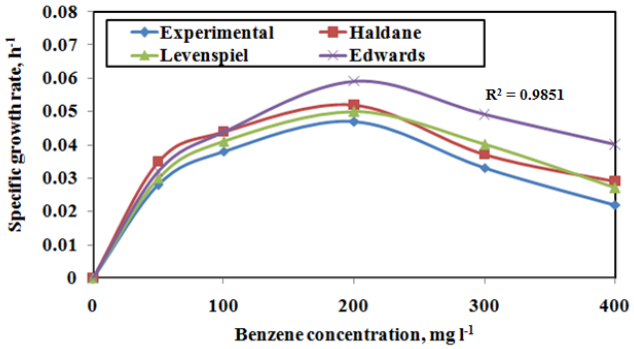


Fig. 4 Experimental and model predicted profile of specific growth rate for benzene degradation

TABLE I
KINETIC PARAMETERS EVALUATED FROM SUBSTRATE INHIBITION MODELS

Models	μ_{max} (h^{-1})	K_S (mg l^{-1})	K_I (mg l^{-1})
Haldane	0.053	14	155
Levenspiel	0.071	20	230
Edvards	0.076	21	275

TABLE II:
COMPARISON OF SUBSTRATE INHIBITION MODEL PARAMETERS FOR BENZENE BIODEGRADATION WITH LITERATURE VALUES

Primary Microorganisms	Benzene concentration range, mg l^{-1}	μ_{max}, h^{-1}	K_s mg l^{-1}	K_I mg l^{-1}	References
Pure culture	15 - 90	0.50	10.11	-	Robledo-Ortiz et al., 2011
Activated sludge	100 - 1000	0.03	54	230	Priya and Philip, 2013
Activated sludge	25 - 600	0.076	45	443	Padhi and Gokhale, 2016
Cow dung compost	50 - 400	0.071	20	230	Present study

B. Microbial Characterization

Isolation study was carried out from cow dung compost. It consists of macroorganisms and microorganisms which are used to take interest of changing temperature, moisture, oxygen content and pH also contains nutrients which are helpful for the microbial growth. For three weeks, culture was enriched in MSM using benzene. After biodegradation, the strains were survived via benzene degraders. According to Bergey's manual, morphological and biochemical characterization identified for the isolated strains. The results of the standard biochemical tests are summarized in Table 3a and 3b for benzene degrading microorganism, which gives positive or negative results obtained with different samples

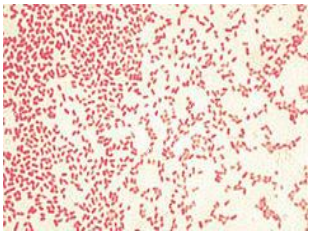
TABLE III:
STANDARD BIOCHEMICAL TEST FOR BENZENE DEGRADERS

Sample	Indole test	MR-VP Test	Citrate Utilization test	Urease test	Nitrate reduction test	Triple – sugar ion agar test	Starch Hydrolysis Test	Motility test	Catalase test	Oxidase test	Gram staining
1.	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(+)	(+)	(+)	Gram positive (Rod shaped)
2.	(+)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(-)	Gram positive (Rod shaped)
3.	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(+)	Gram positive (Rod shaped)

TABLE IV: SUGAR UTILIZING TESTS FOR BENZENE DEGRADING MICROORGANISMS

Sample	Glucose	Xylose	Arabinose	Galactose	Fructose	Lactose	Mannose	Rhamnose	Sucrose
1.	(+)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
2.	(+)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
3.	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)

All the cultures showed catalase activity but some was able to hydrolyze starch. Most of them had urease activity, citrate utilization and nitrate reduction ability. The morphological characteristics of the isolates from different samples were observed using an optical microscope (Novel). It was observed that most of the microorganisms were rod shaped. It was observed that most of the isolates had a wide range of sugar utilization capacity with gas production in several cases. However, same isolates showed some limitations in utilizing xylose, arabinose, rhamnose and mannose. Microscopic views of microorganisms degrading benzene are shown in Fig. 5. From then on, comparison of these results with bergey's manual, benzene degrading microorganisms were found to be (1) *Pseudomonas species* (2) *Bacillus species* (3) *Escherichia Coli*. Among these microorganisms, *pseudomonas species* was found to be predominant in degrading benzene.



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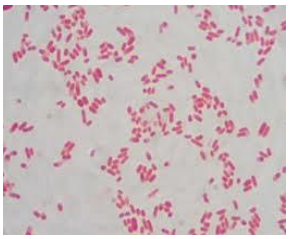

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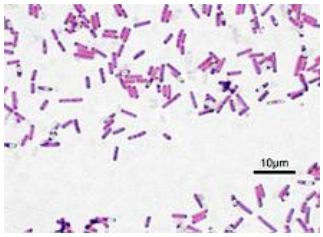

(3)

Fig. 5 Microscopic views of microorganisms degrading benzene (1) *Pseudomonas* s*pecies* (2) *Bacillus* species (3) *Escherichia coli*

IV. CONCLUSION

The present study dealt with the removal of environmentally significant benzene using acclimatized mixed culture in a batch system. The significant findings of this study are reported as below,

- The biodegradation of benzene was utilized to mixed culture in batch experiments at different initial concentration ranging from 50 - 400 mg l^{-1}. The cultures were able to grow well with benzene as the sole carbon source at the concentration ranges; despite the prevalence of inhibition. The maximum specific growth rate was found to be 0.062 h^{-1}
- The microbial profile of the mixed culture treating benzene was analyzed and found *Pseudomonas spices*, *Bacillus spices and Escherichia Coli* are the predominant species.
- Levenspiel inhibition model indicates a high degree of fit with the experimental data (R^2=0.9851).

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