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MICROBIAL CONSORTIA FORMULATION FOR THE EFFECTIVE BIODEGRADATION OF BENZENE, TOLUENE, XYLENE AND PHENOL

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ABSTRACT

Monoaromatic hydrocarbons such as benzene, toluene, xylene and phenol (BTXP) represent an important class of environmental contaminants because of their recognized toxicity to different organisms. Development of microbial consortia was attempted for the biodegradation of the mixture of these compounds. *Alcaligenes sp* d_2, a phenol degrading microorganism reported earlier, was found to degrade all the compounds individually and also as a mixture. Three more novel bacterial isolates, *Enterobacter aerogenes*, *Raoultella sp* and *Bacillus megaterium*, were selected by soil enrichment technique and identified by 16S rDNA analysis. Phylogenetic analysis was performed in Molecular Evolutionary Genetics Analysis4 based on Unweighted Pair Group Method with Arithmetic mean to infer the phylogeny across the data. The isolates could grow in Mineral Salt media supplemented individually with a maximum concentration of 1.36 mM Benzene, 1.09 mM Toluene, 0.923 mM Xylene and 1.22 mM Phenol as the sole carbon source. Degradation studies were conducted in 100 ml Mineral Salt media containing the mixture of all the four compounds. The ether extracted cell-free medium was analyzed using Fourier transform infrared spectroscopy. The primary formulation of the microbial consortia for the degradation of the mixture of BTXP was done using the Fourier transform infrared spectroscopy data. This is the first report on the biodegradation potential of *Bacillus megaterium SBS3*on both phenol and benzene. Hence this strain can be considered as a novel isolate with immense degradation potential. The consortium of *Alcaligenes sp d2*, *Enterobacter aerogenes*, *Bacillus megaterium*, and *Raoultella sp* formulated through this attempt could effectively degrade the mixture of BTXP and application of this consortium can result in the development of strategies for the bioremediation of Benzene, Toluene, Xylene and Phenol.

Keywords: Biodegradation, Fourier transform infrared spectroscopy, Microbial consortium, Mineral Salt media

Abbreviations: BTXP - Benzene, Toluene, Xylene, Phenol, FT/IR - Fourier transform infrared spectroscopy, MEGA4 - Molecular Evolutionary Genetics Analysis UPGMA - Unweighted Pair Group Method with Arithmetic mean, GC-MS - Gas chromatography–Mass spectrometry, NMR - Nuclear magnetic resonance spectroscopy

INTRODUCTION

The carbon cycle in nature operates on the assumption that all biosynthetic organic compounds are biodegradable. Biodegradation has been proved to be economic, versatile and ecologically acceptable method for the removal of toxic organic pollutants. The efficiency of biodegradation is influenced by the type of organic pollutants, nature of the organism, type of enzyme involved, mechanism of degradation and the nature of influencing factors like light, water, oxygen and temperature.

Benzene, toluene, xylene and phenol are the dominant chemicals widely used in different chemical industries (**Lin *et al.*, 2010**). They occur in petroleum products such as diesel, in many household products like kerosene, medicines, fertilizers, foodstuffs, plastic ware, paints etc. These aromatic hydrocarbons are released into the environment in abundance, resulting in extensive water and soil pollution. Among the contaminants present in gasoline benzene, toluene, ethylbenzene, xylene and phenol (BTEXP) are classified as priority pollutants because of their high mobility and toxicity. Different industries use various treatment methods for the removal of these hydrocarbons which includes chemical clarification, membrane filtration, bubble separation, photocatalytic oxidation, granular activated carbon filtration, and reverse osmosis (REF). But all these methods are quite expensive with high capital and operating costs. Mostly these methods remove the contaminants from the environment without transforming them, thereby resulting in the accumulation of toxic residues. The use of microbial metabolic potential for elimination of environmental pollutants provides a safe and economic alternative to their disposal in the waste dumping sites. The use of microbial catalysts in the biodegradation of organic compounds has advanced significantly during the past three decades.

Industrial wastes carry varieties of organic pollutants. Hence it is more appropriate to develop an effective method for the removal of mixture of the organic compounds rather than for the removal of a single compound. A single microorganism is usually incapable of degrading mixture of organic compounds. It has been found that large number of microorganisms co-exists in almost all natural environments. Hence a consortium designed in a proper way can degrade the mixture of organic compounds. Several authors state that a consortium of different species of microorganisms including algae, bacteria, fungus and protozoan usually drives biodegradation. Many pure cultures of bacteria, including various strains of *Pseudomonas putida*, have been evaluated for their BTX biodegradation potential (**Jean *et al.*, 2002, 2008**). However, biodegradation of BTEX can be enhanced with the use of bacterial consortium (**Littlejohns *et al.*, 2008**). **Liu *et al.* (2010)** reported that co-culture of three *Bacillus species* L_4, N_3 *and* N_6 is more efficient than individual *Bacillus sp*. for effective biodegradation of BTX contaminants.

In this study we report the development and formulation of an efficient bacterial consortium for the simultaneous and effective degradation of BTXP. Further subject study aims for the optimization of biodegradation parameters for the better biodegradation of BTXP. The metabolites of biodegradation would be analysed by GC-MS and NMR. This study will be advanced to find out whether the biodegradation is mediated by plasmids or chromosomes through molecular studies.

MATERIAL AND METHODS

Soil enrichment technique

BTXP degrading bacteria were screened through a soil enrichment technique (**Nair *et al.*, 2007**). The soil extract collected from the detergent contaminated area was progressively enriched with benzene, toluene, xylene and phenol. The enrichment was initiated at a concentration of 0.136 mM benzene, 0.109 mM toluene, 0.093 mM xylene and 0.122 mM phenol in 100 ml soil extract and enriched up to a maximum growth limiting substrate concentrations of 0.545 mM benzene, 0.437 mM toluene, 0.372 mM xylene and 0.489 mM phenol. The culture was kept on a shaker at 150 rpm at room temperature up to 5 days. The isolates which could use these compounds at the maximum growth limiting substrate concentrations were selected.

Identification of BTXP degrading isolates

Three novel bacterial isolates were screened through the soil enrichment technique with BTXP. The three isolates *Enterobacter aerogenes*, *Bacillus megaterium*, and *Raoultella sp* were identified by performing various morphological and biochemical tests according to Bergey's manual of systematic bacteriology (**Bergey *et al.*, 1974**). Their identity was confirmed by 16S rDNA sequence analysis using the forward primer sequence (5´-AGA GTT TGA TCM TGG CTC-3´) and the reverse sequence (5´-AAG GAG GTG WTC CAR CC-3´). The final concentration of the reagents were 1 mM $MgCl_2$, 200 µM dNTP, 100 pmol primers and 50 ng DNA (**Chun *et al.*, 1995**). Polymerase Chain Reaction (PCR) was carried out in Mycycler™ (Bio-Rad, USA) with the following PCR Cycle: one cycle at 94ºC for 2 min, followed by 35 cycles at 94ºC for 1 min, 55ºC for 1 min, 72ºC for 2 min, followed by final 2 min incubation at 72ºC and the PCR products were sequenced at Scigenome labs, Pvt Ltd, Cochin, Kerala. *Alcaligenes sp* d_2 reported earlier as a phenol degrading strain was collected from the culture collection of School of Biosciences Mahatma Gandhi University, Kottayam, Kerala.

The sequence similarity was analysed by sequences available in the National Center for Biotechnology Information (NCBI) database using BLAST (Basic Local Alignment Search Tool) analysis and isolates were identified on the basis of the best match in the database. Sequences of BTXP degrading isolates and reference sequences from NCBI GenBank were aligned using the multiple sequence alignment program ClustalW2. Using the alignment file generated by ClustalW2, phylogenetic analysis was performed in MEGA4 (Molecular Evolutionary Genetics Analysis) (**Tamura *et al.*, 2007**). UPGMA (Unweighted Pair Group Method with Arithmetic mean) (**Sneath *et al.*, 1973**) was used to infer the phylogeny across the data. Bootstrap analysis (1000 replicates) was also performed to check the reliability of the phylogram (**Felsenstein, 1985**).

Inoculum Preparation

One loopful of each of the selected cultures was individually inoculated to 50 ml nutrient broth containing 50 µl phenol, benzene, xylene and toluene and the flasks were incubated over night at room temperature at 150 rpm. From the culture the cells were harvested by centrifugation. The pellets were collected and suspended in physiological saline (0.85 % NaCl) to obtain the inoculum of 1.OD concentration.

Biodegradation studies

Submerged biodegradation was conducted using a defined medium with the following compositions (g/l): KH_2PO_4 -1, $(NH_4)_2SO_4$ -1, $MgSO_4.7H_2O$ -0.5 and $CaCl_2$ -0.01, benzene-1.36 mM/ toluene-1.09 mM / xylene-0.93 mM/ phenol-1.22 mM (BTXP) at a pH of 7 at room temperature on a rotary shaker at 150 rpm. The inoculum prepared for each culture was used individually and also in combination with the mineral salt medium carrying Benzene/Toluene/Xylene/Phenol. The biodegradation was continued up to 48 hrs. After removing the cells by centrifugation at 10,000 rpm for 10 minutes, the supernatant was subjected to solvent extraction with diethyl ether followed by FT/IR analysis.

Consortia development

The primary formulation of the microbial consortia for the degradation of a mixture of BTXP was done on the basis of the FT/IR data of the individually degraded samples**.** *Alcaligenes sp* d_2 capable of bringing structural transformation to all the benzene, toluene, xylene and phenol was selected as the primary member in the consortium. Three more strains *Enterobacter aerogenes, Raoultella sp* and *Bacillus megaterium*, were individually selected on the basis of their degradation efficiency. No other strains could be revealed through the soil enrichment technique. The selected bacterial isolates along with *Alcaligenes sp* d_2, have the capability to grown at this concentration of BTXP and no inhibitory effects were shown among the strains. Individual bacteria were grown in nutrient broth and the flasks were incubated over night at room temperature at 150 rpm. From the culture the cells were harvested by centrifugation and resuspended in sterile saline to yield an absorbance reading of 0.5 at 540 nm (**Ghazali *et al.*, 2004**). The consortium was constituted by mixing equal proportions of *Alcaligenes sp* d_2 with *Enterobacter aerogenes*, *Raoultella sp* and *Bacillus megaterium*.

RESULTS

Isolation and Identification of BTXP degrading isolates

In our study, four different isolates of bacteria were selected for the biodegradation of mixture of organic compounds including benzene, xylene, toluene and phenol (BTXP). *Alcaligenes sp* d_2 (**Nair *et al.*, 2004**), a phenol degrading microorganism available in the culture collection centre of School of Biosciences, Mahatma Gandhi University, was found to degrade all the compounds in the mixture of organic compounds. In an attempt to screen BTXP degrading strains through soil enrichment technique, three isolates viz., *Strains* SBS1, SBS2 and SBS3 were selected as the potent degraders of phenol and benzene, toluene and xylene, and phenol and benzene respectively. All three isolates were identified by performing various morphological and biochemical tests according to Bergey's manual of systematic bacteriology. The Isolate SBS1 was identified as *Enterobacter aerogenes*, SBS2 was identified as *Raoultella sp and* SBS3 was identified as *Bacillus megaterium*. Their identity (Figure 1) was confirmed by 16S rDNA sequencing. The sequence data of newly isolated strains are available in the GenBank with accession numbers KC758848, KC758849 and KC758850 respectively for *Enterobacter aerogenes, Raoultella* sp and *Bacillus megaterium*.

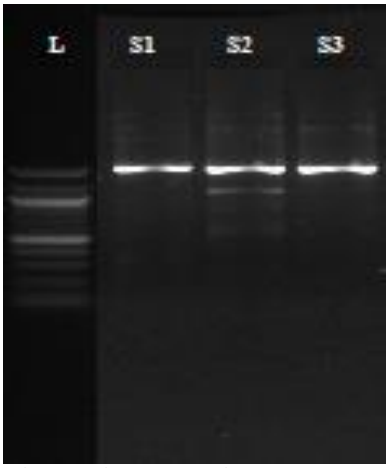

Figure 1 PCR amplification of 16S rDNA of the isolates
Legend: L: 100 base pair ruler; S1: *Enterobacter aerogenes;* S2: *Raoultella sp;* S3: *Bacillus megaterium*

Genetic diversity among the BTXP degrading isolates were studied using 16S rDNA sequences and the sequence obtained were compared with the highest score reference sequences from the NCBI GenBank database. The dendrogram was constructed for illustrating possible relationships among the isolated BTXP degrading bacteria (Figure 2). Phylogenetic analysis was conducted in MEGA4 based on UPGMA method. Evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). The isolates, SBS1, SBS2 and *Alcaligenes sp.* were clustered together with very high bootstrap value (100%), where the isolate SBS3 formed the out group. Even though the distinct sequence variation was observed among the isolates SBS1, SBS2 and *Alcaligenes* sp. and the more similar isolates SBS1 and SBS2 grouped in to one cluster. The isolates SBS1 and SBS2 showed 100% similarity with the reference isolate *Enterobacter sp* NCCP 755 (AB 715352) and *Raoultella sp*. HSL78C (HM461206) respectively. The isolate SBS3 showed distinct sequence variation from other isolates. The variation with the reference isolate *Bacillus megaterium* TAUC4 (HQ914779) was also predictable from the dendrogram.

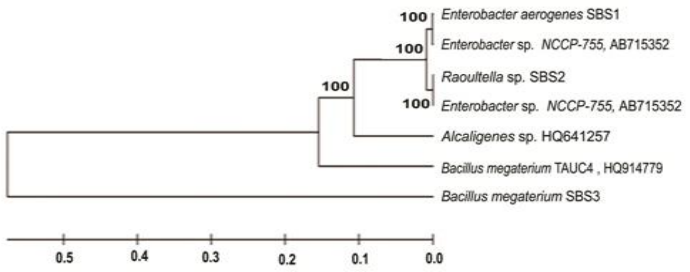

Figure 2 Phylogenetic tree expressing the relationships of identified BTXP degrading isolates based on the 16S rDNA sequences
Numbers above each node are confidence levels (%) generated from 1,000 bootstraps. The scale bar is in fixed nucleotide substations per sequence position. *Enterobacter aerogenes* SBS1, *Raoultella sp*. SBS2, *Bacillus megaterium* SBS3 *and Alcaligenes sp. were used in this study; the* AB 715352, HM 461206 *and* HQ 914779 *are from GenBank database as reference strain.*

Biodegradation studies

The three selected bacterial isolates along with *Alcaligenes sp* d_2 were used for the biodegradation of BTXP compounds. All these isolates could grow in mineral salt media up to a maximum concentration of 1.36 mM Benzene, 1.09 mM Toluene, 0.923 mM Xylene and 1.22 mM Phenol as the sole carbon source. FT/IR analysis of the ether extracts of individually degraded compounds through bacterial isolates strongly supported the fact that *Alcaligenes sp* d_2 could effectively degrade all the four compounds. *Enterobacter aerogenes* and *Bacillus megaterium* degraded phenol and benzene, and *Raoultella sp* could degrade xylene and toluene effectively.

FT/IR analysis of the individual biodegradation of benzene, toluene, xylene, and phenol

FT/IR analysis of the mineral salt benzene medium inoculated with *Alcaligenes sp* d_2, *Enterobacter aerogenes* and *Bacillus megaterium* showed the disappearance of the specific bands represents benzene (Table 1). The structural changes in C-H stretch, C=C stretch and C-H bends after biodegradation supported the effective degradation of benzene by *Alcaligenes sp* d_2, *Enterobacter aerogenes* and *Bacillus megaterium*. *Raoultella sp* did not show any prominent difference in the spectrum of benzene extract after incubation.

Table 1 Fourier transform infrared spectroscopy analysis of Benzene biodegradation after 48 hrs of incubation at room temperature with the selected isolates

Absorption Frequency Ranges (cm^{-1}) Of Functional Groups	Peaks at Relevant wave numbers (cm^{-1})				
	Control	Samples			
		*Alcaligenes sp d_2	*Enterobacter aerogenes	*Bacillus megaterium	*Raoultella sp*
C-H stretch 3100-3000	3037,3032, 3013	Absent	Absent	Absent	Present
C=C Stretch 1600-1450	1598,1578, 1477	Absent	Absent	Absent	Present
C-H Bend 1000-650	958,796, 742,670	Absent	Absent	Absent	Present

*Degrades the compound
Legend: Control – ether extracted uninoculated mineral salt benzene medium, Samples – ether extracted inoculated mineral salt benzene medium after 48 hrs of incubation with the selected isolates

FT/IR analysis of the mineral salt toluene medium inoculated with *Alcaligenes sp* d_2 and *Raoultella sp* showed the disappearance of the relevant bands (Table 2) representing toluene. The structural changes in the functional groups C-H stretch, C=C stretch and C-H bends during biodegradation supported the fact that *Alcaligenes sp* d_2 and *Raoultella sp* could degrade toluene. But *Enterobacter aerogenes* and *Bacillus megaterium* did not show any difference in the spectra and so these strains were considered ineffective for the degradation of toluene.

Table 2 Fourier transform infrared spectroscopy analysis of Toluene biodegradation after 48 hrs of incubation at room temperature with the selected isolates

Absorption Frequency Ranges (cm^{-1}) Of Functional Groups	Peaks at Relevant wave numbers (cm^{-1})				
	Control	Samples			
		*Alcaligenes sp d_2	Enterobacter aerogenes	Bacillus megaterium	*Raoultella sp
C-H Stretch 3100-3000	3086,3072, 3061,3027	Absent	Present	Present	Absent
C=C Stretch 1600-1450	1593,1521, 1495,1459	Absent	Present	Present	Absent
C-H Bend 1000-650	895,785, 725,692	Absent	Present	Present	Absent

*Degrades the compound
Legend: Control –ether extracted uninoculated mineral salt toluene medium, Samples –ether extracted inoculated mineral salt toluene medium after 48 hrs of biodegradation with the selected isolates

FT/IR analysis of the mineral salt xylene medium inoculated with *Alcaligenes sp* d_2, and *Raoultella sp* showed the disappearance of the specific bands (Table 3) representing O-Xylene. The structural changes in the functional groups C-H stretch, C=C stretch and C-H bend after biodegradation, indicated that these strains could degrade xylene. *Enterobacter aerogenes* and *Bacillus megaterium* could not show any prominent change in the FT/IR spectra and it was concluded that these strains were not effective in xylene degradation.

Table 3 Fourier transform infrared spectroscopy analysis of O- Xylene biodegradation after 48 hrs of incubation at room temperature with the selected isolate

Absorption Frequency Ranges (cm^{-1}) Of Functional Groups	Peaks at Relevant wave numbers (cm^{-1})				
	Control	Samples			
		**Alcaligenes sp d_2*	*Enterobacter aerogenes*	*Bacillus megaterium*	**Raoultella sp*
C-H Stretch 3100-3000	3088,3067, 3027,3008	Absent	Present	Present	Absent
C=C Stretch 1600-1450	1599,1577, 1516,1495, 1453	Absent	Present	Present	Absent
C-H Bend 1000-650	964,904, 795,769,696	Absent	Present	Present	Absent

*Degrades the compound

Legend: Control –ether extracted uninoculated mineral salt xylene medium, Samples –ether extracted inoculated mineral salt xylene medium after 48 hrs of biodegradation with the selected isolates

FT/IR analysis of the mineral salt phenol medium individually inoculated with *Alcaligenes sp d_2*, *Enterobacter aerogenes* and *Bacillus megaterium* showed the disappearance of the specific bands represents phenol (Table 4). This disappearance indicated the structural changes in the functional groups H bonded O-H stretch, C-H stretch, C=C stretch, C-O stretch, C-H bend. This clearly indicated the effective degradation of phenol by *Alcaligenes sp d_2*, *Enterobacter aerogenes*, and *Bacillus megaterium.* But *Raoultella sp* could not show any prominent difference in the spectrum, therefore was not effective for the degradation of phenol.

Table 4 Fourier transform infrared spectroscopy analysis of phenol biodegradation after 48 hrs of incubation at room temperature with the selected isolates

Absorption Frequency Ranges (cm^{-1}) of Functional Groups	Peaks at Relevant wave numbers (cm^{-1})				
	Control	Samples			
		**Alcaligenes sp d_2*	**Enterobacter aerogenes*	**Bacillus megaterium*	*Raoultella sp*
H bonded O-H stretch 3600-3100	3304,3286	Present	Present	Present	Present
C-H stretch 3100-3000	3045	Absent	Absent	Absent	Present
C=C Stretch 1600-1450	1594,1500, 1473	Absent	Absent	Absent	Present
C-O Stretch 1300-1000	1229,1100, 1070	Absent	Absent	Absent	Present
C-H bend 1000-650	813,752,691	Absent	Absent	Absent	Present

*Degrades the compound

Legend: Control – ether extracted uninoculated mineral salt phenol medium, Samples –ether extracted inoculated mineral salt phenol medium after 48 hrs of biodegradation with the selected isolates

FT/IR spectral analysis strongly supported the fact that these four isolates *Alcaligenes sp d_2, Enterobacter aerogenes*, *Raoultella sp* and *Bacillus megaterium* could be used for the formulation of an effective microbial consortium for the biodegradation of BTXP mixture. A consortium was prepared by mixing equal volumes of all the four isolates for the biodegradation of BTXP.

FT/IR analysis of Biodegradation by Consortium

FT/IR analysis of uninoculated BTXP medium (Table 5) showed specific bands representing B, T, X and P. FT/IR analysis of consortium inoculated medium showed the absence of many of the specific bands of BTXP on biodegradation. The structural changes indicated in the representation of C-H stretch, C=C stretch, C-O stretch and C-H bends in the FT/IR analysis spectra (Figure 3(a, b) strongly supported the fact that the microbial consortium prepared with the four organisms *Alcaligenes sp d_2, Enterobacter aerogenes*, *Raoultella sp* and *Bacillus megaterium* could effectively degrade the mixture of BTXP. In this consortium *Alcaligenes sp d_2* could effectively degrade all the four compounds; whereas *Enterobacter aerogenes* and *Bacillus megaterium* supplemented the activity by degrading phenol and benzene, and *Raoultella sp* could degrade xylene and toluene.

Table 5 Fourier transform infrared spectroscopy analysis of Benzene, Toluene, Xylene, and Phenol biodegradation after 48 hrs of incubation at room temperature with formulated consortium

Absorption Frequency Ranges (cm^{-1}) of Functional Groups	Peaks at Relevant wave numbers (cm^{-1})	
	Control	Sample
H bonded O-H stretch 3600-3100	3329	Present
C-H Stretch 3100-3000	3043	Absent
C=C Stretch 1600-1450	1594,1498,1472	Absent
C-O Stretch 1300-1000	1217,1167,1069,1023	Absent
C-H bend 1000-650	999,885,809,749,688	Absent

Legend: Control – ether extracted uninoculated mineral salt BTXP medium, Samples – ether extracted inoculated mineral salt BTXP medium after 48 hrs of biodegradation with the formulated consortium

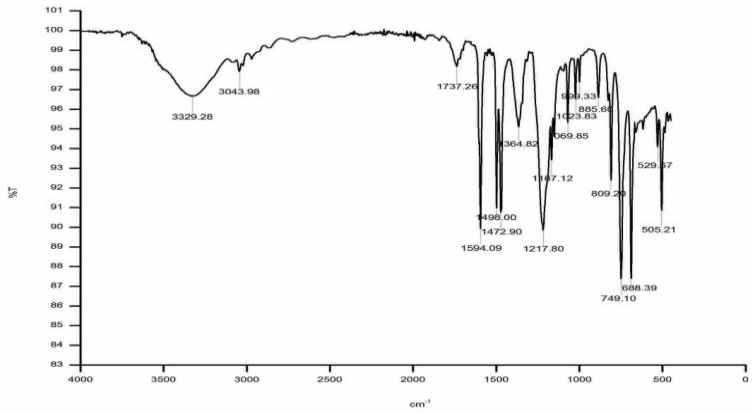

Figure 3(a) Fourier transform infrared spectroscopy analysis of the mixture of the compounds Benzene, Toluene, Xylene, and Phenol- Consortium Control

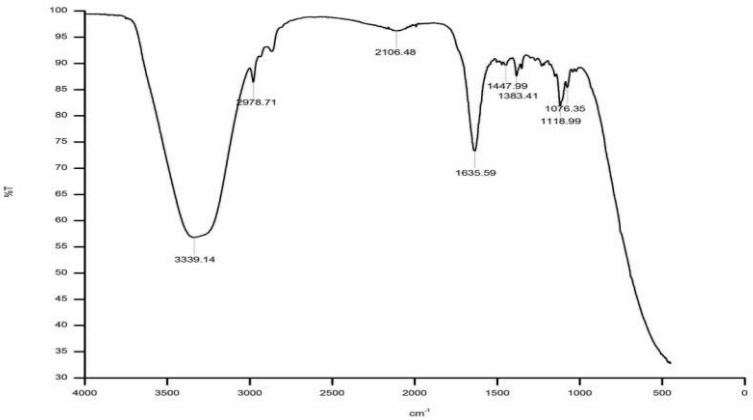

Figure 3(b) Fourier transform infrared spectroscopy analysis of the mixture of the compounds Benzene, Toluene, Xylene, and Phenol after 48 hrs of degradation with the novel bacterial consortium – Test

DISCUSSION

The environment has been continuously polluted by a large array of hazardous chemicals discharged through various industrial activities. Among these organic compounds, aromatic compounds such as benzene, toluene, xylene and phenol are severe contaminants. Because of their low water solubility, acute toxicity, genotoxicity and their ability to bio-accumulate through the food chain, these compounds have been considered as significant pollutants. Thus, it is important to develop methods to accelerate the removal of these components from contaminated environments and biodegradation has been considered as a remedial option. This bioremediation technique that uses the microbial ability to degrade and to detoxify chemical substance is of relatively low cost, with simple technology, and with high public acceptability resulting in number of research works on the degradation of single pollutants (**Machinicka *et al.*, 2001**). But less works have been conducted in the development of the consortium of microorganisms for the degradation of mixture of organic compounds. In the present study a formulated consortium was prepared by mixing equal concentrations of all the four isolates - *Alcaligenes sp* d_2, *Enterobacter aerogenes, Raoultella sp* and *Bacillus megaterium* - for the biodegradation of BTXP. This is the first report on the biodegradation potential of *Bacillus megaterium SBS3* on both phenol and benzene and the phylogenetic studies showed the genetic diversity of the isolate from the reference isolate. Hence this strain can be considered as a novel isolate with immense degradation potential.

There are many reviews about the aromatic biodegradation capacities of *Enterobacter sp.* and *Raoultella sp.* According to **Toledo *et al.* (2008)**, co-culture of *Bacillus subtilis, Alcaligenes faecalis* and *Enterobacter sp.* capable of emulsifying n-octane, toluene, xylene, mineral oils and crude oil, appeared promising for bioremediation application. *Raoultella terrigena* and *Pantoea agglomerans* degraded 93% of phenol content present in the olive washing waste water **(Maza *et al.*, 2013**). *Bacillus megaterium* is not an active participant like *Enterobacter sp.* and *Raoultella sp.* in BTX biodegradation and very few reports has been published on the hydrocarbon degrading ability of *Bacillus megaterium* till date.

FT/IR analysis of the degradation studies in all the individual cases of B, T, X, P indicated the introduction of a ketonic group which was represented in the wave number range 1625-1750 cm^{-1}. The formation of this ketonic group in the initial stages strongly supported the fact that the consortium follows aerobic degradation pathways. Studies on metabolic pathways for BTXP removal under aerobic condition have revealed that BTXP compounds are degraded by aerobic pathway to a substituted catechol. Benzene is degraded to catechol while toluene is degraded via separate pathways to 3-ethylcatechol with 3-methylcatechol as intermediate product. The xylenes are metabolized to monomethylated catechols. The consortium of the selected bacterial isolates in the presently formulated consortium could utilize BTXP compounds more effectively than degradation by an individual isolate of the same consortium. This may be attributed to the production of different catabolic enzymes involved in the degradation of BTXP compounds, by mixed microbial population. In aerobic degradation pathway the initial step involves the addition of a carbonyl group by the enzymes oxygenase and dio-oxygenase. Aerobic degradation can take place mainly by two pathways, viz. the meta pathway of degradation or the ortho pathway of degradation. Further molecular studies with GC-MS (Gas chromatography–Mass spectrometry) and NMR (Nuclear magnetic resonance spectroscopy) analysis of the degradation products may throw light into the exact pathway followed for the degradation of these organic compounds.

CONCLUSION

In conclusion, Biotechnological application for hazardous waste management requires the development of a mixed biological system for the detoxification, degradation or decontamination of environmental pollutants. The consortium of *Alcaligenes sp* d_2, *Enterobacter aerogenes*, *Bacillus megaterium*, and *Raoultella sp* formulated through this attempt could effectively degrade 1.36 mM benzene, 1.09 mM toluene, 0.92 mM xylene and 1.22 mM phenol in 48 hrs. Further investigation into the application of this consortium can result in the development of strategies for the bioremediation of Benzene, Toluene, O-Xylene and Phenol from polluted environments.

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