IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 11, Issue 9 Ver. II (September. 2017), PP 31-37
www.iosrjournals.org

Biodegradation of Benzene by Alkaliphilic bacteria under Aerobic conditions

Ahmed T. Ahmed[1], Shaif M. Kasem Saleh[2], Adel A. Mohamed[3] Saeed
[1](Department of Chemistry, Faculty of science, University of Aden, Yemen)
[2](Department of Chemistry, Faculty of science, University of Aden, Yemen)
[3](Department of Chemistry, Faculty of science, University of Aden, Yemen)
ahmedbiochemgmail.com

Abstract: *The aerobic biodegradation of benzene in laboratory cultures have been well studied, by using alkaliphilic bacteria strain Bacillus badius D1, The bacterial strain Bacillus badius D1 could complete degradation of benzene at a concentration of 5 ml/100 ml at pH 9.0 within 48 hrs, The metabolites of aerobic degradation of benzene by this strain were identified as Catechol, 2-hydroxymuconic semialdehyde and 2-hydroxypenta-2,4-dienoic acid. The structural determination of the intermediates of benzene degradation was carried out by spectroscopic analysis like GC-MS chromatography and FTIR spectra. A tentative pathway of benzene degradation is also reported. To our knowledge, this is the first report that the isolated bacterial culture of Bacillus badius D1can capable to degraded benzene under alkaline conditions.*
Keywords :*Bacillus Badius, Benzene, Biodegradation, Alkaliphilic, Metabolite*

Date of Submission: 09-09-2017 Date of acceptance: 23-09-2017

I. Introduction

Biodegradation of organic compounds under aerobic conditions most often occurs when bacteria catalyze the breakdown of these molecules and then recover some of this chemical as sole of carbon and energy source, which is necessary for maintenance of the bacterial cell. The aerobic bacterial degradation of aromatic compounds is usually initiated by dioxygenases that incorporate two hydroxyl groups into the aromatic substrate [1]. Monoaromatic hydrocarbons, e.g., Benzene, Toluene, Ethylbenzene and Xylene (BTEX compounds) extensively used in industrial solvents and the major components of gasoline [2]. Due to their hypopholicity and their toxicity [3] BTEXs components are classified as priority pollutants by the U.S. Environmental Protection Agency [4], because these components are difficult to remedy and can rapidly once introduced into aquifers. BTEXs have been the focus of a large number and variety of biodegradation and bioremediation studies. The reviewed evidence includes both field and laboratory investigations for which aerobic degradation of the BTEXs by microbial action. There is evidently a much greater risk of exposure to high concentrations of BTEX in contrast with the other hydrocarbon contaminants in environmental, because of widespread occurrence of leakage from storage tanks and leaks of petroleum production wells, pipelines, and distribution terminals [5]. This exposure risk is greater than before by the fact that benzene is considered a human carcinogen [3] and [6].

Benzene is the simplest aromatic hydrocarbon, maybe the most recalcitrant of the BTEX compounds, and of greatest regulatory concern because of its associated health impacts. Benzene is released to the environment by both natural and industrial sources.

Biodegradation, principally under aerobic conditions, is an important environmental fate process for water- and soil-associated benzene. Aerobic degradation of benzene is usually initiated by progressive oxidation of the aromatic ring to produce catechols. Catechol is transformed to substrates of the citric acid cycle through cleavage of the aromatic ring. The first step of benzene oxidation is a hydroxylation catalyzed by a dioxygenase. The product, a diol, is then converted to catechol by a dehydrogenase. These initial reactions hydroxylation and dehydrogenation are also common to pathways of degradation of other aromatic hydrocarbons [7].

Many aerobic benzene degrading bacteria was isolated have been obtained and examined include, the genera Pseudomonas[8-13] and [14], Burkholderia [8],[13] and [14], Ralstonia [8]and [13] Achromobacter [15], Pseudoxanthomonas [12], Hydrogenophaga , Rhodococcus and Arthrobacter [16], Alicycliphilus [17] and Acinetobacter [18]. In this report we present a demonstration of complete mineralization of benzene to carbon dioxide and water by alkaliphilic organisms. But there is no reported illustrated that ability of alkaliphilic organisms to degraded benzene. Microbial degradation pathways of PAHs containing up to three rings as anthracene by alkaliphilic bacteria have been proposed [1]. The term "Alkaliphilic" is used for microorganisms that grow optimally or very well at pH values above 9, often between 10 and 12, but cannot grow or grow only slowly at the near-neutral pH value. In our present studies the bacteria strain *Bacillus badius* D1 is isolated from

alkaline Crater Lake [1]. In this present study, attempt has been made to examine the degradative potential of alkaliphilic bacteria towards benzene.

II. Materials and methods

2.1 Chemicals

Benzene was obtained from the Department of Chemistry, University of Pune, India. Bacteriological media chemical from HI media, Mumbai, India and solvents were purchased from SRL Mumbai, India.

1.2 Microorganism and cultivation

The alkaliphiles bacteria strain *Bacillus badius* D1was isolated from Pristine Crater Lake of Lonar, Buldana, Maharashtra state. India. Isolation of pure culture of this organism was done by using serial dilution and pour plating methods under aerobic condition. This bacterial strain were grown at 37ºC in nutrient broth (NB) medium in 500 ml conical flasks, containing 100 ml of the following medium 0.5% Yeast, 0.5% Peptone,0.5% NaCl, KH_2PO_4 170 mg, Na_2HPO_4 980 mg, $(NH_4)_2SO_4$ 100 mg, $MgSO_4$ 4.87 mg, MgO 0.1mg $FeSO_4$ 0.05 mg, $CaCO_3$ 0.20 mg, $ZnSO_4$ 0.08 mg, $CuSO_4.5H_2O$ 0.016 mg, $CoSO_4$ 0.015 mg, H_3BO_3 0.006 mg, distilled water 100 ml, drops of 0.1N NaOH to adjusted media to pH-9.0 for alkaliphilic bacterial strain.

1.3 Biodegradation experiments

Biodegradation was performed by adding 15 ml of benzene to 100 ml of the 24 hrs grown culture (during the log phase). The conical flasks were placed on a rotary platform incubator shaker at 100 rpm at 37ºC and incubated for 12, 18, 24, 36 and 48 hrs. The culture media of each flask was then centrifuged at 10,000xg for 15 min in cold centrifuge, Du Pont Instruments SORVALL RC-5B to separate the bacterial cell mass, and the resulting supernatant was preserved for the extraction of biodegradation products. The supernatant was extracted by dichloromethane (DCM) and then dried over sodium sulphate anhydrous. The solvent was evaporated at 40ºC to obtain the residue. The purified residue was subjected to thin layer chromatography (TLC) to confirm the number of metabolites. The recovered compounds were subjected to further analysis on Fourier transform infrared spectroscopy (FTIR) and the analysis was carried out using the KBr pellet technique in the wavelength rang of 400-4000cm^{-1} on the shimadzu-8400, FTIR spectrophotometer. Gas Chromatography Mass (GC-MS) analysis was performed using a GB5 column with a 15-20 min runtime on a shimadzu-GC-MS-QP5050.

III. Result

Addition of 15 ml /100ml of benzene to the 24 hrs grown culture has resulted in complete degradation of benzene within 48 hrs of incubation by alkaliphilic organism under shaking condition.

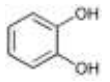
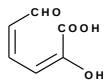
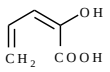
3.1 Evaluation of Optimum Condition for Benzene Degradation

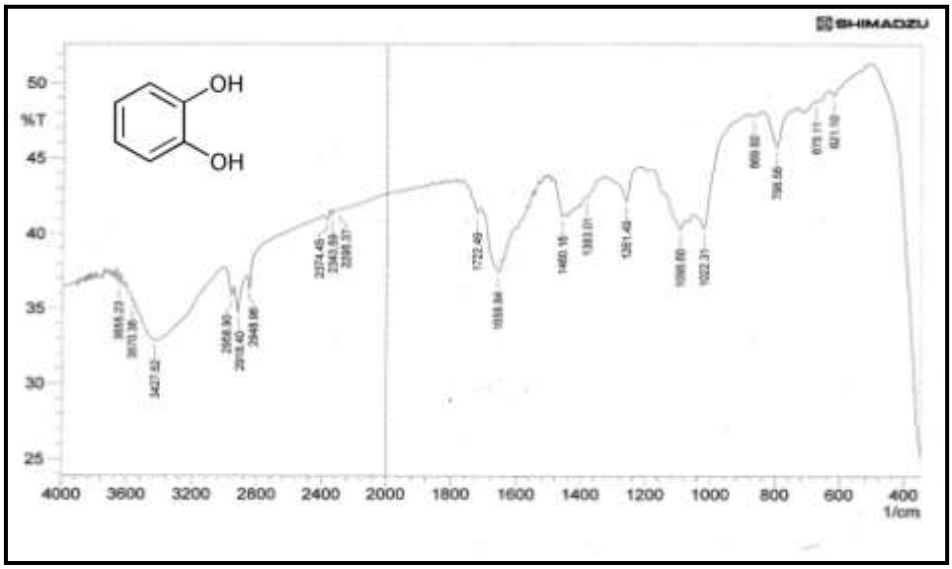
After 48 hrs of incubation at 37°C and pH 9.0, it was found that complete degradation of benzene was observed under shaking condition. The degradation pattern was checked after every 12 hrs of incubation. The growth of bacteria and utilization of benzene were measured the increase of optical density (O.D) at 300nm (data not shown).

1.4 *Identification of benzene degradation products*

The structural analysis of the intermediates/metabolites of benzene collected at interval of each 12 hrs is determined by various spectroscopic techniques like FTIR and GC-MS and summarized in the Table (1). The biodegradation pathway of benzene by alkaliphilic bacterial strain *Bacillus badius* D1shown in "Fig" (4) has been established. The GC-MS chromatography illustrated that. The first metabolite is elucidated in "Fig" (1) with retention time (12.033) and a molecular ion at an m/z of 110 and fragmentation ions 81[M+-29, CHO], 66 [M+-44, C_2H_4O], 53 [M^+-58, C_3H_6O] this compound is identified as Catechol, (benzene oxidation is a hydroxylation catalyzed by a dioxygenase) these initial reaction of hydroxylation. FTIR spectra of this metabolite is shown in "Fig" (1) the broad peak of hydroxyl group at 3427.62 and the peak at 1658.84 belong to group of C=C. The second metabolite is shown in "Fig" (2) was identified as 2-hydroxymuconic semialdehyde by GC-MS analysis with retention time (13.825). The compound had a molecular ion at an m/z of 142 and fragmentation ions at m/z of 113 [M+-29, CHO] 97 [M+-45, COOH], 85[M^+-57, C3H4O]. An FTIR spectrum of this metabolite is shown in Figure (2) the broad peak at 3427.62 and 3398.69 belong to hydroxyl group and the sharp peak at 1670.41 for carbonyl group. The third metabolite is shown in "Fig" (3) also was observed at retention time (12.458) and had a molecular ion at m/z of 114 and fragmentation ions at m/z 99 [M+-15, CH3] 85 [M+-29, CHO] 69 [M+-45, COOH] this intermediate identical to that of 2-hydroxypenta-2, 4-dienoic acid. FTIR, shown the carbonyl group at 1707.06, C=C group at 1660.77 and hydroxyl group at 3396.69 "Fig" (3).

Table (1) Metabolite extracted during biodegradations of benzene by alkaliphilic bacteria *Bacillus badius* D1

Metabolites	Chemical structural	Time/hrs	GC-MASS Chromatography			FTIR Spectra
			Retention time/mints	Fragmentation ion (m/z)	Molecular weight (M/w)	
Catechol		18	12.033	81, 66, 53	110	3427.62 (OH group) 1658.84 (C=C)
2-hydroxymuconic semialdehyde		24	13.825	113, 97, 85	142	3427.62 & 3398.69 (OH group),1670.41 (Carbonyl group
2-hydroxypenta-2,4-dienoic acid		36	12.458	99, 85, 96	114	3396.69 (OH group),1707.06 (Carbonyl group), 1660.77 (C=C)



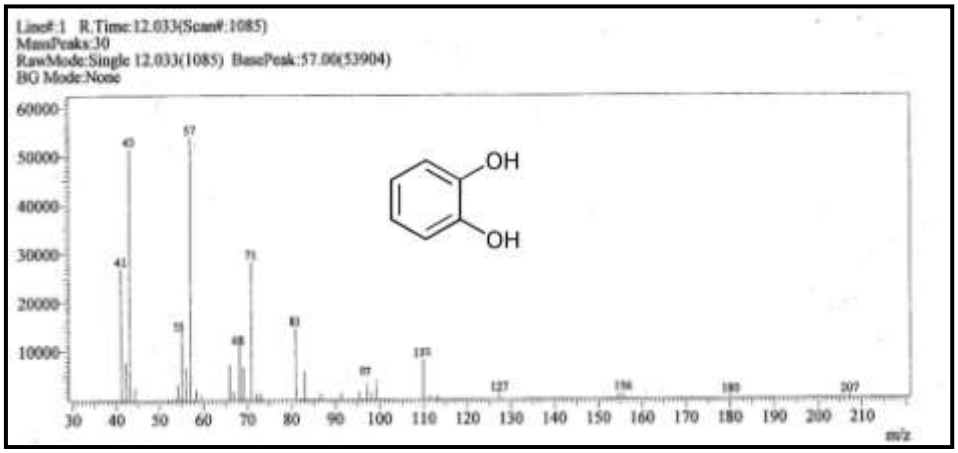
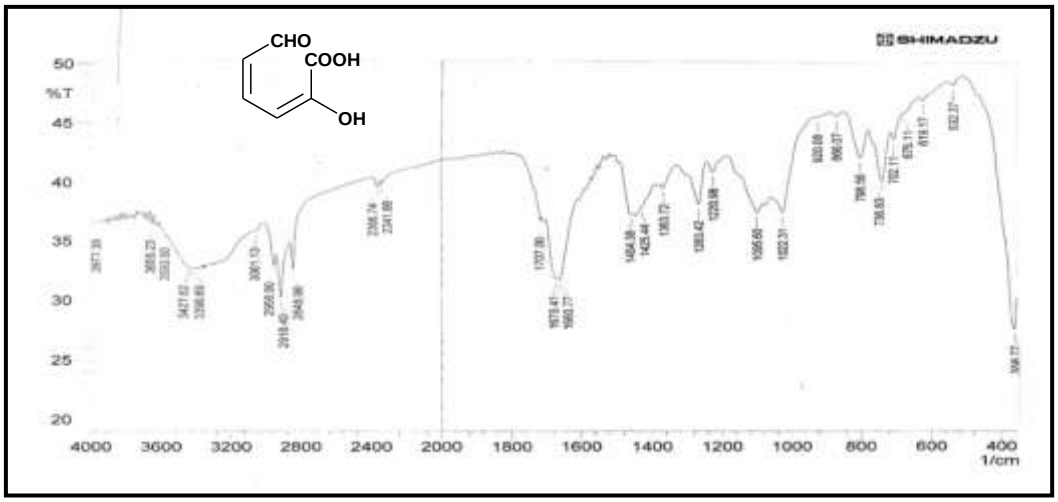


Figure 1: FTIR and GC-MS of Catechol produced from Benzene degradation by alkaliphilic bacteria *Bacillus badius* D1 after 18 hrs of incubation



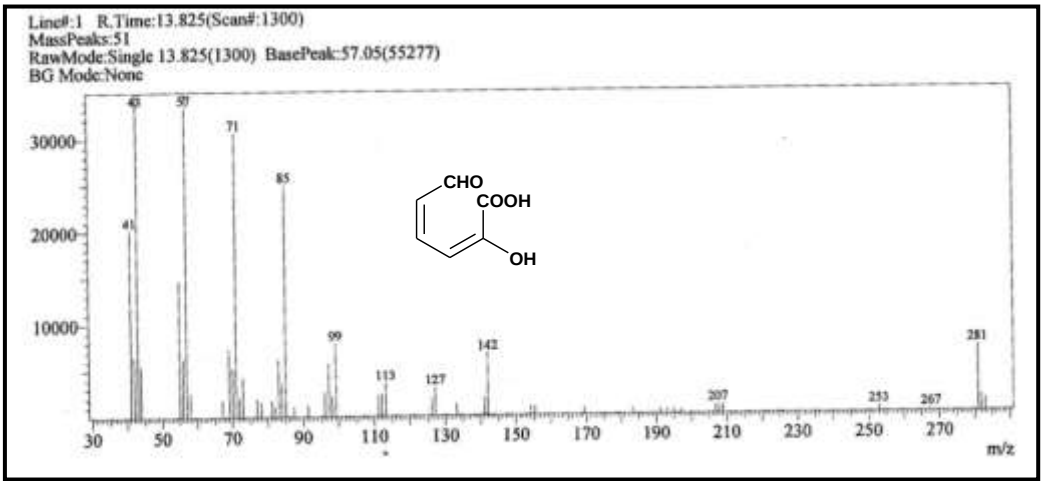
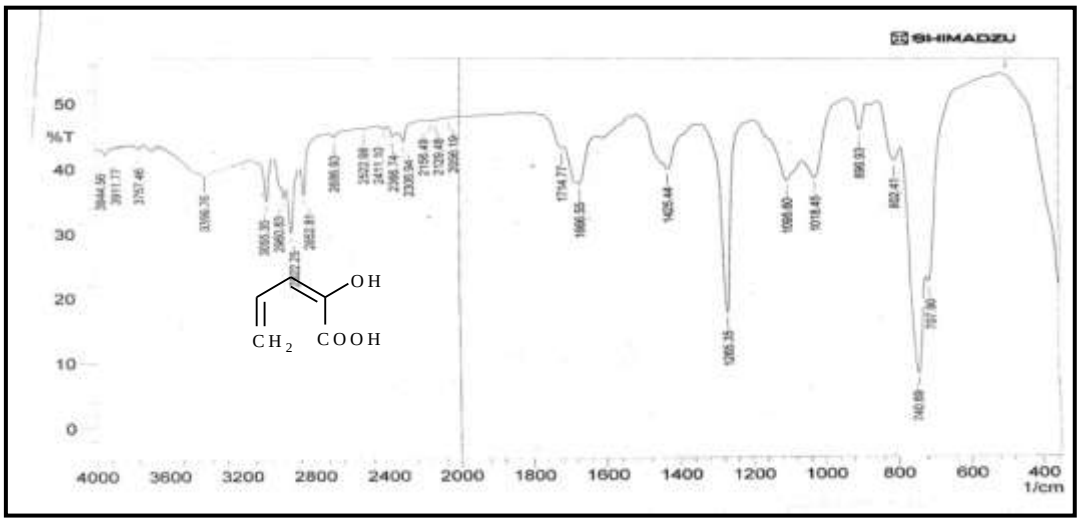


Figure 2: FTIR and GC-MS chromatography of 2-hydroxymuconic semialdehyde produced from benzene degradation by alkaliphilic bacteria *Bacillus badius* after24 hrs of incubation



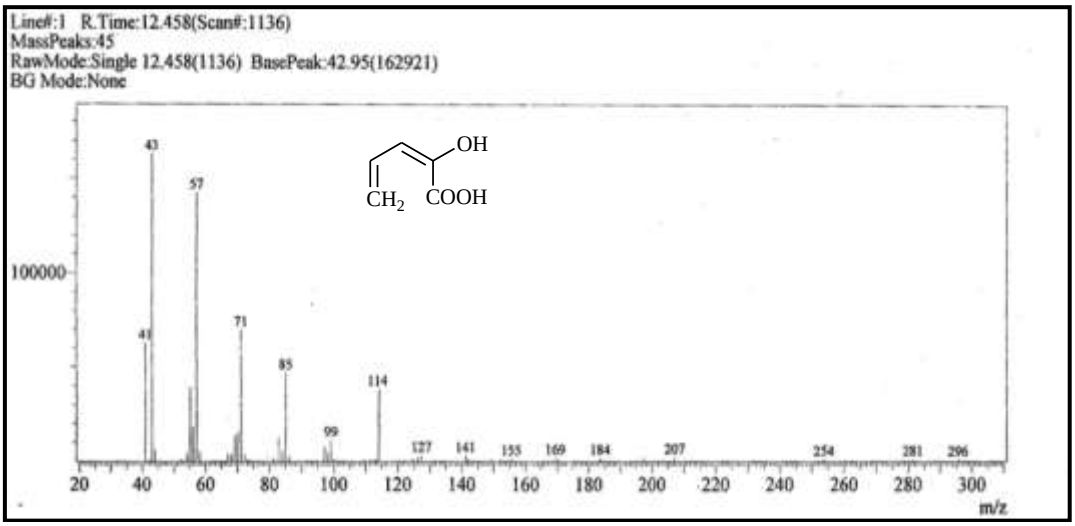


Figure 3: FTIR and GC-MS chromatography of 2-hydroxypenta-2, 4-dienoic acid produced from benzene degradation by alkaliphilic bacteria *Bacillus badius* D1 after36 hrs of incubation

IV. Discussion

The benzene biodegradative bacterial strain was isolated from alkaline Crater Lake of Lonar Buldana, M.S, India. Growth of the strain was observed at optimum temperatures 37°C. The isolated pure bacterium of *Bacillus badius* D1 was examined for benzene degradation in liquid cultures under aerobic conditions. The bacterial strain *Bacillus badius* D1 completely degraded benzene at a concentration of 15 ml/100 ml at pH 9.0 with in 48 hrs. The structural determination of the intermediates of benzene degradation was carried out by spectroscopic analysis like FTIR and GC-MS. A tentative pathway of benzene degradation by *bacillus badius* D1 is also reported "Fig" (4). Only few reports on bacteria capable of attacking benzene have been published Smith, [19], also Hassan, et al [20] have reported the isolation of *Alcanivorax* sp. HA03 from soda lakes in Wadi E1Natrun capable of degrading benzene, toluene, and chlorobenzene as the sole sources of carbon [21] reported that *Halobacterium*, and *Halococcus* isolated from a hypersaline Arabian Gulf coast was mineralized of benzene. Nicholson, et al [22] have reported the degradation of benzene by *halophilic* and *halotolerant* bacteria under aerobic conditions. The first step of benzene oxidation is a hydroxylation catalyzed by Enzymes called a dioxygenase. It is observed that several dioxygenases are present in alkaliphilic bacteria *Bacillus badius* D1 [1]. The results show that alkaliphilic *Bacillus badius* D1 utilized benzene as sole carbon and energy source. The isolation and characterization of the major initial oxidation and ring fission products have suggested multiple routes of enzymatic attack [1].The product, a diol, is then converted to catechol by a dehydrogenase. These initial reactions, hydroxylation and dehydrogenation, are also common to pathways of degradation of other aromatic hydrocarbons. Molecular oxygen serves a reactant in the pathway for benzene degradation. In this reaction, both oxygen atoms become incorporated into the benzene ring. This is accomplished by a dioxygenase-catalyzed reaction between benzene and molecular oxygen, resulting in production of benzene dihydrodiol. Aromaticity is restored by a dehydrogenase-catalyzed conversion of benzene dihydrodiol to catechol (i.e., 1, 2-dihydroxybenzene), which is the ring cleavage substrate. The reactions leading to Catechol and then Catechol either is oxidized by the intradiol *ortho*-cleavage, or the extradiol *meta*-cleavage. Both ring cleavage reactions are catalyzed by specific dioxygenases. The product of the *o*-cleavage *cis, cis*-muconic acid which transfer by many reactions to acetyl-CoA and succinate, the oxygenolytic *m*-cleavage yields 2- hydroxymuconic semialdehyde, which is metabolized by the hydrolytic enzymes to form acetaldehyde, and pyruvate. These are then utilized in the central metabolism. The degradation of aromatic compounds occurs by a limited number of reactions, which include hydroxylation, ring cleavage, isomerization, and hydrolysis [23]and[24]. Finally this report showed the ability of alkaliphilic bacteria to degrade of monoaromatic compounds under alkaline conditions.

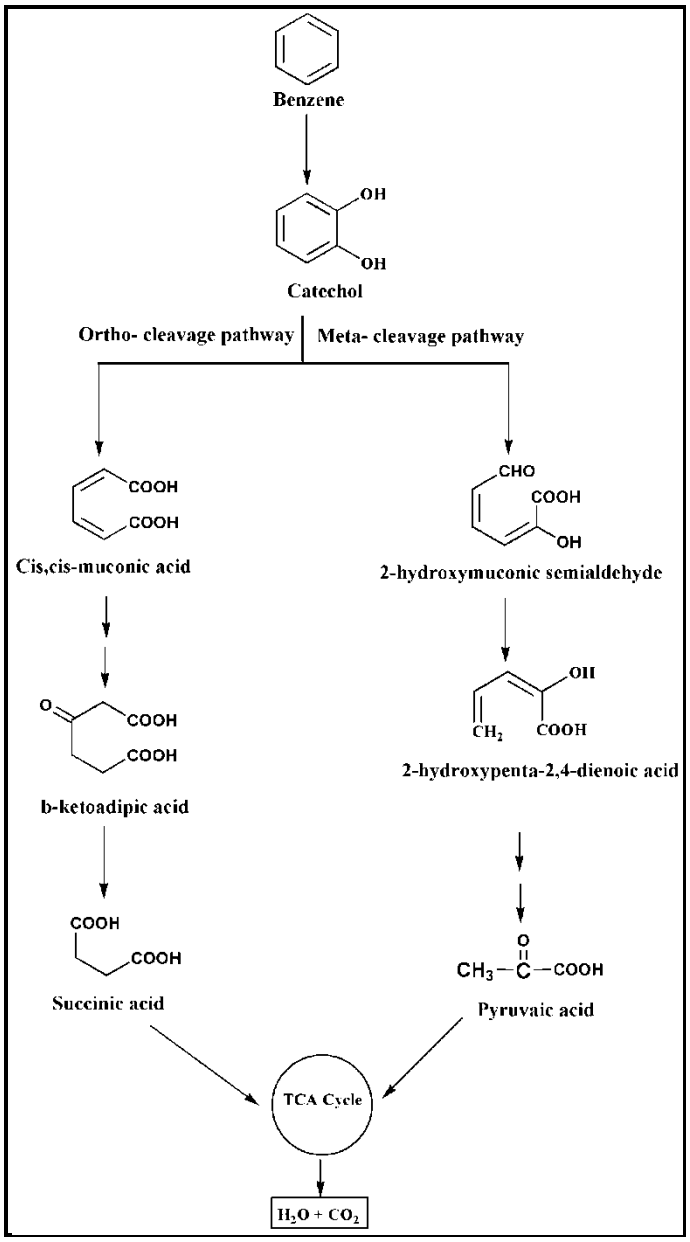


Figure 4: Proposed pathway of Benzene degradation by ortho or meta cleavage by alkaliphilic bacteria *Bacillus badius* D1

V. Conclusions

This study provides the data on the ability of microorganisms capable of degrading contaminated compounds which has accumulated in the environment. Biodegradation processes aid the elimination of pollutant aromatic compounds from the environment because microorganisms have enzymatic systems that degrade and utilize different chemical compounds as source of carbon and energy. In this study, it has concluded that the alkaliphilic bacterial strain *bacillus badius* D1 has a promising potential to degrade Benzene by different ways, either by reduction, oxidation or by induction of some enzymes that degrade these toxic compounds. Also it is concluded that the degradation of Benzene as a model of monoaromatic hydrocarbons compounds degradation under alkaline condition. Also, a better knowledge of the variety of catabolic pathways would certainly bring important information for the development of safety bioremediation processes.

Acknowledgements

Ahmed T. Ahmed thanks to Aden University, Republic of Yemen for awarding scholarship and Department of Chemistry, University of Pune, India, for infrastructure facilities.

References

[1]. A. T. Ahmed, A.O. Munif, D. S. Vasudeo, K. R. Gawai, Degradation of Anthracene by Alkaliphilic Bacteria *Bacillus badius*. *Environment and Pollution*, Vol. 1, No. 2, (1212). 97-104.

[2]. C.D. Cunha, S.G.F. Leite , Gasoline biodegradation in different soil microscoms. *Braz. J. Microbiol.*, 31, , (2000). 45-49.

[3]. B.J. Dean, Recent findings on the genetic toxicology of benzene, toluene, xylene and phenols. *Mutat. Res.*, 145, (1985). 153-181.

[4]. M. Eriksson, A. Swartling, G. Dalhammar, Biological degradation of diesel fuel in water and soil monitored with solid-phase microextraction and GC-MS. *Appl. Microbiol. Biotechnol.*, 50, (1998). 129-124.

[5]. M.R. Fries, J. Chee-Zhou, J. Sauford, J.M. Tiedje, Isolation, characterization, and distribution of denitrifying toluene degraders from a variety of habitats. *Appl. Environ. Microbiol.*60, (1994),2802–2810.

[6]. K. Hughes, M.E. Meek, S. Bartlett, Benzene: Evaluation of risks to health from environmental exposure in Canada. *J. Environ. Science and Health*. 19, (2008). 161-171

[7]. E. Jindrová, M. Chocová, K. Demnerová, V. Brenner, Bacterial aerobic degradation of benzene, toluene, ethylbenzene and xylene. *Folia Microbiol*, 47(2): (2002). 83-93.

[8]. G. Alagappan, R. Cowan, Substrate inhibition kinetics for toluene and benzene degrading pure cultures and a method for collection and analysis of respirometric data for strongly inhibited cultures. *Biotechnol Bioeng* 83: z798–809.

[9]. G. Bertoni, F. Bolognese, E. Galli, P. Barbieri, Cloning of the genes for and characterization of the early stages of toluene and o-xylene catabolism in Pseudomonas stutzeri OX1. *Appl Environ Microbiol* 62: (1996). 3704–3711.

[10]. S. Irie, S. Doi, T. Yorifuji, M. Takagi, K. Yano, Nucleotide sequencing and characterization of the genes encoding benzene oxidation enzymes of Pseudomonas putida. *J. Bacteriol* 169: (1987). 5174–5179.

[11]. D.J. Kim, J.W. Choi, N.C. Choi, B. Mahendran, C.E. Lee, Modeling of growth kinetics for Pseudomonas spp. during benzene degradation. *Appl Microbiol Biotechnol* 69: (2005). 456– 462.

[12]. J. M. Kim, N.T. Le, B.S. Chung, J.H. Park, J.W. Bae, E.L. Madsen, C.O. Jeon, Influence of soil components on the biodegradation of benzene, toluene, ethylbenzene, and o-, m-, and p-xylenes by the newly isolated bacterium Pseudoxanthomonas spadix BD-a59. *Appl Environ Microbiol* 74: (2008). 7313–7320.

[13]. J.G. Leahy, K.D. Tracy, M.H. Eley, Degradation of mixtures of aromatic and chloroaliphatic hydrocarbons by aromatic hydrocarbon-degrading bacteria. *FEMS Microbiol Ecol* 43: (2003). 271–276

[14]. K.F. Reardon, D.C. Mosteller, J.B. Rogers, N.M. DuTeau, K.H. Kim, Biodegradation kinetics of aromatic hydrocarbon mixtures by pure and mixed bacterial cultures. *Environ Health Perspect* 110: (2002). 1005–1011

[15]. S.H. Yeom, Y.J. Yoo, Removal of benzene in a hybrid bioreactor. *Process Biochem* 34: (1999). 281–288

[16]. A. Fahy, A.S. Ball, G. Lethbridge, K.N. Timmis, T.J. McGenity, Isolation of alkali-tolerant benzene-degrading bacteria from a contaminated aquifer. *Lett Appl Microbiol* 47: (2008). 60–66.

[17]. S.A.B. Weelink, N.C.G. Tan, H.ten Broeke, C. van den Kieboom, W. van Doesburg, A.A.M. Langenhoff, J. Gerritse, H. Junca, A.J.M. Stams, Isolation and characterization of Alicycliphilus denitrificans strain BC, which grows on benzene with chlorate as the electron acceptor. *Appl Environ Microbiol* 74: (2008) 6672–668.

[18]. J.M. Kim, C.O. Jeon, Isolation and characterization of a new benzene, toluene, and ethylbenzene degrading bacterium, Acinetobacter sp. B113. *Curr Microbiol* 58: (2009). 70–75.

[19]. M. R. Smith, The biodegradation of aromatic hydrocarbons by bacteria. *Biodegradation* 1: (1990). 191 -206.

[20]. H.A. Hassan, N.M.H. Rizk, M.A. Hefnawy, A.M. Awad, Isolation and characterization of halophilic aromatic and chloroaromatic degrader from Wadi El-Natrun Soda lakes. Life Sci J 9: (2012). 1565-1570.

[21]. D.M. Almailem, N.A. Sorkhoh, H. Al-Awadhi, M. Eliyas, S.S. Radwan, Biodegradation of crude oil and pure hydrocarbons by extreme halophilic archaea from hypersaline coasts of the Arabian Gulf. Extremophiles 14: (2010). 321- 328.

[22]. C.A. Nicholson, B.Z .Fathepure, Biodegradation of benzene by halophilic and halotolerant bacteria under aerobic conditions. Appl. Environ. Microbiol. 70: (2004). 1222-1225.

[23]. A. Cebron, M.P. Norini, T. Beguiristain, C. Leyval, Real-Time PCR quantification of PAH ring hydroxylating dioxygenase (PAH-RHDα) genes from Gram positive and Gram negative bacteria in soil and sediment samples. *Journal of Microbiological Methods* 73: (2008). 148-159.

[24]. J.S. Seo, Y.S. Keum,. Q.X. Li, Bacterial degradation of aromatic compounds. *International Journal Environmental Research and Public Health* 6: (2009). 278-309.

Ahmed T. Ahmed. "Biodegradation of Benzene by Alkaliphilic bacteria under Aerobic conditions ." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), vol. 11, no. 9, 2017, pp. 31–37.