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O- cresol Biodegrdation by Bacillus badius D1 isolated from Salt Lake Lonar (MS), India.

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Abstract: *Cresols are known to be dominant lethal mutations in germ cells in male mice. Cresols may persist in extremely oligotrophic water bodies, and/or those under anaerobic conditions, such as in some sediments and groundwater aquifers The Strain Bacillus badius D1 was employed for biodegradation of o-cresol at higher concentration isolated from Lonar Crater Lake Buldana (MS), India. It has degraded 350 mg/L concentration within 30 hr. The optimum pH and temperature observed was 9.00 and 32 ^{0}C respectively. The intermediates of catabolism were trapped as salicylic acid and catechol. This organism can be employed for bioremediation at polluted site or in bioreactors.*

Key Words: *Bacillus badius, alkaliphile, o-cresol, biodegradation*

I. Introduction:

Cresols are widespread in nature, occurring in petroleum, coal tar, crude oil and volcanic actions. They are emitted from municipal incinerators, during coal and wood combustion, with vehicle exhaust, from oil refineries and cigarette smoke. Cresols are also products of the photo oxidation of toluene.

These could be absorbed through the skin, inhalation and are distributed throughout the body. These are toxic to respiratory and gastrointestinal tract. The p-cresol on oxidation leads to a reactive quinine methide. Cresols are known to be dominant lethal mutations in germ cells in male mice. Cresols may persist in extremely oligotrophic water bodies, and/or those under anaerobic conditions, such as in some sediments and groundwater aquifers [1]. m -Cresol, p-cresol o-cresol have high (21.5 - 24.4 g/L) water solubility [2] at 25°C.Hence the risk of toxicity increases in water bodies.

Biodegradation is the dominant mechanism responsible for the fast breakdown of cresols in soil and water. Although bio-degradation of cresol has reported by various researchers using neutrophiles but there is no report of o-cresol degradation by an alkaliphile. An attempt was made to study the o-cresol bio-degradation in alkaline condition.

II. 2 Methodology:

2.1 Chemicals: Yeast extract, peptone was purchased from Hi-media Mumbai. Other chemicals were taken from SRL chemicals. Pure o-cresol was obtained by the curtsy of Department of Chemistry, Pune University.

2.2 Biodegradation study:

Six 500 ml conical flasks containing sterilized 250 ml alkaline broth of pH-9.00 were inoculated by 1 % Bacillus badius D1 culture possessing 1.6 OD at 600 nm aseptically [3]. These culture flasks were incubated for 24 hrs at 37 ^{0}C with shaking on Orbital shaker at 110 rpm. The 24 hrs grown culture flasks were induced by adding concentration (150 -350) mg/L of o-cresol. These flasks were removed sequentially from 0 to 30 hours by 6 hr. interval. The removed flasks were used for OD at 600 nm to check the growth and then spun to DuPont Sorvoll Cold centrifuge at 10000 x g. Similarly one another flask was kept as abiotic control by adding experimental concentration of cresol. The residual concentration was investigated using 4 aminoantipyrine method using Jasco Varian -630 as our early report [4,5,6]. These experiments were repeted thrice. The study was extended for various concentration, pH and temperature. The residual sample was given for GCMS analysis after solvent extraction. The study was extended to the bio-catalytic induction using standard methods like Omura and Sato[7,8] for cytochrome P450.Catechol 1, 2 dioxygenase activity was studied by Guzik Urszula, Gren Izabela et al [9] and catechol 2, 3 dioxygenase activity was studied by J.M Sala Trepat and W.C. Evans[10,11] . The enzyme activity noted as µM/ min/ mg of protein. The enzyme activity was expressed as µM / min / mg of protein.

2.3 Spectrophotometric pattern of biodegradation:

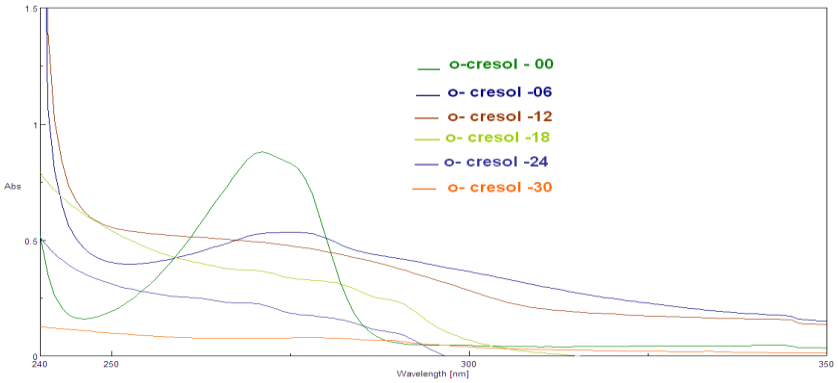


Fig.1 Spectrophotometric pattern of biodegradation by Bacillus badius D1

The **(Fig. 1**) showed that UV-Vis spectrum of o-cresol. Initially the spectrum was observed at 270 nm at 00 hr. Later on it changed to various levels suggested o-cresol has undergone several metabolic transformations.

2.4 Percentage degradation of cresol at various concentration :

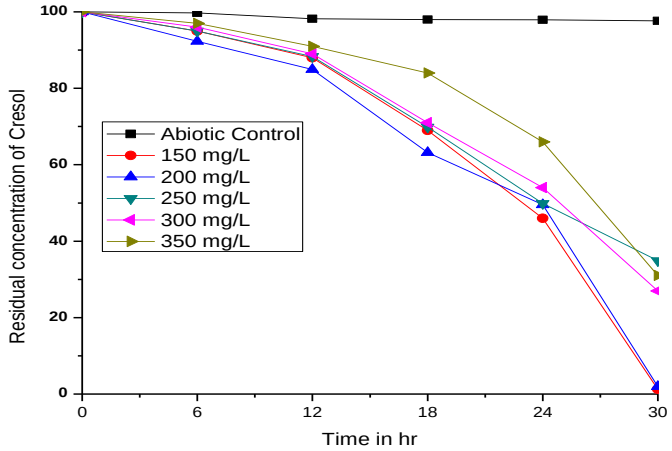
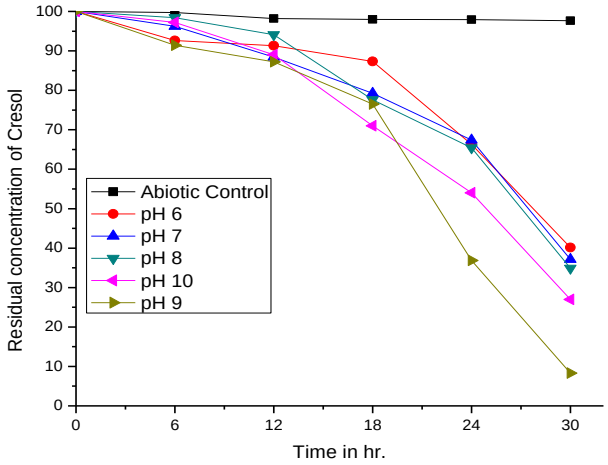


Fig.1.1 Percentage degradation of cresol at various concentration

As all microbes grow at pH 7.00 and 37 ^{0}C.The experiments were carried at the same parameters and the residual concentration of cresol at various time intervals has shown in (**Fig 1.1**). It indicates that various concentrations ranging from (150 mg/L -350 mg/L) was almost degraded by Bacillus badius D1 within 30 hr. At concentrations 150 mg/L and 200 mg/L no residual concentration observed after 30 hrs while at higher concentrations more than 75% degradation was observed.

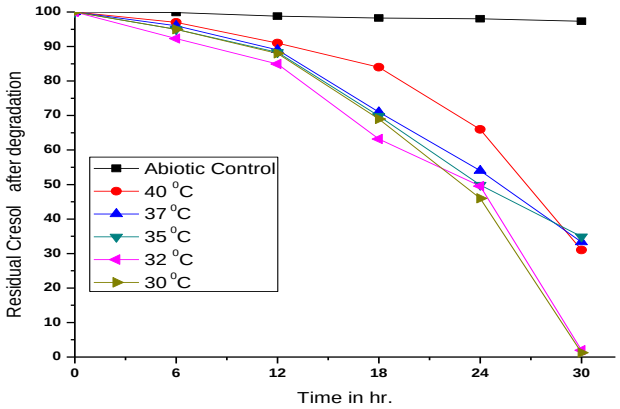
2.5 Percentage degradation of cresol at various pH:



Percentage degradation of cresol at various pH (**Fig 1.2**)

The (**Fig. 1.2**) is self explanatory. It showed that the pH 9.00 was more convenient for degradation as compared to other pH. The highest (350 mg/L) experimental concentration observed 92% degraded at pH 9.00.

2.6 Percentage degradation of cresol at various temperatures:



Percentage degradation of cresol at various temperatures (**Fig 1.3**)

The (**Fig 1.3**) suggested that the optimum temperature 32 ^{0}C was optimum. At this temperature and pH 9.00 the highest experimental concentration was completely degradaed.

2.7 GCMS fragmentation data of Salicylic acid

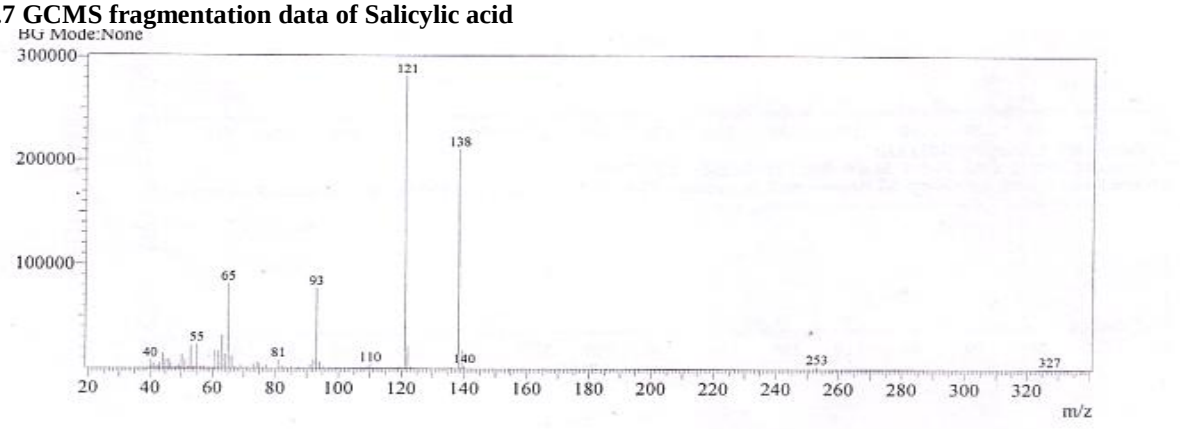


Fig.14 GCMS fragmentation pattern of Salicylic acid

The **(Fig.1.4**) showing fragmentation pattern was of salicylic acid had been confirmed by software GC Solution.

2.8 GCMS fragmentation data of catechol:

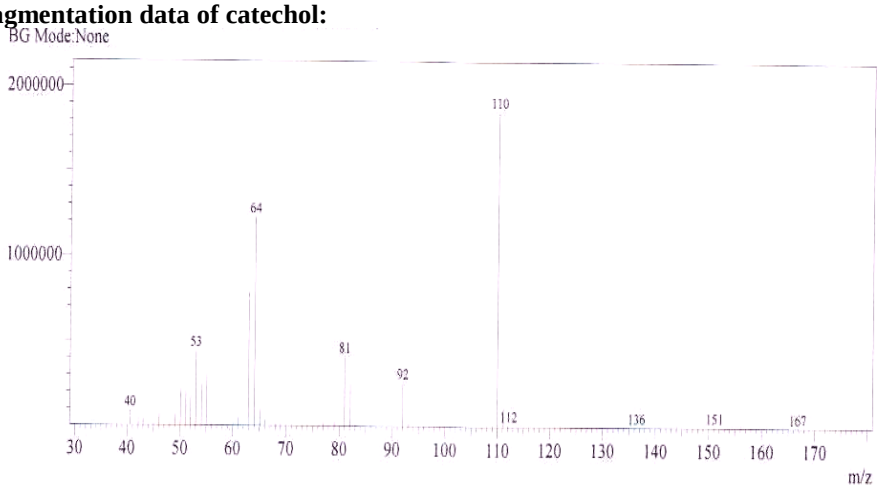


Fig.1.5 GCMS data of catechol as an intermediate metabolite

The **(Fig.1.4** and **1.5**) is showing fragmentation pattern had been confirmed by software GC Solution as Salycylic acid and Catechol respectively.

2.9 Enzyme induction Study:

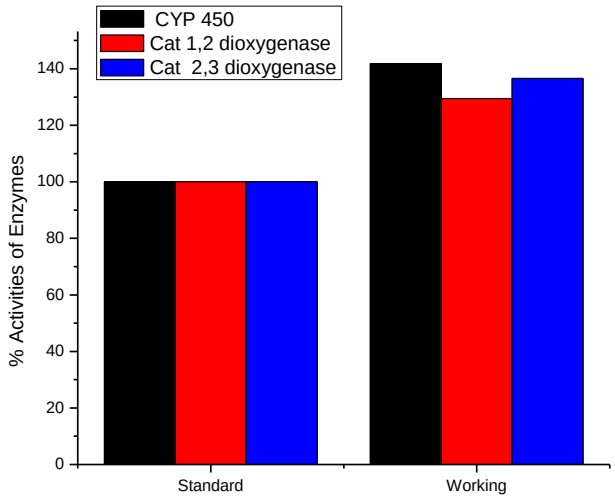


Fig. 1.6 Enzyme induction Study

CYP 450 and Catechol dioxygenase induction after addition of experimental concentration of cresol has shown in (**Fig 1.6**). It indicated the induction of CYP 450, Cat 1,2 dioxygenase and Cat 2,3 dioxygenase. Comparative to Cat 1,2 dioxygenase Cat 2,3 dioxygenase activity observed more.

III. Discussion:

Cresols are natural components of crude oil and coal tar. These are obtained by fractional distillation as well as synthesized in laboratory. During these processes cresols are released in atmosphere by natural and anthropogenic activities like manufacture, use, transport, and storage [12].

O-cresol has been used in disinfectants, fumigants in dyes, odors, in photographic film developers, pesticides etc [13]. It is carcinogenic and affects the kidney; lungs and also the nervous system [14].Most of all phenolic compounds including cresols are harmful to both humans and our environment. The compounds are highly stable and soluble in water hence, create hazards to the environment [15]. Although other

photocatalytic or physicochemical methods are available to remove the pollutants from water bodies but these methods are harsh and costly. Therefore biodegradation is very good alternative to these methods.

There are several reports regarding the bio-degrdation of cresols by neutrophilic organisms like bacteria and fungi but very scare or no reports found on alkaliphilc biodegradation of cresol. Therefore an attempt was made to investigate the percent degradation of o- cresol by alkaliphilic strain Bacillus badius D1. Several changes in o-cresol original spectrum showed that it has undergone different bio-transformations (**Fig.1**)

The degradation of aromatic compounds by mesophilic micro-organisms and the oxidative breakdown of phenolic compounds have been studied in case of aerobic and anaerobic bacteria as well as yeasts [16]. In case of anaerobic biodegradation of o-, p-, and m- cresols; p-cresol was preferentially degraded while m-isomer and o- isomer remain persistent **[17, 18].** In case of Alkaligen eutrophus phenol and cresol reduced from higher concentration to lower from soil [19, 20]. Similarly our strain Bacillus badius D1 degraded high concentration of o-cresol. (**Fig 1.1**) in very less time.The study was also extended to other parameters like variable pH (**Fig.1.2**) and temperature (**Fig.1.3**). It made to know the optimum pH 9.00 and temperature 32 ^{0}C for biodegradation.

The attempt was also made to isolate few of the of metabolites by solvent extraction and GCMS. The metabolites identified from fragmentation pattern were salicylic acid and catechol (**Fig 1.4 and Fig 1.5**). Earlier report shown 4-Hydroxybenzylsuccinate in culture flasks of anaerobic Desulfobacterium cetonicum feeding on p-cresol [21]. The observations regarding enzyme studies showed the enzyme activities of CYP 450, cat 1,2 dioxygenase and Cat 2,3 dioxygenase indicated that Bacillus badiusD1 actively involved in o-cresol degradation.(**Fig. 1.6**). CYP 450 and Cat 2,3 dioxygenase activity was more than Cat 1,2 dioxgense in case of Bacillus badius D1.The earlier report of aromatic hydrocarbon degradation using archaebacteria [22] noted the induction of enzymes catachol 1,2 dioxygenase ,protocatechuate 3,4 dioxygenase, phenol hydroxylase, acetanilide hydroxylase etc [23, 24].

Although; other reports [25, 26, 27, 28, 29] were observed on biodegradation of cresols the Strain Bacillus badius D1 showed higher degradationof o-cresol in very less time. It is nonpathogenic alkaliphile. Photocatalytic and physic-chemical methods are being employed in detoxification of cresols parallelly using nano particles [30, 31, 32]. Few other strains like Lysinibacillus cresolivorans [33] Staphylococcus aureus [34] Aspergillus fumigates [35] were exploited for biodegradation recently.

IV. Conclusion:

Bacillus badius D1 is nonpathogenic alkaliphile have high pollution remediation measure and it could be employed at bioremediation site as a potent strain.

References:

[1]. OVERVIEW, Potentiall Forr Human Exposure, pp- 147-186, 2006.
[2]. OECD SIDS m- / p-CRESOL, pp-1-337, 2003
[3]. V.D. Sarwade, Biodegradation of Benzidibe by Alkaliphilic Strain Bacillus badius D1, IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) e-ISSN: 2319-2402, Volume 8, Issue 11 PP 73-80, 2014.
[4]. V. D. Sarwade1, K.R. Gawai, Biodegradation of phenol by alkaliphiic Bacillus badius D1, IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 8, PP 28-35, 2014
[5]. P.Balamurugan, B.Preetha, Statistical Evaluation of Biodegradation of O-Cresol using Aspergillus fumigatesInternational Journal of ChemTech Research CODEN (USA): IJCRGG ISSN : 0974-4290 , Vol.6, No.12, pp 4934-4939, 2014
[6]. Rafael G. Dolatto, Iara Messerschmidt, Determination of Phenol and o-Cresol in Soil Extracts by Flow Injection Analysis with Spectrophotometric Detection, J. Braz.Chem. Soc, Vol. 23,(5), pp - 970-976, 2012.
[7]. Omura T. and Sato T.R., The Carbon Monoxide-binding Pigment of Liver Microsomes, J. Of Biol. Chem, 239, pp - 2370-2378, 1964
[8]. Suk-Jung Choi, Mira Kim, Sung-Il Kim and Joong-Kyun Jeon, Microplate Assay Measurement of cytochrome P450-CarbonMonoxide Complexes, Journal of Biochemistry and Molecular Biology, pp-332-335,36 (2003).
[9]. Guzik Urszula; Gren Izabela; Wojcieszyska Danuta; abuek Sylwia, Isolation and characterization of a novel strain of Stenotrophomonas maltophila possessing various dioxygenases for monocyclic aromatic hydrocarbon degradation, Brazilian Journal of Microbiology 40 (2009) 285-291.
[10]. M. Grekova-Vasileva, Y. topalova, Enzyme activities and shifts in microbial populations associated with activated sludge treatment of textile effluents, Biotechnol. & Biotechnol. eq. 23(2009)1136-1142.
[11]. Jose M. Sala -Trapet and W. Charles Evans, The meta Cleavage of Catechol by Azotobacter Species 4-Oxalocrotonate Pathway, Eur. J. Biochem. 20, pp - 400- 413. 1971
[12]. Husein Yemendzhiev, Anna Terziyska, Degradation of mixed aromatic pollutants by Trametes verisicolr Strain 1, Biotechnol & Biotechnol. Eq. ISS.No. 1310-2818(print) pp-39- 40, 2011.
[13]. United Nations Environmental Program (UNEP). Division of Technology, Industry and Economics Chemicals Branch. (2006).
[14]. Agency For Toxic Substances and Disease Registry (ATSDR).. Toxicological profile for cresols. U.S. Department of Health and Human Services, Public Health Service, Atlanta. (2008)
[15]. Wojciech Zmudziński, Removal of o-Cresol from Water by Adsorption Photocatalysis, Polish J. of Environ. Stud. Vol. 19, No. 6, pp- 1353-1359, 2010.
[16]. J. A. Buswell, D. G. Twomey, Utilization of Phenol and Cresols by Bacillus steavothevmophilus Strain PH24, Journal of General Microbiology 87, pp -377-379,1975.
[17]. Walter J. Smolenski, Jseph M. Suflita, Biodegradation of Cresol Isomers in Anoxic Aquifers, Vol.53, No. 4 Applied and environmental microbiology, pp. 710-716, 1987.

[18]. Kathleen L. Londry, Phillip M. Fedorak, Anaerobic Degradation of m-Cresol by a Sulfate- Reducing Bacterium, Applied and environmental microbiology, Vol. 63, No. 8, pp- 3170– 3175, Aug. 1997
[19]. Robert Evangelista, Treatment of phenol and cresol contaminated soil,Journal of Hazardous materials 25, pp-343-360. 1990.
[20]. P.Y. Aneez Ahamad and A.A.M. Kunhi, Degradation of high concentrations of cresols by Pseudomonas sp. CP4, World Journal of Microbiology & Biotechnology 15: 321±323, 1999.
[21]. Jochen A. Muller, Alexander S. Galushko, Initiation of Anaerobic Degradation of p-Cresol by Formation of 4-Hydroxybenzylsuccinate in Desulfobacterium cetonicum, Journal of Bacteriology, , pp- 752–757 Vol. 183, No. 2, Jan. 2001
[22]. Sevim Feyaza,Aromatic hydrocarbon degradation by Halophilic archaea isolated from Camalti Saltern,Turky,Water air, soil pllut 224,1449 DOI 10.1007/s 11270-013-1449-9, 2013.
[23]. Hyusein Yemendzhiev, Maria Gerginova, Phenol and cresol mixture degradation by Aspergillus awamori Strain: Biochemical and kinetic substrate interactions, Proceedings of ECO pole Vol. 2, No. 1,pp- 153- 160, 2008
[24]. Ahmed T. Ahmed, Munif A. Othman, Degradation of Anthracene by Alkaliphilic Bacteria Bacillus badius, Environment and Pollution; Vol. 1, No. 2; 2012 ISSN 1927-0909 E-ISSN 1927- 0917, 2012.
[25]. Rania M. A. Abedin, Khouloud M. I. Barakat, Evaluation of medium components using Plackett-Burman design for phenol degradation by marine degrading Fusarium oxysporum, African Journal of Microbiology Research Vol. 7(7), pp. 541-550, 12 February, 2013
[26]. P. Balamurugan, B.Preetha, Statistical Evaluation of Biodegradation of O-Cresol using Aspergillus fumigates, International Journal of ChemTech Research CODEN (USA): IJCRGG, ISSN : 0974-4290 Vol.6, No.12, pp 4934-4939,October 2014.
[27]. Pichiah Saravanan, Kannan Pakshirajan, Prabirkumar Saha, Kinetics of phenol and m-cresol biodegradation by an indigenous mixedmicrobial culture isolated from a sewage treatment plant, Journal of Environmental Sciences 20, 1508–1513,2008.
[28]. I.H. Farooki, F. Basheer, Studies on biodegradation of phenols and m - cresol by upflow anaerobic sludge blanket and aerobic sequencial Batch reactor, Global NEST Journal, Vol 10, No 1, pp 39-46, 2008
[29]. Yan Jiang, Xun Cai, Biodegradation of phenol and m-cresol by mutated Candida tropicalis, Journal of EnvironmentalSciences 22 (4) pp- 621–626, 2010.
[30]. Wojciech Zmudziński, Removal of o-Cresol from Water by Adsorption/Photocatalysis, Polish J. of Environ. Stud. Vol.19, No. 6, pp -1353-1359, 2010.
[31]. Yadollah Abdollahi, Abdul Halim Abdullah, Photodegradation of m-cresol by Zinc Oxide under Visible Light irradiation, International Journal of Chemistry, Vol. 3; pp -31-43. 2011
[32]. Yadollah Abdollahi, Abdul Halim Abdullah, Semi-empirical study of o-cresol photo degradation in manganese-doped zinc oxide nanoparticles suspensions, Chemistry Central Journal, 6:88,pp- 2-8, 2012.
[33]. Haiyan Yao, Yuan Ren, Biodegradation characterisation and kinetics of m-cresol by Lysinibacillus cresolivorans, Water SA Vol. 37 No. 1 January,2011, ISSN 0378-4738,pp 2-8
[34]. Naresh Butani, Vaishali Saliya, Aerobic Micorbial degradation of m-cresol, Multi Disciplinary Edu Global Quest (Quarterly), Volume 2, Issue 1 -5, January 2013
[35]. P. Balamurugan, B.Preetha, Statistical Evaluation of Biodegradation of O-Cresol using Aspergillus fumigates, International Journal of Chem Tech Research, IJCRGG, ISSN: 0974-4290, Vol.6, No.12, pp- 4934-4939, October 2014