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Phenol Biodegradation by *Bacillus aryabhattai* k13 From Paper Mill Effluent Under Optimized Conditions

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Abstract

The environment has been largely contaminated by variety of toxic compounds. Especially through discharge of industrial effluents. Phenol is an organic compound extremely toxic to microorganisms, plants, fish and animals. Therefore, it is necessary to eliminate from the environment. The present investigation is aimed to isolate phenol degrading bacteria, its characterization and treatment of paper mill effluent with selected bacteria *Bacillus aryabhattai* k13 under optimized conditions of p^H, temperature, and concentration of carbon and nitrogen sources, shaking speed. The isolated bacterial strain from paper mill effluent was found to be excellent for phenol degradation. The isolate *B.aryabhattai* k13 from paper mill effluent found to utilize phenol as a source of carbon and energy. The optimized p^H for phenol degradation was neutral and temperature of 35°C -37°C at 48-96 hrs. The culture medium supplied with 0.5 gm/L of urea has stimulated the phenol degradation. Maltose at concentration of 1.0 gm/L was more suitable for phenol degradation. The bacterial strain was able to degrade phenol concentration of1200mg/L at 144hrs of incubation under 150rpm shaking condition. The results indicate that isolate *Bacillus aryabhattai* k13 has much potentiality strain to use in bioremediation of phenol contaminated soils.

Keywords

B.aryabhattai, phenol degradation, potential strain, optimization.

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INTRODUCTION

With the advent of rapid rate of industrialization and contamination of natural sources by toxic chemicals has become a serious problem. A huge quantity of xenobiotics especially phenols and its derivatives are released into the ecosystem from production of agricultural, industrial and pharmaceutical products. Accumulation of these compounds resulted in environmental pollution and contributed to many deleterious effects to the living system [1]. The compounds are bio-non degradable and persistent in soil [2]. Phenol is extremely toxic to aquatic species and adds odour to drinking water [3].Forty percent of hazardous wastes on the environmental surfaces are contaminated with organic and heavy metal pollutants that may cause health hazards to human

and wild life. Major organic pollutant at these sites include phenol, it causes irritation of eye, redness, swelling and finally leads to blindness. High concentration of phenol ingestion causes hepatic damage, vomiting and central nervous system disorders. Liquid form of phenol easily penetrate through the skin; it may accumulate in the liver, lungs and kidney too. [4], it is also suspecting that exposure to phenol for a long period may cause paralysis and cancer [5, 6].

As the toxicity of phenolic compounds is important, their concentration (up to several grams per liter) unfortunately inhibits or even reduces microorganisms in municipal biological wastewater treatment plant [7]. Removal of phenol pollutants from contaminated sites has been a major environmental concern today. A wide variety of microorganisms are known to be capable of metabolizing or mineralizing phenol under aerobic and/or anaerobic conditions. Metabolic processes are governed by the action of enzymes [8]. Many microbes belonging to the genus of *Pseudomonas* have been reported as good degraders of phenol. Among the various species *Pseudomonas putida is* the most popular organism for degradation of phenol as this species uses phenol as carbon source [9]. It has been reported that the *Pseudomonas* species follows a typical Meta cleavage pathway for metabolizing phenol at relatively low concentrations [10].

Among the various techniques available for removal of phenol, biological treatment has been proved to be economical, most promising and versatile approach because of its potential leading to complete mineralization of organic compounds. Aerobic degradation of phenol with pure cultures especially *Pseudomonas putida* has been widely studied [11]. However, a mixed community of microbes would be necessary for complete mineralization of a pollutant. Removal of phenol by solvent extraction, adsorption, chemical oxidation, incineration and other non biological methods have serious drawbacks such as expensive and production of hazardous byproducts whereas biological degradation of phenol is more preferable due to possibility of complete mineralization and cost effective.

The present investigation was aimed to isolate and characterize phenol degrading bacteria *B.aryabhattai* k13 to understand phenol degrading ability with selected physicochemical parameters. The efficiency of phenol degradation was checked at optimized conditions for pH, temperature, nitrogen sources, carbon sources, substrate concentration and aeration conditions.

MATERIAL AND METHODS:

Sample collection: Various effluent samples &soils were collected in sterile plastic containers from random sites of industry located at Ballarsha, Maharashtra, India. Collected samples were filtered through ordinary filter paper to remove coarse particles. Samples were transferred to laboratory for analysis of various parameters.

Enrichment of effluent sample: Enrichment of samples was carried out in laboratory condition by addition of 10mg/L of phenol and glucose was added as additional carbon source to 100ml of effluent sample. The flasks kept on rotary shaker at 120rpm for seven days. 10ml of enriched sample was transferred to the fresh media, containing phenol.

Isolation and screening of phenol degrading microorganisms:

An industrial effluent/soil samples were used by enrichment method; samples were cultured in Erlenmeyer flask. After sterilization of media phenol (10mg/l) was added as carbon source under aseptic conditions. The flasks were incubated at 32°C temperature at 120 rpm for seven days. 10ml of enriched effluent was transferred to 100ml fresh media, containing phenol and glucose as additional carbon source. After enrichment bacterial cultures were streaked on nutrient agar plate containing phenol and glucose as a carbon source containing mineral salt medium with following composition in (mg/L) KH_2po_4- 17,K_2Hpo_4-85 $Mgso_4$-30, $Feso_4.7H_2O$-30, $Cacl_2$-30, $MnSO_4.4_{H2o}$-30, $(NH4)_2SO_4$-17 Trace element solution-1ml ,purified agar18g , at p^H7.5 . All the Petri plates were incubated at 32°C for two days, regular observations were made. The bacterial isolates, which showed consistent growth on the medium, were screened for further study.

Screening for phenol tolerance was done using nutrient enrichment technique in mineral salt media (MSM) amended with different concentrations of phenol i.e., at 100, 200, 300, 400, 500, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200 mg/l and 1% glucose was added (w/v) as additional carbon source. The plates were incubated at 32°C for five days. On the basis of their phenol tolerance efficient strains were selected for further studies.

Identification of bacterial isolate:

The selected isolates were identified from Xcelris laboratory Ahmadabad, India. Bacterial strains were identified based on their colony morphology, physiological a biochemical test, including staining

procedures [12], checked it by referring Bergey's manual of determinative bacteriology [13].

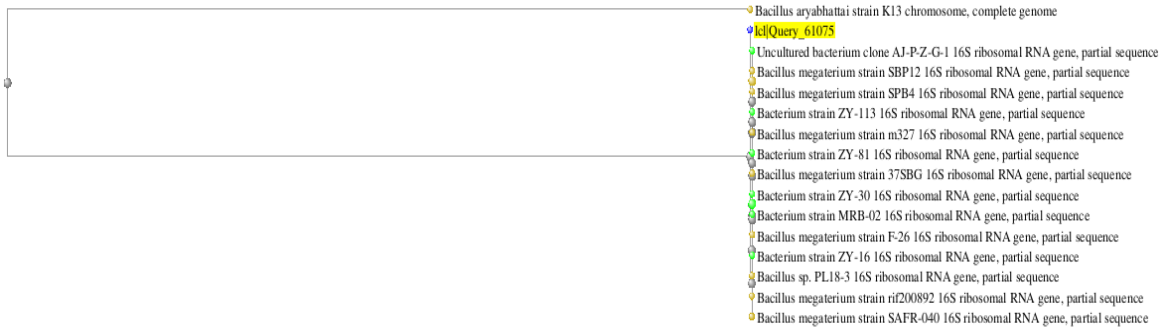

FIG1. phylogenetic analysis based on the 16s r-DNA sequences of phenol degrading strain *Bacillus aryabhattai k 13* using Mega 5 analytical software

Standardization of various physicochemical parameters for phenol removal:

Keeping all other parameters constant, the ability of phenol degradation was studied at temperature of 25^{o}C, 27, 29, 31, 33, 35, 37, 39, 41^{o}C, pH of 5, 6, 7, 8, 9, 10 and 11. To assess the effect of pH on phenol degradation, the pH of the medium was maintained by adding 0.2M buffer solutions: acetate buffer (PH 4.0-6.5) phosphate buffer (PH 7.0and 7.5), Tris- HCL buffer (P^{H} 8.0) and glycine- NaoH buffer (pH 8.5-12.0). The effect of aeration on phenol degradation was studied by incubating the cultures on rotary incubating shaker at 50-200 rpm shaking speed. The effect of nitrogen sources with two different concentrations of urea and ammonium chloride 0.5, 1.0 on phenol degradation along with carbon sources viz maltose, sucrose, glucose, and lactose were studied**.**

Estimation of phenol biodegradation

Residual phenol concentration was estimated using the 4-aminoantipyrine colorimetric method based on standard methods for the examination of water and wastewater [14] with some modifications (wavelength 500nm). The reaction mixture prepared by chemicals including 0.3ml of 2% aqueous 4-aminoantipyrine solution and 1ml of 2N NH_4OH, 1ml of 2% of potassium hexacyanoferrate. Absorbance of red color produced is measured and compared with absorbance of standard solution of phenol.

Analytical method RP-HPLC:

For the determination of phenol degradation performance of *Bacillus aryabhattai* k13, the samples were incubated at 30±1 °C at 150 rpm for 7 days. Sample volumes of 1.5 mL were taken from each flask for alternate days. Later samples were centrifuged at 12000xg at 4 °C for 10 min to remove biomass. Phenol degradation was measured by analyzing the concentration of phenol in the samples by performing reverse phase HPLC (Shimadzu LC-20AD) using C18 column (dimensions: 150 mm x 4.6 mm; particle size: 5 µ) with photo diode array (PDA) detector at 25 °C. Acetonitrile: water (60:40 v/v) was used as mobile phase at flow rate of 0.6 mL/min. Standard phenol was used as reference to determine the retention time of phenol in the samples. Broth without microbial growth was used as control.

RESULTS AND DISCUSSION:

Screening of phenol degrading isolates:

In our study, the industrial effluents collected from paper industry (sarapaka, Bhadrachalam,) BILT graphics paper industry (Ballarsha), textile industry (Warangal)paint industry (Bangalore), leather industry (Deshaipet, Warangal), Cardboard industry (ookal) India, were used for isolation of bacterial strains. A total of twenty strains were isolated, and incubated for further study, among which four isolates (MBVS-1MBVS-2, MBVS-3, MBVS-9) found to be more efficient and faster in phenol degradation (2200mg/L) these can be exploited for further bioremediation studies. Whereas four isolates (MBVS-4, MBVS-5, MBVS-7, MBVS-8, MBVS-20) could degrade phenol up to2000mg/L, remaining isolates able to degrade moderate amount of phenol (1400-1800mg/L). The present findings reveals that (MBVS-1, MBVS-2, MBVS-3, MBVS-9) strains have more efficiency in degradation of phenol as compare to the earlier reports [15, 16, 17]. They have reported a *pseudomonas* strain phDV1 isolated from samples of petroleum contaminated soils of Denmark, capable of degrade phenol up to concentration of 200 mg/l. Among all the potent phenol degraders MBVS-3 bacterial strain isolated from paper mill industry effluents of Ballarsha was identified as *Bacillus*

aryabhattai k13. The degradation performance of selected strain was studied at different culture conditions.

Bacterial strains	MBVS-1	MBVS-2	MBVS-3	MBVS-4	MBVS-5	MBVS-6	MBVS-7	MBVS-8	MBVS-9	MBVS-10
Degradability (mg/L)	2200	2200	2200	2000	1800	2000	2000	2200	1800	1800
Bacterial strains	MBVS-11	MBVS-12	MBVS-13	MBVS-14	MBVS-15	MBVS-16	MBVS-17	MBVS-18	MBVS-19	MBVS-20
Degradability (mg/L)	1800	1800	1600	1600	1600	1000	1400	1800	1800	1600

Optimization of various physicochemical parameters for phenol degradation

Effect of substrate concentration on phenol degradation:

The isolate *Bacillus aryabhattai k13* was subjected to phenol concentration, range from 200-1200 mg/L in mineral salt medium, samples were tested at different time intervals and phenol degradation was studied. The experimental organism was able to degrade phenol up to 98.23 % of 1200 mg/L at 144 hrs of incubation, whereas it could degrade 94.11% of 600mg/L, 97.05 % of 400 mg/L at 96 hrs of incubation time. The results indicated that even at high substrate concentration, phenol degradation ability was remarkable, and the organism has much potential to remove phenol up to 1200mg/L when compare to the previous reports [18] on bacterial strains isolated from phenol contaminated sites of ankleshwar.

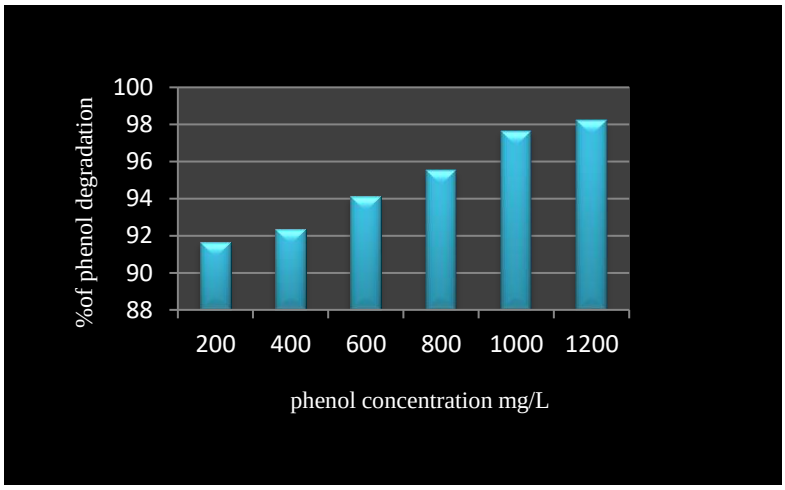

Fig.2 Effect of substrate concentration on phenol degradation by *B.aryabhattai* K 13

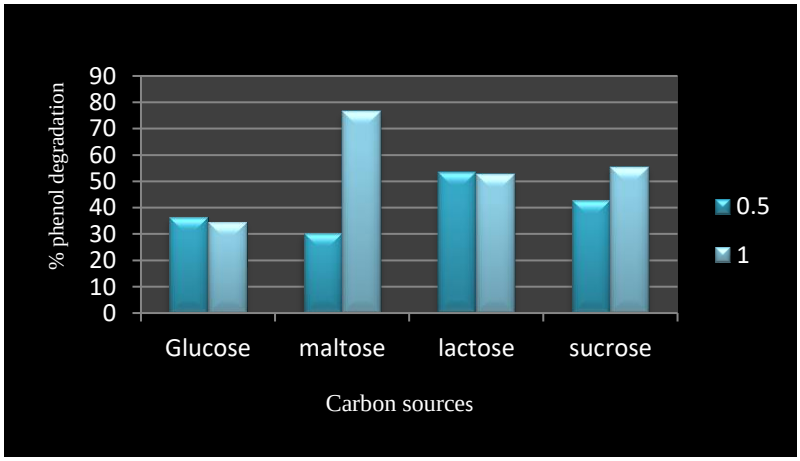

Fig.3 Effect of carbon sources on phenol degradation by *B. aryabhattai* K13

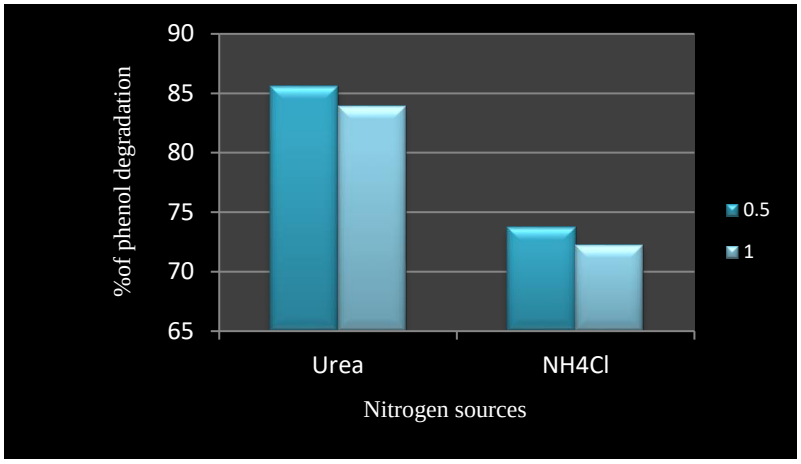

Fig.4Effect of nitrogen sources on phenol degradation by *B. aryabhattai* K13

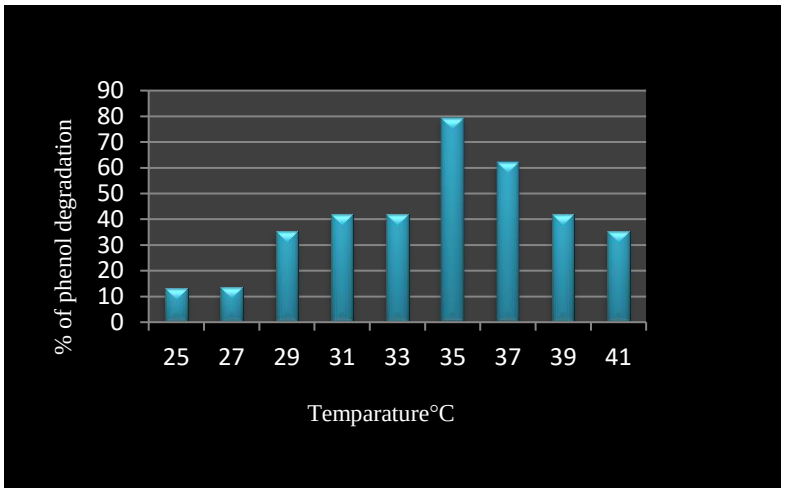

Fig.5 Effect of temperature on phenol degradation by *B. aryabhattai* K13

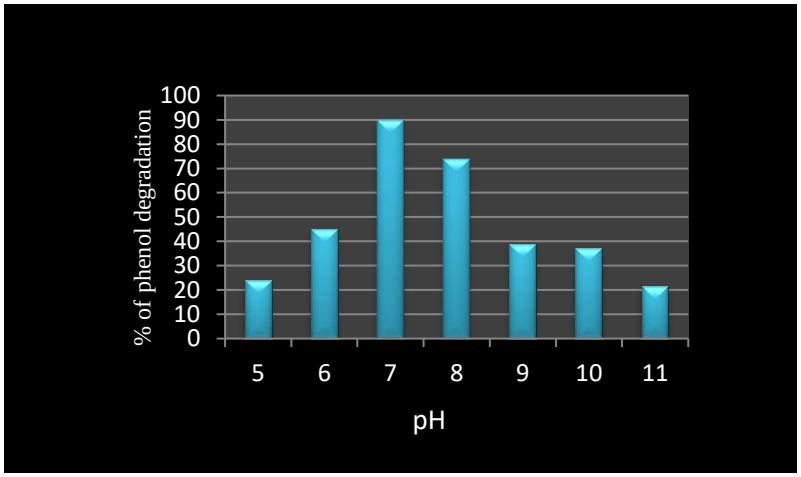

Fig.6.Effect of PH on phenol degradation by *B. aryabhattai* K13

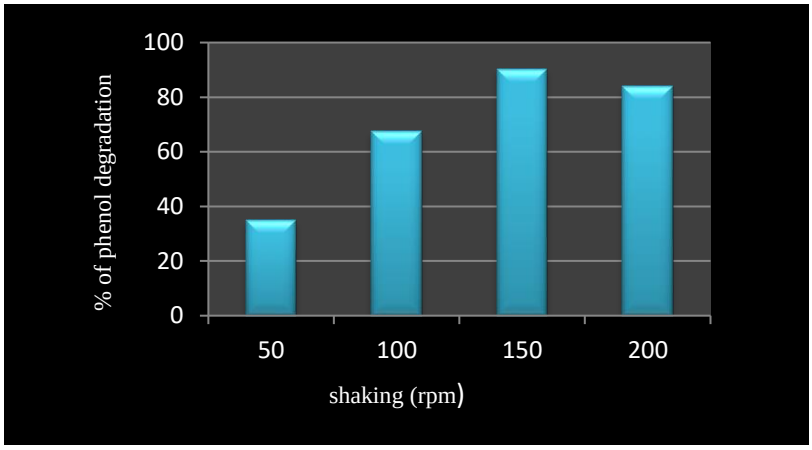

Fig.7 Effect of aeration on phenol degradation by *B. aryabhattai* K13

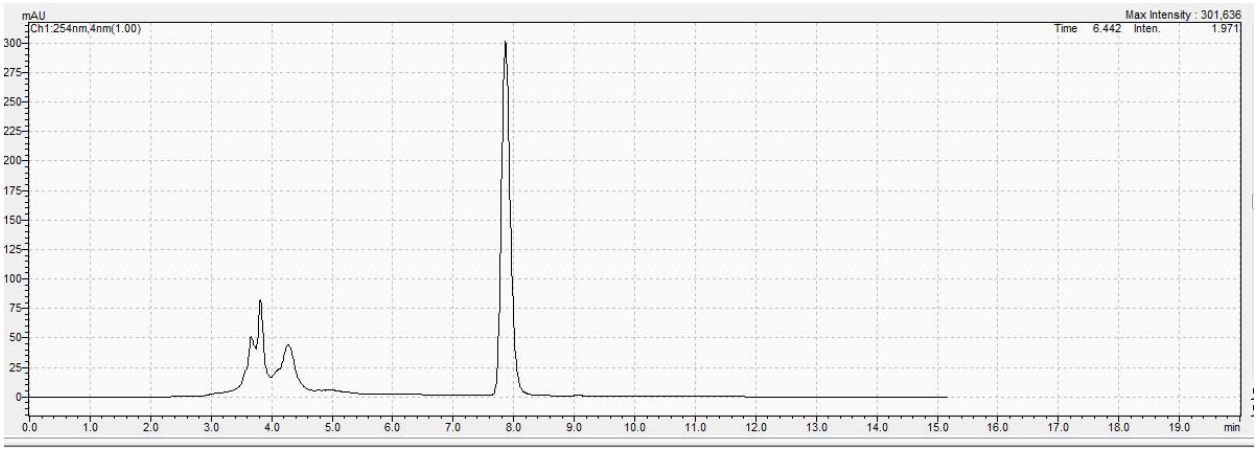

Fig: 8HPLC analysis of phenol by Control showing maximum intensity of phenol

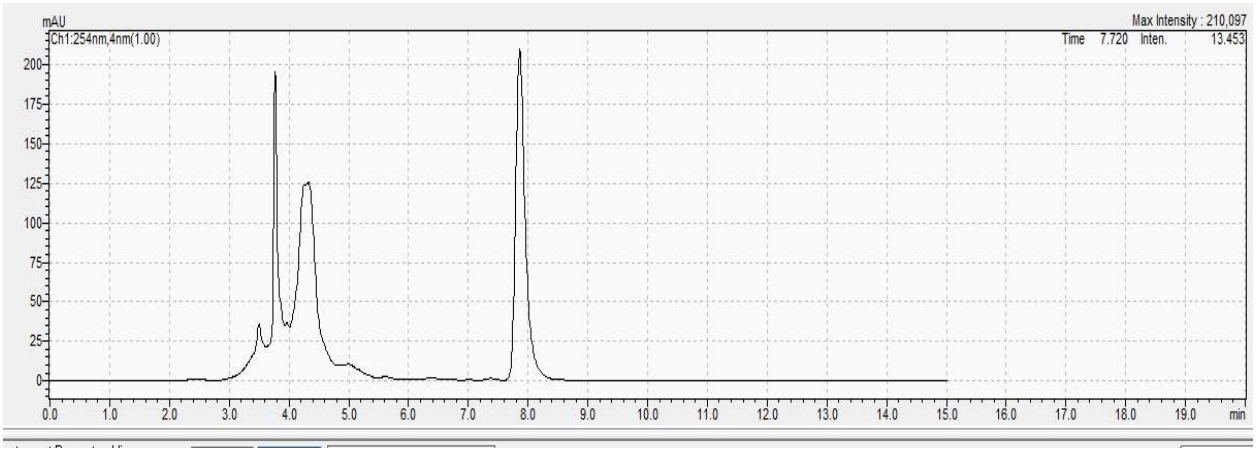

Fig: 9 HPLC analysis of phenol by *Bacillus aryabhattai k13* at different time intervals showing maximum phenol degradation

Effect of carbon sources on phenol degradation

Four different additional carbon sources (glucose, maltose, sucrose, lactose) were tested for maximum phenol degradation by the selected strain (Fig.3).The strain was capable of removing high amount of phenol in presence of maltose at 1.0gm/L concentration (76.44%), where as it opted 1.0gm/ L sucrose to degrade the phenol of 55.44% and 52.55%of phenol degraded in presence of lactose. The present study indicated that maltose at high concentration 1.0gm/L influence the rate of phenol degradation. whereas 0.5gm/L of glucose and lactose stimulated phenol degradation.[19] also reported that glucose supports growth and phenol degradation, the rate of degradation decreased with increase in glucose concentration. According to earlier reports by [18] the 0.5%of glucose was optimum for maximum amount of degradation of phenol by *staphylococcus aureus*. This may occur due to catabolic repression by glucose as reported by [20] because presence of glucose could inhibit utilization of target substrate

Effect of nitrogen sources on phenol degradation

Two nitrogen sources (urea and ammonium chloride) were tested on degradation of phenol by the bacterial strain *B.aryabhattai K13* (Fig.4). Results indicated that strain has potential to remove 85.6% of phenol at concentration of 0.5gm/L of a urea by 96hrs of incubation whereas it could degrade 72.22% of phenol in presence of 1.0gm/L of ammonium chloride.

The rate of phenol degradation was slightly decreased when nitrogen sources were increased from 0.5to1.0mg/L concentration. However, phenol degradation ability increased gradually with incubation period from 24hrs -96hrs.The enhanced rate of phenol degradation at less than 1.0gm/L urea and ammonium chloride can be attributed attenuation of phenol toxicity by nitrogen sources. Similar results were reported by [21].

Effect of temperature on phenol degradation

Optimization of temperature on degradation of phenol was studied by bacterial strain *B.aryabhattai* K13. Results suggested that, though the temperature range of 25-41^{o}C was suitable for complete phenol degradation, it opted temperature of 35 ^{o}C for phenol degradation (Fig.5) [22]. Also reported the optimum temperature of 31 ^{o}C for phenol degradation by microorganisms. Temperature may show crucial role than nutrient availability in degradation of phenol [23]. According to [24] phenol degradation was significantly decreased at 31^{o}C. However, several studies on phenol degradation have revealed that optimum temperature of 30^{o}C [25, 17]. According to [26] when temperature increased to beyond 30 or 34^{o}C no phenol degradation was observed due to cell decay [27]. However, in the present study bacterial strain was able to degrade phenol up to 41^{o}C because of thermo tolerant properties of experimental strain.

Effect of pH on phenol degradation

The PH range from 5.0-11.0was studied on phenol degradation by the bacterial strain *B.aryabhattai K13* (Fig.6). Optimum of p^{H} 7.0& 8.0 was found suitable for the complete degradation of phenol. At low (pH 5.0) and at high (P^{H}9.0) phenol degradation was drastically decreased, at these pH values acid and bases can penetrate into the cells easily, because they exist in undissociated form under these conditions and electrostatic force cannot prevent them from entering cell [28,29] were also reported same kind of results showing maximum phenol degradation at p^{H}7.0

Effect of aeration on phenol degradation

Effect of aeration on selected strain was studied by incubating bacterial culture at different shaking speed (50,100,150,200rpm) for 4 days. A speed of 150rpm was found to be optimum and requiring 96hrs for 90.24% of degradation as compared to static condition. However, at 200rpm 83.23% of phenol degradation was observed at 96hrs (Fig.7) of incubation. Similar type of results were also revealed by other researchers [30, 31, 32]

HPLC analysis:

Upon analyzing the control, phenol peak was recorded at7.8 min (retention time of phenol) with peak intensity of ~300 mAU (milli absorbance units). Whereas the sample (broth incubated with *B.aryabhattai* K13) has shown peak intensity of ~195 mAU. This difference in the peak intensity of phenol between the control and *B.aryabhattai K13* incubated samples indicate the degradation of phenol.

CONCLUSION:

Results from this study have revealed that *B.aryabhattai K13* was capable to degrade phenol was greatly influenced by the culture conditions such as temperature, pH , aeration as well as type and concentration of carbon and nitrogen sources supplied to the culture. Results of optimization studies suggest that the strain can survive under harsh environmental conditions. *B.aryabhattai K13* can be exploited as a potential strain for bioremediation of phenol in industrial effluents.

REFERENCES

[1]. Mailin, M. and Firdausi, R., High Performance Phenol Degrading Microorganisms Isolated from Wastewater and Oil-Contaminated Soil, *Malaysian Journal of Microbiology*, 2(2), 32-36 (2006)

[2]. Nandish, M.S., Microbial Degradation of Phenol and Pentachlorophenol, A thesis. University of Agricultural Sciences, Dharwad (2005).

[3]. Adav S.S, Chen M.Y, Lee D.J, Ren N. Q., Degradation of Phenol by Aerobic Granules and Isolated Yeast Candida tropicalis, Biotechnology and Bioengineering96, 844-852 (2006)

[4]. Kafilzadeh F., Farhangdoost M.S., Tahery Y. Isolation and identification of phenol degrading bacteria from lake Parishan and the growth kinetic assay. Afr. J. Biotechnol., 9(40): 6721-6726 (2010).

[5]. Christopher M. D., Using Stable Isotopes probing to identify soil microbial populations involved in phenol metabolism at an agricultural field site, A thesis cornell university (2007).

[6]. Nair C. I., Jayachandran K., Shashidhar S. (2008) Biodegradation of phenol. African Journal of Biotechnology, 7 (25), 4951-495

[7]. Gernjak W., Krutzler T., Malato A., Caceres J., Bauer R., and Fernandez-Alba A., Photo-Fenton treatment of water containing natural phenolic pollutants, *Chemosphere*, 50, 71–78 (2003).

[8]. Agarry S, Durojaiye A and Solomon B. O., Microbial degradation of phenols: a review, International Journal of Environment and Pollution, 32, 12-28 (2008).

[9]. Mohite, B.V.; Jalgaonwala, R.E.; Pawar, S.; Morankar, A., (2010). Isolation and characterization of phenol degrading bacteria from oil contaminated soil. Innov. Romanian Food Biotech., 7: 61-65 (5 pages)

[10]. Banerjee, A.; Ghoshal, A.K., (2010). Phenol degradation by Bacillus cereus: Pathway and kinetic modeling. Bioresource Tech., 101(14): 5501-5507 (7 pages).

[11]. Kumar A., Kumar S., Kumar S., Biodegradation kinetics of phenol and catechol using Pseudomonas putida MTCC 1194, Biochemical Engineering Journal, 22, 151-159 (2005)

[12]. Aneja K. R. (2001) Experiments in Microbiology plant pathology and Biotechnology 4th edition.102, 106,112, 245-275,278

[13]. Brenner, D.J., Krieg, N.R., Staley, J.T. & Garrity, G.M. (eds.,2005). Bergey's Manual of Systematic Bacteriology, 2nd ed., vol. 2, parts A, B and C, Springer-Verlag, New York, NY.

[14]. American Public Health Association (APHA) (1998). Standard methods for the examination of water and wastewater (19th ed). Washington, DC.

[15]. Chung TP, Tseng HY, Juang RS (2003) Mass transfer effect and intermediate detection for phenol degradation in immobilized *Pseudomonas putida* systems. Process Biochem 38:1497–1507

[16].Abuhamed T, Bayrakter E, Mehmetoglu T, Mehmetoglu U, Kinetics models for growth of *pseudomonas putida*F1 during benzene toluene, and phenol biodegradation. Process Biochem, 2004, 39,983-988.

[17].Paraskevi NP, Euripides GS (2005) Effect of temperature and additional carbon sources on phenol degradation by an indigenous soil Pseudomonad. Biodegradation 16:403–413

[18]. B. Naresh, P. Honey, and S. Vaishali, Biodegradation of phenol by a bacterial strain isolated from a phenol

Contaminated site in India, Res J Environmental Sci, 1(1), 2012, 46-49.

[19]. Loh KC, Wang SJ (1998) Enhancement of biodegradation of phenol and a nongrowth substrate 4-chlorophenol by medium augmentation with conventional carbon sources. Biodegradation 8:329–338

[20]. Chandra, R., Raj, A., Purohit, H. J., & Kapley, A. (2007). Characterization and optimization of three potential aerobic bacterial strains for Kraft lignin degradation from pulp paper waste. *Chemosphere, 67*, 839–846.

doi: 10.1016/j.chemosphere.2006.10.011.

[21]. Lob KC, Tar PP (2000) Effect of additional carbon sources on biodegradation of phenol. Bull Environ Contam Toxicol 64:756–763

[22]. S. Chakraborty, T. Bhattacharya, T. Patel, and K. Tiwari, Biodegradation of phenol by native microorganisms isolated from coke processing wastewater, Journal of Environmental Biology, *31(3)*, 2010, 293.

[23]. Agneta, B., Maraha, N., & Jansson, J. K. (2004). Impact of temperature on the physiological status of potential bioremediation inoculants, Arthrobacter chlorophenolicus A6. Applied and Environmental Microbiology*, 5*, 2952–2958.

[24]. Cort, T, Bielefeldt, A 2000Effects of surfactants and temperature on PCP biodegradation J. Environ. Eng.126635643

[25]. Annadurai G, Juang RS, Lee DJ (2002) Microbiological degradation of phenol using mixed liquors of *Pseudomonas putida* and activated sludge. Waste Manage 22:703–710

[26]. Chandra, R., Raj, A., Purohit, H. J., and Kapley, A. (2007). Characterization and optimization of three potential aerobic bacterial strains for Kraft lignin degradation from pulp paper waste. Chemosphere, 67*,* 839–846.

doi: 10.1016/j.chemosphere.2006.10.011.

[27]. Prieto M.B, Hidalgo, A, Rodriguez-Fernandez, C, Sera, J L and Llama, M, J 2002. Biodegradation of of phenol in synthetic and industrial wastewater by *Rhodococcus erythropolis* UPV-1 immobilized in an air-stirred reactor with clarifier. Appl. Biotechnol, 58:853-859

[28]. O. Aysha, and K. Mumtaj, Degradation of phenol by selected strain of *Bacillus spp.* isolated from marine water, *American* Journal of Pharmacy and Health Care, 2(6), 2014, 41-56.

[29]. Z.U.R. Awan, A. Shah, and M. Amjad, Microbial degradation of phenol by locally isolated soil bacteria, Global Adv Res J Microbiol, 2(4), 2013, 072-079.

[30]. Arrojo B, Mosquera-Corral A, Garrido JM, Mendez R (2004) Aerobic granulation with industrial wastewater in sequencing batch reactors. Water Res 38:3389–3399

[31]. Li ZH, Kuba T, Kusuda T (2006) Selective force and mature phase affect the stability of aerobic granule: An experimental study by applying different removal methods of sludge. Enzyme Microb Technol 39:976–981

[32]. Lopes FA, Vieira MJ, Melo LF (2000) Chemical composition and activity of a biofilm during the start-up of an airlift reactor. Water Sci Technol 41:105–111