

Bio-degradation of phenol from waste water of coastal sources in Bengal bay in India

Ahmad Fazilat

M.Sc in Department of Biotechnology, Andhra University, Visakhapatnam, India.

ABSTRACT: - Phenol, an organic compound, is very toxic upon ingestion, contact or inhalation and is lethal to living organism even at low concentration. Phenolic compounds are present in varying concentration in waste water of synthetic resins, plastic and polymer manufacturing, dye and dye intermediate manufacturing and pharmaceutical industries. Among various techniques available for removing phenol, biological treatment has been proved to be economical and most promising and versatile approach. The present investigation was under taken to assess the biodegradation of phenol by native bacterial strain from effluent from phenol contaminated site of coastal region in Bengal bay, in India. A selected Gram positive bacterial strain is isolated and has been used to study biodegradation of phenol in shake flask culture. Various physicochemical parameters are optimized for the maximum biodegradation of phenol, viz., pH, and temperature, initial concentration of phenol, additional carbon sources and additional nitrogen sources. Complete phenol biodegradation was achieved after 4 days in 1000 ppm solution. The isolated Gram positive bacterium can be exploited as a candidate of choice for the bioremediation of phenolic effluent.

KEYWORDS: Phenol degradation, Biodegradation and temperature.

INTRODUCTION

Organic pollutants comprise a potential group of chemicals which can be dreadfully hazardous to human health. Many of them are resistant to degradation. As they persist in the environment, they are capable of long range transportation, bioaccumulation in human and animal tissue and biomagnification in food chain. Biodegradation is used to describe the complete mineralization of the starting compound to simpler ones like CO₂, H₂O, NO₃ and other inorganic compounds (Atlas and Bartha, 1998). The term has been proposed for describing the ultimate degradation and recycling of an organic molecule to its mineral constituents. According to Alexander (1965) no natural organic compound is totally resistant to biodegradation provided that the environmental conditions are favourable. This is known as the principle of microbial infallibility. Microbiologists have hardly dipped below the surface of the natural pool of microbial diversity. When new organisms have been isolated with biodegradation efficiency, their biochemical versatility has been found to be immense. Attempts to determine microbial diversity in natural environments like soil are limited by the inability of the microbiologists to culture specific microbes present in a particular environmental sample. However, the isolation of those microbes will often require a targeted intelligent approach to screen the biosphere for its presence (Wackett and Hershberger, 2001).

The massive mobilization of compounds in natural resources or the introduction of xenobiotics into the biosphere leads to unidirectional fluxes, which result in the persistence of a number of chemicals in the biosphere and thus constitute a source of contamination. Phenol and its higher homology are aromatic molecules. Containing hydroxyl group attached to the benzene ring structure. The origin of phenol in the environment is both industrial and natural. Phenol pollution is associated with pulp mills, coal mines, refineries, wood preservation plants and various chemical industries as well as their wastewaters (Paula and Young, 1998). Natural sources of phenol include forest fire, natural run off from urban area where asphalt is used as the binding material and natural decay of lignocellulosic material. The presence of phenol in water imparts carbolic odor to receiving water bodies and can cause toxic effects on aquatic flora and fauna (Ghadhi and Sangodkar, 1995). Phenols are toxic to human beings and affect several biochemical functions (Nuhoglu and Yalcin, 2005). Phenol is also a priority pollutant and is included in the list of EPA (1979).

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Chemistry of Phenol

Phenols (C_6H_6O) contain an OH group attached directly to an aromatic ring. They may be colourless solids or thick liquids, often contains a pink tint owing to the presence of oxidation products. Phenol is a hygroscopic, crystalline solid with distinctive odour and is acidic. Molecular weight of phenol is 94.11, the density is 1.072 and the boiling point is $181.9^\circ C$.

Toxicity of Phenol

Acute exposure of phenol causes central nervous system disorders. It leads to collapse and coma. Muscular convulsions are also noted. A reduction in body temperature is resulted and this is known as hypothermia.

Mucus membrane is highly sensitive to the action of phenol. Muscle weakness and tremors are also observed. Acute exposure of phenol can result in myocardial depression. Phenol causes a burning effect on skin. Whitening and erosion of the skin may also result due to phenol exposure. Phenol has an anaesthetic effect and causes gangrene. Renal damage and salivation may be induced by continuous exposure to phenol. Exposure to phenol may result in irritation of the eye, conjunctival swelling, corneal whitening and finally blindness. Other effects include frothing from nose and mouth followed by headache. Phenol can cause hepatic damage also. Chronic exposure may result in anorexia dermal rash, dysphasia, gastrointestinal disturbance, vomiting, weakness, weightlessness, muscle pain, hepatic tenderness and nervous disorder. It is also suspected that exposure to phenol may cause paralysis, cancer and genotoxicity. Phenol and its derivatives are toxic and classified as hazardous materials (Zumriye and Gultac, 1999). These phenolic compounds possess various degrees of toxicity and their fate in the environment is therefore important (Bollag et al., 1988). In recent years, a great deal of research work has been directed toward the development processes in which enzymes are used to remove phenolic contaminants (Ghiourelotis and Nicell, 1999). Phenol is an antiseptic agent and is used in surgery, which indicates that they are also toxic to many microorganisms.

Mechanism of Phenol biodegradation

Generally aromatic compounds are broken down by natural bacteria. However, polycyclic aromatic compounds are more recalcitrant. Derivatization of aromatic nuclei with various substituents particularly with halogens makes them more recalcitrant. There are reports on many microorganisms capable of degrading phenol through the action of variety of enzymes. These enzymes may include oxygenases, hydroxylases, peroxidases, tyrosinases and oxidases (Table 2). Oxygenases include monooxygenases and dioxygenases. The critical step in the metabolism of aromatic compounds is the destruction of the resonance structure by hydroxylation and fission of the benzoid ring which is achieved by dioxygenase-catalysed reactions in the aerobic systems. Based on the substrate that is attacked by the ring cleaving enzyme dioxygenase, the aromatic metabolism can be grouped as catechol pathway, gentisate pathway and protocatechaute pathway. In all these pathways, the ring activation by the introduction of hydroxyl groups is followed by the enzymatic ring cleavage. The ring fission products, then undergoes transformations leading to the general metabolic pathways of the organisms. Most of the aromatic catabolic pathways converge at catechol. Catechols are formed as intermediates from a vast range of substituted and nonsubstituted mono and poly aromatic compounds. Aerobically, phenol also is first converted to catechol, and subsequently, the catechol is degraded via ortho or meta fission to intermediates of central metabolism. The initial ring fission is catalysed by an ortho cleaving enzyme, catechol 1, 2 dioxygenase or by a meta cleaving enzyme catechol 2,3 dioxygenase, where the product of ring fission is a cis-muconic acid for the former and 2-hydroxy muconic semi aldehyde for the latter (Gurujeyalakshmi and Oriel, 1988). *Streptomyces setonii* (ATCC 39116) degraded aromatic compounds such as phenol or benzoate via an ortho cleavage pathway using catechol 1, 2 dioxygenase (An et al., 2001). These dioxygenases are highly labile enzymes and there requires a detailed investigation into its structural properties. A bacterial strain, *Serratia plyniuthica* was able to tolerate phenol up to a concentration of 1050 mg/L. Phenol was degraded through ortho pathway and the crude extract showed the presence of ring cleaving enzyme catechol 1, 2-dioxygenase (Nilotpala and Ingle, 2007). Catechols are cleaved either by ortho-fission (intradiol that is carbon bond between two hydroxyl groups) or by a meta-fission (extradiol that is between one of the hydroxyl groups and a non-hydroxylated carbon). Thus the ring is opened and the open ring is degraded (Cerniglia, 1984). As a general rule, most of the halo aromatics are degraded through the formation of the respective halocatechols, the ring fission of which takes place via ortho-mode. On the other hand, most of the non halogenated aromatic compounds are degraded through meta pathway.

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Material and Methods

Chemicals and reagents: Phenol, lactose, glucose, sucrose, 4-amino antipyrine, NH_4OH , K_3FeCN_6 and other chemicals were of analytical grade and purchased from Himedia Laboratories Pvt. Limited (India).

Sample collection: Various effluent samples were collected from water samples in sterile plastic containers from various regions across Bengal Bay. Collected samples were filtered through ordinary filter paper to remove coarse particles. Samples were immediately transferred to laboratory for analysis of various parameters.

Enrichment of effluent sample: Enrichment was carried out in laboratory condition by addition of 100 ppm of phenol and glucose as additional carbon source to the 100 ml of effluent sample. The flask was kept on rotary shaker at 100 rpm. At every five days, 10 ml of enriched effluent was transferred to 100 ml fresh effluent sample, containing phenol and glucose as additional carbon source.

Isolation and partial characterization of phenol degrading microorganism: From the enriched sample, a loop full of suspension was streaked on Bushnell Haas (BH) Agar plate having (g l⁻¹) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; CaCl_2 , 0.02; KH_2PO_4 , 1; K_2HPO_4 , 1; NH_4NO_3 , 1; FeCl_3 , 0.05; Agar, 30; and containing 100 ppm of phenol. BH agar plate was incubated at 37°C for 24 hr. Next day only one type of pigmented colony was found to be abundant in the plate. Partial characterization of isolated microorganism was carried out by studying their morphological, cultural and biochemical characteristics. Isolated microorganisms were used in further study.

Microorganism and culture condition: This culture was grown initially in 250 ml Erlenmeyer flask containing 100 ml of BH (Bushnell Haas) media containing phenol. Then the flasks were incubated on shaker for incubation. At different time intervals, sample is removed and flask was observed for the color change and which is measured with the colorimetric method.

Optimization of various physicochemical parameters for phenol removal: Phenol was added as the substrate to the basal medium having a concentration range from 100 ppm to 2000 ppm and was inoculated with selected strain. Samples were removed at different time intervals and phenol degrading activity was determined (16,17). Three additional carbon sources i.e. glucose, lactose, sucrose and two additional nitrogen sources i.e. organic nitrogen source (urea), inorganic nitrogen source (ammonium chloride) were tested for the degradation of phenol at various concentrations i.e. 0.2, 0.5 and 1.0% (w/v). 1.5 ml of inoculum was inoculated in 100 ml BH medium containing phenol and different concentration of additional carbon and nitrogen source. All flasks were incubated at 37°C on shaker. Sample was removed for the estimation of degraded phenol at different time intervals. Effect of temperature and phenol was studied in which BH medium containing phenol was maintained at different pH i.e. 5, 6, 7, 8, 9, 10, 11 and temperature i.e. 25°C, 27°C, 29°C, 31°C, 33°C, 35°C, 37°C, 39°C, 41°C inoculated with a selected strain. Aliquots were removed for estimation of degraded phenol at different time intervals.

Analytical method (rapid colorimetric method): To 50 ml of diluted sample, 0.3 ml of 2% aqueous 4-amino antipyrine solution and 1 ml of 2N NH_4OH were added. After mixing the content thoroughly 1 ml of 2% K_3FeCN_6 is added. Absorbance of red colour produced is measured and compared with absorbance of standard solution of phenol (18).

RESULT AND DISCUSSION

By enrichment of the effluent, the potent phenol removing organism was isolated. On the basis of morphological, cultural and biochemical characteristics the *Streptomyces setonii* (ATCC 39116) was identified as a well potential bacterium for phenol biodegradation.

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Table1: Staphylococcus aureus

Morphological and Biochemical characteristics			Carbohydrate fermentation		
Sr. No.	Characteristics	Result	Sr. No.	Carbohydrate	Result
1	Gram reaction	Positive	1	Glucose	Positive
2	Cell morphology	Cocci	2	Sucrose	Positive
3	Motility	Non motile	3	Lactose	Positive
4	Pigmentation	Yellow	4	Mannitol	Positive
5	Spore formation	Negative	5	Maltose	Positive
6	Urea hydrolysis test	Positive	6	Xylose	Negative
7	In dole production test	Negative	7	Cellobiose	Negative
8	Methyl red test	Positive	8	Trehalose	Positive
9	Voges Proskaur test	Negative	9	Arabinose	Negative
10	Gelatin hydrolysis test	Negative	10	Raffinose	Negative

Microbial removal of phenol: The isolated microorganism was tested for its potential to remove phenol from effluent. Phenol was added to BH medium at concentration of 1000 ppm as a sole source of carbon and nitrogen. The result indicated that the strain has potential to remove phenol up to 800 ppm within 7days.

Optimization of culture condition: Three different additional carbon sources (glucose, lactose, and sucrose) were tested for maximum phenol removal by the selected strain. The strain is capable of removing the phenol in the presence of glucose at various concentrations. And the best phenol removal (1000ppm) observed when there was addition of 0.5% of glucose (figure-2a). There was no increase in rate of removal of phenol when lactose and sucrose were added as compared to glucose(figure-2b and 2c).Two nitrogen sources (urea and ammonium chloride) were tested for better removal of phenol by selected strain (figure-2d and 2e). Results suggest that selected strain has potential to remove maximum phenol at a concentration (0.2%) of urea and ammonium chloride. And the maximum removal of phenol (985ppm) was observed at 0.2% of ammonium chloride. Optimization of temperature and pH for the removal of phenol was tested. Result suggested that at a temperature of 37°C was optimum for maximum phenol removal (995 ppm) (figure-2f).And optimum pH for maximum phenol removal (993 ppm) was 7.00.

CONCLUSION

On the basis of the data it is concluded that the isolated organism i.e. *Staphylococcus aureus* has a good potential to remove phenol. Complete removal of phenol up to a maximum concentration of 950 to 1000 ppm was obtained.

REFERENCES

- [1] Kumar Praveen G.N. and Sumangala K.B., Fungal Degradation of Azo dye- Red 3BN and Optimization of Physico-Chemical Parameters, ISCA Journal of Biological Sciences, 1(2), 17-24 (2012)
- [2] Muftah H.E., Shaheen A.A. and Souzan M., Biodegradation of phenol by *Pseudomonas putida* immobilized in polyvinylalcohol (PVA) gel, Journal of hazardous material, 164, 720-725 (2009)
- [3] Hayashi D., Hoeben W.F.L.M., Veldhuizen E.M., Rutgers W.R. and Kroeson G.M.W., In-situ study for the reaction pathway of aqueous phenol degradation by pulsed coronadischarges. Proceedings of international conference on phenomena in ionized gases, Germany (2003)
- [4] Kumar S.R., Kumar R. S. and Kumar R. J., Decolorization of Reactive Black HFGR by *Aspergillus sulphureus*, ISCA Journal of Biological Sciences, 1(1), 55-61 (2012)
- [5] Baroniya M., Baroniya S.S. and Jain M., Operation and Maintenance of Water Treatment Plant at BNP Campus Dewas, India: A Case Study, ISCA Journal of Biological Sciences, 1(1), 83-86 (2012)
- [6] Murhekar G.H., Trace Metals Contamination of Surface Water Samples in and Around Akot City in Maharashtra, India, Research Journal of Recent Sciences, 1(7), 5-9 (2012)
- [7] Nor S.Y., Ariff A., Rosfarizan M., Ahmed S.A., Abdul L. I., Norazah M.N. and Shukor M. Y. A., Optimization of parameter for phenol degradation by *Rhodococcus* UKM-Pin shake flask culture, Proceedings of the World Congress on Engineering, 1, (2010)



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- [8] Pawar M.J., Nimbalkar V.B., Synthesis and phenoldegradation activity of Zn and Cr doped TiO₂ Nanoparticles, Research Journal of Chemical Sciences, 2(1), 32-37 (2012)
- [9] Frieda O. and Nava N., Characteristics of organic removal by PACT simultaneous adsorption and biodegradation, Water Research, 31(3), 391-398 (1997)
- [10] Ruy S.J., Wen C.H. and Ya H.H., Treatment of phenol in synthetic saline wastewater by solvent extraction and two phase membrane biodegradation, Journal of Hazardous Materials, 164(1), 46-52 (2009)
- [11] Wang Y.T., Effect of chemical oxidation on anaerobic biodegradation of model phenolic compounds, Water Environment Research, 64(3), 268-273 (1992)
- [12] Azin I. and Katayon S., Degradation of phenol in wastewater using anolyte produced from electrochemical generation of brine solution, Global Nest: International Journal, 4(2-3), 139-144 (2002)
- [13] Sally N.J., Extraction of Phenol from Industrial Water Using Different Solvents, Research Journal of Chemical Sciences, 2(4), 1-12 (2012)
- [14] Pichiah S., Kannan A.P. and Prabirkumar S., Kinetics of phenol and m-cresol biodegradation by an indigenous mixed microbial culture isolated from a sewage treatment plant, Journal of Environmental Sciences, 20, 1508-1513 (2008)
- [15] Garrity G.M., Bergey's Manual of Systematic Bacteriology, part B 2nd Verlag, 2 (2005)
- [16] Hank D., Saidani N., Namane A. and Hella A., Batch phenol biodegradation study and application of factorial experimental design, Journal of Engineering Science and Technology Review, 3(1), 123-127 (2010)
- [17] Indu C.N. and Shankar S., Microbial degradation of phenol by a species of Alcaligenes isolated from a tropical soil, Ipyhmoehabcmbo, 5(3-4), 47-51 (2004)
- [18] Martin R.W., Rapid Colorimetric estimation of phenol, Analytical chemistry, 21(11), 1419-1420 (1949)