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Short Communication

Biodegradation of Phenol by a Bacterial Strain Isolated From a Phenol Contaminated Site in India

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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 Amla Khadi, Ankleshwar. A selected, Gram positive, bacterial strain so isolated, 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, 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.

Key words: Effluent, phenol, biodegradation.

Introduction

Pollution of environment is one of the major challenges of today's civilization[1]. Phenolic compounds are environmental pollutants because of their wide distribution and detection in the effluent of various industrial operations, which include petrochemicals, textiles, dyeing, phenolic resin manufacturing and steel plant. Phenol is one of the most widely used organic compounds in existence. These phenolic compounds have high toxicity to most of the micro organisms, plants, fish and animals which can cause considerable damage to the environment. Phenols may be fatal by ingestion or skin absorption, since it quickly penetrates the skin and may cause severe irritation to eyes and respiratory tracts. It is listed among priority organic pollutants by the US environmental protection agency[2]. Phenol is potential human carcinogen and is of considerable health concern, even at a low concentration. Hence, the treatment of wastewater containing phenol is necessary. The removal of such hazardous organic pollutants from wastewater is a growing issue[3,4]. As water is precious commodity[5,6], according to the standard set by United State Environmental Protection Agency (USEPA) surface water must contain less than 1 microgram/ liter phenol[7]. In this regard; industrial effluents containing phenols require proper treatment prior to discharge into the environment[8]. Many technologies have been investigated for removing and degradation of phenolic compounds in wastewater. These include adsorption[9], solvent extraction[10], activated carbon adsorption, chemical oxidation[11] and bio degradation[12]. But methods such as solvent extraction[13], activated carbon adsorption and chemical oxidation often suffer from serious draw back including high cost and formation of hazardous byproducts. Among various methods available, biodegradation is economical[14] and environmental friendly. Biological treatment of phenol has therefore been an increasingly important process in pollution prevention.

In our present study the ability of microorganism to degrade phenol was evaluated. Various physicochemical parameters were also optimized for maximum degradation of phenol.

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 Mumbai-400086, India and Astron chemicals (India).

Sample collection: Various effluent samples were collected in sterile plastic container from random sites of 'Amla Khadi' located in Ankleshwar, India. 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 day, 10 ml of enriched effluent was transferred in 100 ml fresh effluent sample, containing phenol and glucose as additional carbon source. Such five transfers were carried out.

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}) $MgSO_47H_2O$,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 incubate at 37C 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[15].

Microorganism and culture condition: This culture was grown initially in 250 ml Erlenmeyer flask containing 100ml of BH (Bushnell Haas) media containing phenol. Then the flasks were incubated on shaker for incubation. At different time interval sample is removed and flask were 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 at 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 concentration i.e. 0.2, 0.5 and 1.0% (w/v). 1.5 ml of inoculums was inoculated in 100 ml BH medium containing phenol and different concentration of additional carbon and nitrogen source. All flasks were incubated at 37ºC on shaker. Sample was removed for the estimation of degraded phenol at different time interval. 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. 25ºC, 27ºC, 29ºC, 31ºC, 33ºC, 35ºC, 37ºC, 39ºC, 41ºC inoculated with a selected strain. Aliquots were removed for estimation of degraded phenol at different time interval**.**

Analytical method (rapid colorimetric method): To 50 ml of diluted sample, 0.3 ml of 2% aqueous 4-amino antipyrine solution and 1ml of 2N NH_4OH were added. After mixing the content thoroughly 1ml of 2% K_3FeCN_6 is added. Absorbance of red color 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 organism was identified as *Staphylococcus aureus* (table1).

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 7 days (figure-1).

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 (1000 ppm) 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 (985 ppm) 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 37ºC was optimum for maximum phenol removal (995 ppm) (figure-2f). And optimum pH for maximum phenol removal (993 ppm) was 7.00 (figure-2g).

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.

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Table-1
Morphological and biochemical characteristics of *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	Indole 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

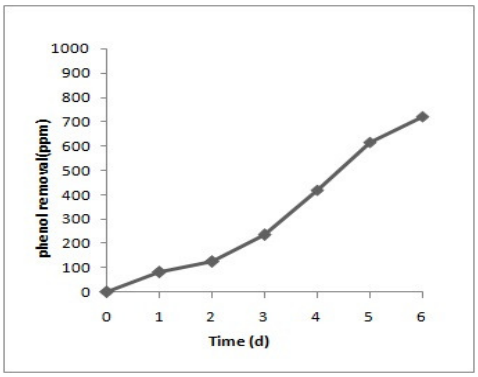

Figure 1
Removal of phenol in BH medium

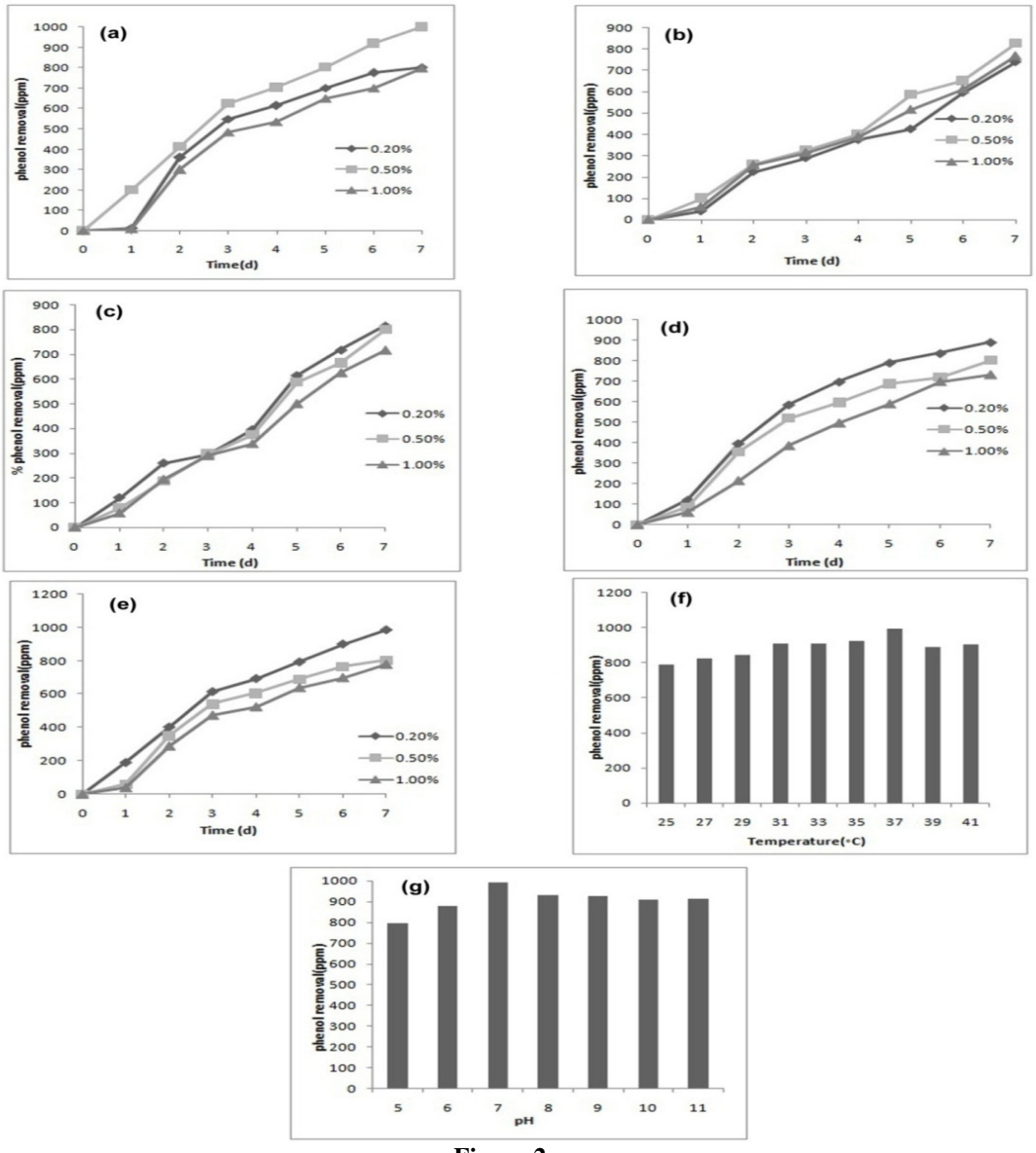

Figure 2:
Effect of various physicochemical parameters on phenol removal (a) glucose, (b) lactose, (c) sucrose, (d) urea, (e) ammonium chloride, (f) temperature, and (g) pH