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Biodegradation of phenol by native microorganisms isolated from coke processing wastewater

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Abstract: *The present investigation was undertaken to assess the biodegradation of phenol by native bacteria strains isolated from coke oven processing wastewater. The strains were designated $ESDSPB_1$, $ESDSPB_2$ and $ESDSPB_3$ and examined for colony morphology Gram stain characters and biochemical tests. Phenol degrading performance of all the strains was evaluated initially. One of the strains namely $ESDSPB_2$ was found to be highly effective for the removal of phenol, which was used as sole carbon and energy source. From an initial concentration of 200 mg l^{-1} it degraded to 79.84 ± 1.23 mg l^{-1}. In turn the effect of temperature (20 to 45^{0}C), pH (5 – 10) and glucose concentration (0, 0.25 and 0.5%) on the rate of phenol degradation by that particular strain was investigated. Observations revealed that the rate of phenol biodegradation was significantly affected by pH, temperature of incubation and glucose concentration. The optimal conditions for phenol removal were found to be pH of 7 (84.63% removal), temperature, 30^{0}C (76.69% removal) and 0.25% supplemented glucose level (97.88% removal). The main significance of the study is the utilization of native bacterial strains from the waste water itself having potential of bioremediation.*

Key words: *Bacterial strains, Phenol degradation, pH, Temperature, Glucose*
PDF of full length paper is available online

Introduction

Phenols are common starting materials and often waste by-products in the manufacture of industrial and agricultural products. Specially phenolic compounds are often found in wastewaters from coal gasification, coke-oven batteries, refinery and petrochemical plants and other industries, such as synthetic chemicals, herbicides, pesticides, antioxidants, pulp-and-paper, photo developing chemicals, *etc.* (Marrot *et al*., 2006; Bodalo *et al*., 2008; Jayachandran and Kunhi, 2008). Now the associated problem due to phenol is that when it is present in waste water even in low concentrations can be toxic to some aquatic species and causes taste and odour problems in drinking water (Rittmann and McCarty, 2001). Inhalation and dermal contact of phenol causes cardiovascular diseases and severe skin damage, while ingestion can cause serious gastrointestinal damage and oral administration into laboratory animals has also induced muscle tremors and death. Even short-term application of phenol to the skin can produce blisters and burns in animals. Therefore, the removal of such chemicals from industrial effluents is of great importance. Current methods for removing phenols from wastewater include hybrid process (Bodalo *et al*., 2008) electrocatalytic degradation (Wang *et al*., 2009) adsorption on to different matrices, chemical oxidation, solvent extraction or irradiation (Spiker *et al*., 1992) which poses other problems like costly process and production of hazardous by-products. One of the cheapest possible solutions to resolve phenol contamination problem is by bioremediation using microbial cells. Many studies on biodegradation of phenol using pure and mixed cultures have been reported (Collins *et al*., 2005; Dursun and Tepe, 2005; Marrot, 2006; Shen *et al*., 2009; Laowansiri *et al*., 2008; Celik *et al*., 2008; Santos *et al*., 2009). In India also a considerable amount of study on phenol biodegradation has been undertaken like the studies of Dhagat *et al*. (2002); Chandra and Rathore (2002); Ambujom and Manilal (2004); Shetty *et al*. (2007); Jayachandran and Kunhi (2008). In these studies there has been considerable interest in the self-cultured functional microorganisms, which are able to thrive on high concentrations of phenol. However there are few reports on the investigation of the role of optimal physical conditions like temperature, pH, additional substrate supplementation on biodegradation efficiency of naturally occurring microbial strains. Such knowledge is desired for improving the efficiency of phenol degradation and its process control. In this study, we investigated the degradation of phenol by a naturally occurring bacterial strain present in coke processing waste water under different growth conditions, including pH, incubation temperature, and additional different carbon sources.

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Materials and Methods

Estimation of phenol: Initial phenol concentration of the waste water samples were measured spectrophotometrically by the method of Yang and Humphrey (1975). Suitable aliquots of the sample were taken and to it 2.5 ml 0.5 (N) NH_4OH solution was added and

pH adjusted immediately to 7.9± 0.1 with phosphate buffer. 1 ml 4-amino antipyrine solution and 1ml $K_3Fe(CN)_6$ solution was added to it and mixed well. After 15 minutes, transferred to cells and absorbance read at 500 nm. Readings were compared with standard phenol.

Isolation and identification of the bacteria from wastewater: Bacteria, thriving in phenolic waste water of coke processing unit (DSP, Durgapore, India) were isolated in Mineral salt agar medium supplemented with phenol concentration of 200 mg l^{-1}. The compositions of Mineral salt medium (MSM) in g l^{-1} were KH_2PO_4 (0.42), K_2HPO_4 (0.375), $(NH_4)_2SO_4$ (0.244), NaCl (0.015), $CaCl_2.2H_2O$ (0.015), $MgSO_4.7H_2O$ (0.05), and $FeCl_3.6H_2O$ (0.054). Microscopic observation and growth characteristics as well as biochemical tests of the isolated bacteria were studied. All the biochemical tests have been performed according to the" Bergey's Manual of Bacteriology ".

Experimental procedure: The isolated bacteria were suspension cultured in nutrient medium containing 3 g l^{-1} beef extract, 5 g l^{-1} peptone, and MSM at pH 7. The suspension cultures of the isolated bacteria were inoculated in MSM containing 200 mg l^{-1} initial concentration of phenol to compare their phenol degrading efficiency. The line of experiment was designed according to a previous study of Wael *et al.* (2003), where he had isolated six pure bacterial strains from a coke processing unit waste water. The major modification was the initial phenol concentration of 200 mg l^{-1}, which he had earlier taken as 100 mg l^{-1}. The residual phenol concentration was monitored at different time intervals spectrophotometrically according to the method described by Yang and Humphrey (1975). The strain degrading phenol to a greater extent within a relatively short time was selected as efficient phenol degrader among the isolates for the optimization studies of the physical environment.

To study the optimum functional pH, temperature and carbon source for maximum degradation, variation in incubation temperature (between 20 to 45°C) with constant initial concentration of phenol (200 mg l^{-1}) and neutral pH in absence of carbon was carried out. Similarly, other parameters were kept constant and pH was varied between 5 and 10. For optimization of glucose as a carbon source, keeping the cultures at pH 7 and 30°C, three different glucose status, *viz.* without glucose, with 0.25% glucose to phenol solution and with 0.5% of glucose was chosen in the media containing bacterial suspension and phenol. The residual phenol concentration was measured at time slots of 6, 12, 18 and 24 hr. All the results were given as a mean with standard deviation (± SD).

Results and Discussion

Estimation of phenol: The initial concentration of phenol in the coke processing waste water of DSP (from which the bacteria were isolated) was found to be between 200-240 mg l^{-1}.

Isolation and identification of the bacteria from wastewater: Three bacterial strains ($ESDSPB_1$, $ESDSPB_2$ and $ESDSPB_3$) were found to be growing in mineral salt medium containing 200 mg l^{-1} of phenol during the early screening for the investigation. When the morphological, Gram stain and biochemical tests were done, two of them were found to be Gram-negative, rod-shaped and the third was Gram-positive, rod-shaped. Different biochemical results were evident for glucose, lactose, sucrose, gas formation from glucose, Simmon's citrate and Voges-Proskauer test (Table 1)

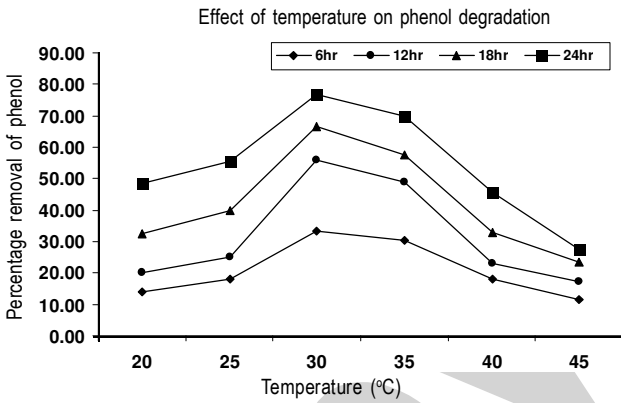

Fig. 1: Effect of temperature on phenol degradation by $ESDSPB_2$

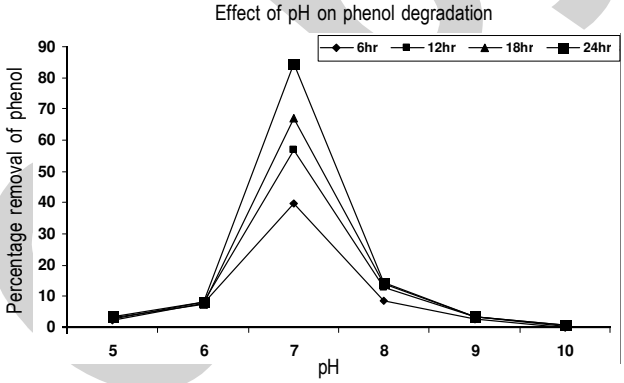

Fig. 2: Effect of pH on phenol degradation by $ESDSPB_2$

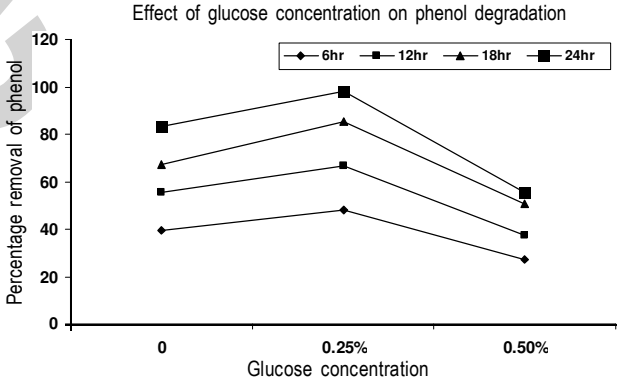

Fig. 3: Effect of glucose concentration on phenol degradation by $ESDSPB_2$

When the performance of phenol degradation was seen by these three strains, strain $ESDSPB_2$ degraded phenol to a greater extent within a relatively short time. From an initial concentration of 200 mg l^{-1} it degraded to 79.84 ± 1.23 mg l^{-1} (Table 2) whereas strains

Table - 1: Microscopic, Gram stain and biochemical characters of the isolated strains

	$ESDSPB_1$	$ESDSPB_2$	$ESDSPB_3$
Microscopic examination	Rod shaped	Rod shaped	Rod shaped
Gram stain	Gram negative	Gram negative	Gram positive
Glucose test	Positive	Negative	Positive
Gas from glucose	Positive	Positive	Positive
Lactose test	Positive	Positive	Positive
Sucrose test	Positive	Positive	Positive
Simmon's citrate test	Negative	Positive	Negative
Voges-Proskauer test	Negative	Negative	Negative

Table - 2: Residual phenol concentration in mgl^{-1} after 6, 12, 18 and 24 hr

Strains	After 6 hr	After 12 hr	After 18 hr	After 24 hr
$ESDSPB_1$	182.57 ± 1.07	174.72 ± 2.03	152.63 ± 2.91	127.12 ± 4.04
$ESDSPB_2$	161.03 ± 2.99	131.41 ± 2.28	90.57 ± 2.62	79.84 ± 1.23
$ESDSPB_3$	191.51 ± 2.42	182.12 ± 2.64	176.42 ± 1.82	163.18 ± 2.78

V = Initial concentration of phenol was 200 mg l^{-1}, N=3

$ESDSPB_1$ and $ESDSPB_3$ showed less degradation. Hence, $ESDSPB_2$ was selected as efficient phenol degrader among the isolates.

Effect of incubation temperature on phenol degradation: The residual phenol estimation data of $ESDSPB_2$ revealed that maximum degradation of 33.46% occurred in cultures placed at 30°C for 6hr (Fig. 1). At 35°C also degradation occurred significantly but less than at 30°C. Degradation was hampered both at low as well as high temperatures. Very less degradation was observed in all the other cases. Similar trend was observed in 12, 18 and 24 hr with slightly higher values of removal rate. At the end of 24 hr 76.69% of phenol was degraded by $ESDSPB_2$ at 30°C and 69.90% at 35°C. While at extreme temperatures of 20°C and 45°C it was only 48.62 and 27.63% respectively. This corroborates with previous studies by Polymenakou and Stephanou (2005) and Rosa *et al*., (2004), on phenol degradation by soil *Pseudomonad*. They recorded maximum degradation rates for phenol to be at 30°C. However temperature of 35°C also showed considerable degradation but level of degradation was much lower than 30°C. Similar results have been reported on the *Pseudomonas pictorum* at 30°C (Gurusamy *et al*., 2007).

Effect of pH on phenol degradation: Increasing the pH of the media at 30°C increased the rate of phenol degradation (Fig. 2) from 5 to 7. On increasing the pH further it had reserved effect on $ESDSPB_2$ phenol removal potentiality. In 6 hr 39.85% phenol was removed at pH 7, while the rest of the pH conditions could not degrade phenol more than 8.42%. Both acidic and alkaline pH had a marked inhibition on phenol removal efficiency. After 12, 18 and 24 hr also analogous result was seen with only 84.63% removal till end at pH 7 at 30°C. At pH 8 phenol removal was maximum upto 14.41%. Neutral pH (pH-7) could degrade phenol at higher rates as compared to the other pH at 30°C. These results substantiate with work by Karigar (2006) on *Arthrobacter citreus*. This may be due to the effect of pH on the ionization and therefore binding and interaction of a myriad of molecular processes, which in turn affect the metabolic pathway. It could even cause denaturing of proteins which might result in lethal toxicity.

Effect of glucose status on phenol degradation: Phenol removal efficiency was determined at different glucose concentrations at a neutral pH of 7 and 30°C temperature for $ESDSPB_2$. The data collected after 24 hr showed that maximum phenol removal efficiency of 97.88% was accessible at 0.25% of glucose concentration (Fig. 3). This might be due to the fact that Glucose acts as a growth substrate in presence of phenol in the wastewater due to its simple structure as compared to phenol. But it was decreased to 55.36% with increasing glucose concentration to 0.5% and also in the absence of the glucose. Media devoid of glucose, at the end of 24 hrs phenol removal was about 83.10%. Previously Kar *et al*. (1996) showed the effect of glucose on phenol degradation and the results indicated that when a mixed substrate (phenol and glucose) was used, phenol acclimatized population showed initial preference for phenol to glucose concentration. A glucose concentration of 0.5% repressed the induction of phenol oxidation though glucose did not fully repress utilization of phenol. Alike results were obtained by Santos *et al*. (2003) and Khaled (2006) in their respective studies.

Therefore it can be concluded that some native bacterial strains isolated from coke oven processing waste water can be good phenol degraders at optimum pH of 7 and an incubation temperature of 30°C. Glucose addition up to a specific low concentration could improve the degradation rate, but impeded the degradation process at higher concentrations. This study can focus on more cost effective applications of native bacterial strains for phenol degradation at large scale in industries, where it pose an alarming problem due to its detrimental health effects on different organisms and human beings.

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