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 ESDSPB1, ESDSPB2, and ESDSPB3, 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 ESDSPB1, 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°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°C (76.59% 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

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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 Maniwal (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.

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) NH₂OH solution was added and
pH adjusted immediately to 7.9 ± 0.1 with phosphate buffer. 1 ml 4-
amino antipyrine solution and 1 ml K$_2$Fe(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$_2$PO$_4$
(0.42), K$_2$HPO$_4$ (0.375), (NH$_4$)$_2$SO$_4$ (0.244), NaCl (0.015),
CaCl$_2$.2H$_2$O (0.015), MgSO$_4$.7H$_2$O (0.05), and FeCl$_3$.6H$_2$O
(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$_3$, and ESDSPB$_5$) were
found to be growing in mineral salt medium containing 200 mg l$^{-1}$ of

![Fig. 1: Effect of temperature on phenol degradation by ESDSPB$_2$](image1)

![Fig. 2: Effect of pH on phenol degradation by ESDSPB$_2$](image2)

![Fig. 3: Effect of glucose concentration on phenol degradation by ESDSPB$_2$](image3)
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\textsubscript{1} 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 the isolates. At pH 8 phenol removal was maximum up to 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 (2007). 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.

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\textsubscript{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.

References


