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RESEARCH ARTICLE

BIOREMEDIATION OF PHENOL USING MICROBIAL CONSORTIUM IN BIOREACTOR

Dipty SINGH and M. H. FULEKAR[1*]

Environmental Biotechnology Laboratory

[1]Professor & Head Department of Life Sciences, University of Mumbai,

Vidyanagari Campus, Santacruz (E) Mumbai-400 098.

Abstract

The aerobic bioremediation of phenol has been carried out in a specially designed bioreactor where activated cow dung slurry was used as a source of microbial consortium containing various concentrations of phenol i.e., 100, 250, 500, 1000 mg/L. A phenol concentration of 1000 mg/L was found inhibitory for cow-dung microbial consortium. Higher concentration (1000 mg/L) of phenol degradation was studied by phenol-acclimated cow dung slurry. The acclimated microbial consortium was found able to degrade higher concentrations of phenol. The present study proved effective in removing phenol of higher concentrations even over a period of 7 days (168 hr). The chemical oxygen demand (COD) has found direct correlation of bioremediation of phenol at concentrations where as decrease in biological oxygen demand (BOD) shows growth and metabolic activity of microorganisms under such conditions the phenol concentration was found decreasing to 100 % at the most in the bioreactor. The technology suggested proved useful for the bioremediation of xenobiotics compounds such as phenol.

Key words: Bioremediation, Phenol, Bioreactor, Microorganisms

Introduction

Phenol, one of the most common environmental pollutants is a simple organic compound possessing a hydroxyl group attached to benzene ring or to another more complex aromatic ring system (Ferhan *et al*, 2002). Alongside its derivates, phenol is widely-spread in the environment as a consequence of its common presence in the effluents of many industrial processes such as oil refineries, petrochemical plants, coal conversion plants and phenolic resin industries (Hinteregger *et al.* 1992). High solubility of phenol in water and the higher content in sewage water testify to a greater probability of the phenol phenomenon acting as a water pollutant, and deteriorating the organoleptic qualities of water (Izmerov, 1984). Phenol is considered to be a toxic compound by the Agency for Toxic Substances and Disease Registry (Agency for Toxic Substance and Disease Registry 2003) and

* Corresponding author : mhfulekar@yahoo.com
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death with ingestion of phenol ranging from 1 to 32 g/L has been reported among adults. Although absorbed rapidly through the lungs, the low volatility of phenol and its affinity with water make oral consumption of contaminated water the greatest risk to humans. Phenol vapors are dangerous in concentrations above 0.001 mg/dm^3. The lungs retain almost 90% of inhaled phenols. The maximum permissible concentrations (MPCs) of phenols range from 0.1 to 0.001 mg/dm^3. Phenol has traditionally been removed from industrial effluents by costly physico-chemical methods, but biodegradation has been studied recently as an alternative (Fulekar, 2005a, Fulekar, 2005b), on account of its lower cost associated with this as well as the possibility of complete mineralization of the xenobiotics.

The present bioremediation technology using the microbial consortium in aqueous environment under controlled environmental conditions in a bioreactor will be useful to treat the hazardous waste containing phenol. In the present study the bioremediation of phenol is carried out in a specifically-designed bioreactor using microbial consortium of various phenol concentrations. This technology is also useful higher phenol concentrations (1000 mg/L).

Material and Method

Microbial biomass preparation and acclimatization

The cow dung has been taken as a source of biomass (Satsangee *et al.* 1990) which is diluted with water in the ratio 1:25 and filtered through sieve (20µm) to remove suspended particles. The prepared cow-dung slurry was aerated and activated in a glass vessel for a week. The physico-chemical (Jackson, 1973) and microbial characteristics of the cow dung have been determined after the activation with 0.1 mg/ml $(NH_4)_2 SO_4$, 0.2 mg/ml Dextrose, 0.1 mg/ml K_2HPO_4, 0.1 mg/ml KH_2PO_4 added as sources of C: N: P for growth and metabolic activity stimulation of microbial biomass. The cow-dung slurry was further acclimated by adding 50 mg/L phenol under continuous aeration and stirring in a glass vessel (Satsangee *et al*, 1996).

Bioreactor system and operation

The bioreactor has been designed for bioremediation of the phenol. The bioreactor is made up of Glass and SS having dimensions of 20cm x 20cm x 25cm. The provision for aeration by pump has been provided to maintain the aerobic condition. The condenser is attached on top of the bioreactor to condense the vaporized organic compounds. The sampling port has been provided on the top of the lid connected to the bottom of the bioreactor. The bioreactor was operated at 250 rpm, 25 ^oC, 12 mg/L dissolved oxygen (DO).

Bioremediation studies

All the bioremediation experiments were carried out in an especially-designed bioreactor**.** In a lab setup 100, 250, 500, 1000 mg/L concentrations of phenol were taken in activated cow-dung slurry. In another experimental set up 1000 mg/L phenol was taken in acclimated cow dung slurry. Two control sets of experiments, one of zero phenol concentration and another set without biomass only phenol in sterile water was set up. The bioremediation conditions were monitored in the bioreactor. Samples were withdrawn initially from 0 hr up to 6 hr then after every 24hr over a period of 168 hr.

Analytical procedure

Samples were centrifuged (5 min, 10000 rpm) to separate cell mass and the supernatant was injected in a HPLC system using UV-VIS Detector and C – 18 Column. The samples were analyzed using the following programme: mobile phase methanol-water 50: 50, wavelength 260 nm, flow rate 1 ml / min, isocratic run for 10 min (Pinto *et al.* 2002).

Results and discussion

The bioremediation of the phenol was carried out in a specially designed bioreactor using activated cow-dung consortium. The varying concentrations of phenol were taken in a separate bioreactor for bioremediation. The physico-chemical characteristics of cow dung were determined after activation. The values of physico-chemical parameters are presented in Table 1.

Table 1. *Physico – chemical characterization of activated cow – dung slurry*

No.	Physico-chemical characterization	Values
1	pH	7.3
2	Dissolved Oxygen, ppm	10.3
3	Temperature, ^{o}C	27.7
4	Organic Carbon, %	0.24
5	Phosphorus, ppm	0.13
6	Kjeldahl Nitrogen, ppm	14
7	Sulphate, ppm	34
8	Calcium, ppm	9.8
9	Biological Oxygen demand (BOD), ppm	23.50
10	Chemical Oxygen demand (COD), ppm	128
11	Total viable count,UFC/100 ml	$1.04 \bullet 10^{9}$

The Table shows the presence of inorganic nutrient in cow dung slurry which is used as a source of nutrients by present microorganisms. The microbial assessment of cow dung shows the presence of bacteria, fungi, and actenomycetes. Table 2 shows the total microbiota of cow dung slurry. The data shows that increase of the temperature indicating bioremediation process which tally with findings that the rate of bioremediation decreases once temperature is decreased (Okoh, 2006).

Table 2. *Cow – dung slurry microbiota*

Bacteria	Fungi	Actinomycetes
Pseudomonas sp.	*Penicillium sp.*	*Nocardia sp.*
Streptococcus sp.	*Rhizopus sp.*	
Sarcina sp.	*Mucor sp.*	
E.coli sp.		

It is also in agreement with research findings of Anthony I Okoh (2006) who reported highest rate of bioremediation in aqueous environment which occurred in range of 20-30 ^{o}C. The pH variation was found near neutrality as biodegradation rate is highest at a pH near neutrality (Salleh *et al.* 2003). Dissolved oxygen was also found decreasing indicates the growth and proliferation of microorganisms. The essential environmental parameters, responsible for bioremediation like pH, temperature, dissolved oxygen and nutrient level (C: N: P), were monitored in bioreactor throughout the experiment (Table 3).

Table 3. *Environmental parameters variation observed during phenol bioremediation*

Parameters	Values
pH	7.2 – 6.5
Temperature, ^{o}C	25 – 28
Dissolved Oxygen, ppm	12 – 9

During the bioremediation the variation of parameter like COD and BOD has been carried out, results are presented in Figure 1 and Figure 2. The Figure 1 demonstrates the decrease in COD levels over a period of bioremediation which indicates the degradation of phenol by microorganisms present in cow dung consortium. The decrease in BOD (Figure 2) values indicates the growth of microorganisms in the varying concentration of phenol.

The present bioremediation study was carried out in a specially-designed and developed bioreactor. In a bioremediation experiment, initial concentrations of phenol (100, 250, 500, 1000 mg/L) were taken in un-acclimated cow-dung slurry.

The experimental findings indicate that in the case of 100 mg/L degradation started immediately i.e., there was no lag phase observed in 100 mg/L concentration; 98.59 % degradation of 100 mg/L of phenol was observed over a period of 24 hr. Similarly, 250 mg/L and 500 mg/L were degraded up to 99.4 and 99.6 % within 72 hr and 96 hr, respectively. Concentration of 1000 mg/L phenol was found inhibitory for cow-dung slurry microbiota as it is not degraded up to 168 hr.

The present bioremediation of phenol shows that the un-acclimated cow-dung consortium can degrade up to the 500 mg/L concentration of phenol completely within 120 hr. The degradation pattern of phenol with time, by un-acclimated biomass, is presented in Figure 3.

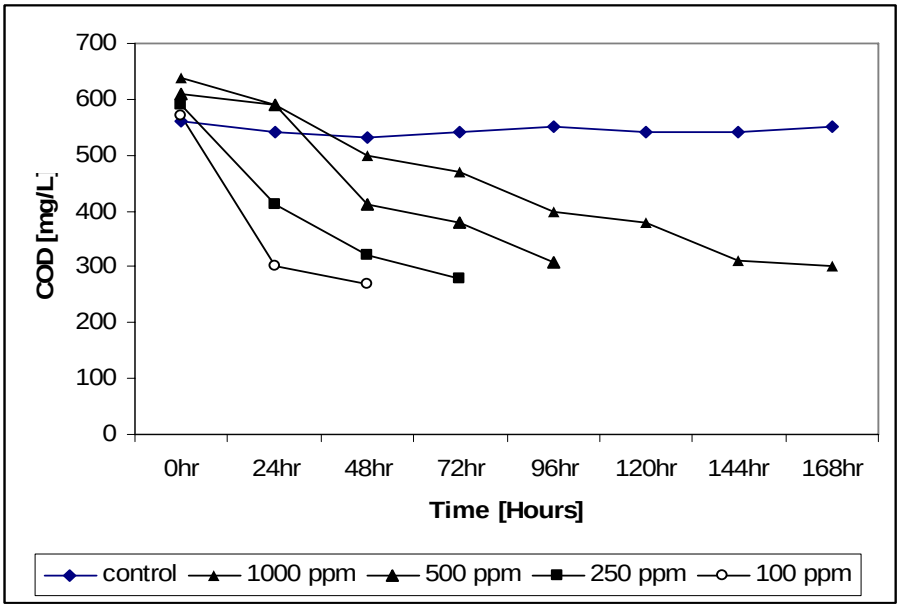

Figure 1. *Chemical oxygen demand (COD) variation during phenol bioremediation by cow-dung slurry*

The degradation of an inhibitory concentration of phenol (1000 mg/L) was carried out by acclimated cow-dung biomass and results were compared to the previous experiment. Cow dung slurry was acclimated by adding 50 mg/L of phenol under continuous stirring and aeration (10 mg O_2/L) in a glass vessel for a period of 96 hr

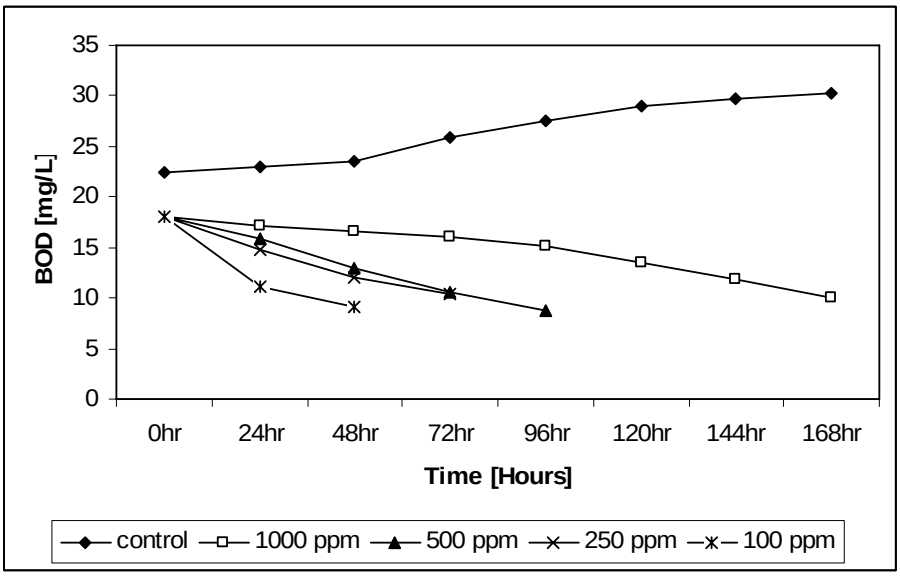

Figure 2. *Biological oxygen demand (BOD) variation during phenol bioremediation by cow-dung slurry*

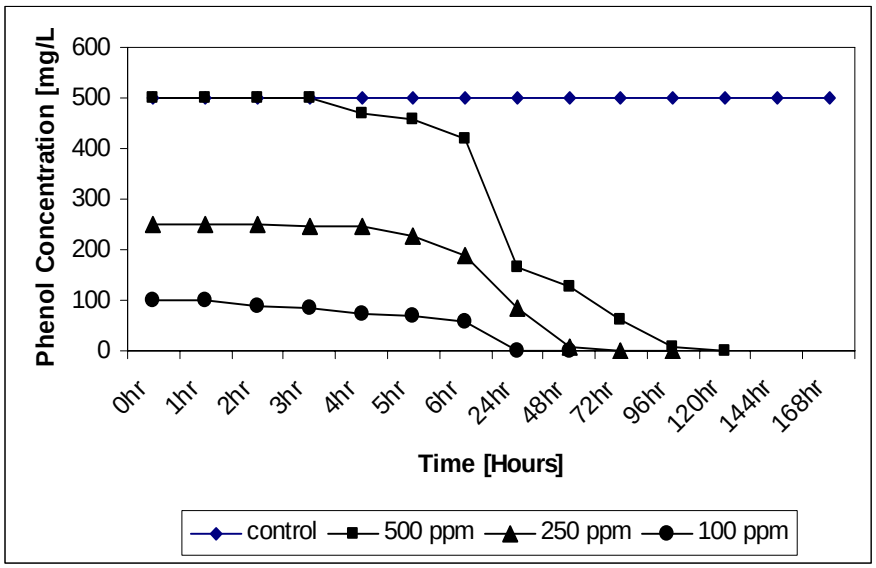

Figure 3. *Time course plot of phenol concentration modification during bioremediation by un-acclimated cow-dung slurry*

Next this acclimated cow dung slurry was taken as a source of biomass for bioremediation study under controlled environmental conditions. The above bioremediation study shows that degradation of 1000 mg/L phenol started within 4hr of experimental set up (Figure 4). Lag phase of only 4 hr was noticed in that case, whereas in un-acclimated slurry lag phase of 3hr was noticed for 500 mg/L phenol (Figure 3). In so doing, the following study reveals that acclimated cow dung slurry is capable of phenol degradation even for higher concentrations. The time course plot of phenol degradation during bioremediation by acclimated cow-dung slurry is shown in Figure 4.

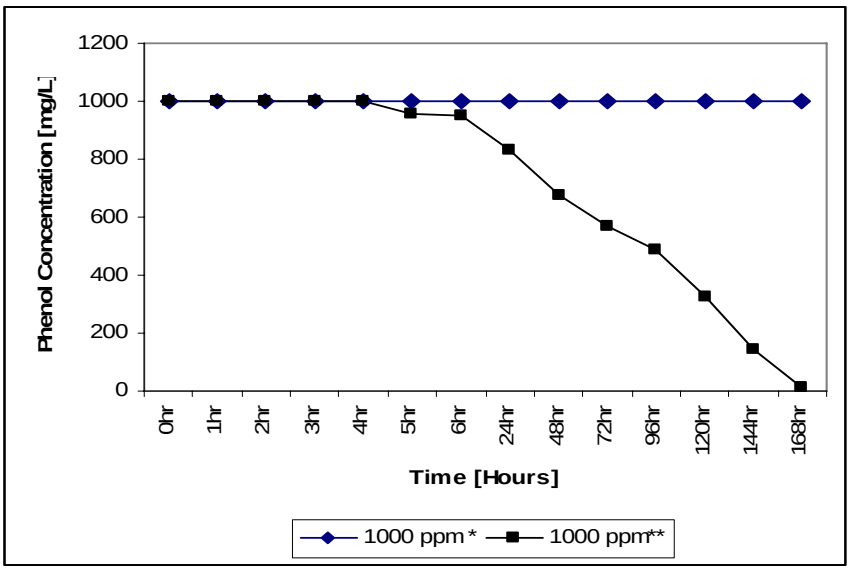

Figure 4. *Time course plot of high concentration (1000 mg/L)] phenol modification during bioremediation by acclimated (*) and un-acclimated (**) cow-dung slurry*
** In un-acclimated cow-dung slurry; ** In acclimated cow-dung slurry*

Conclusion

The present study has been carried out to degrade the phenol in the aqueous environment by use of cow dung consortium. The cow dung consortium (bacteria, fungi and actinomycetes) was found effective in degrading phenol ranging from 100 to 1000 mg/L. The present finding will be useful to treat the waste containing phenol to convert the toxicant into nutrient, biomass and CO_2 via biodegradation through their intermediates. This technology will be useful to the Petrochemical industry and Chemical industry which generates the waste containing compounds such as phenol. The present technology will also be efficient and beneficial to treat the waste generated by chemical industry.

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