DETOXIFICATION STUDIES OF O-CRESOL CONTAMINATED WATER SAMPLE BY IMMOBILIZED CELLS IN A PACKED BED COLUMN REACTOR FOR REMOVAL OF EFFICIENCY

1Dr Korra Amarnath Naik, 1,2Dr Kolluru Sindhuri, 1Dr Saranya Devi Datla

1. Department of Anesthesia, Konaseema Institute of medical Sciences Research Foundation, Amalapuram, Andhra Pradesh, India

*corresponding author: Dr Kolluru Sindhuri
E-Mail: dr.amarnathaikk@gmail.com

Abstract
Increasing population and industrialization have polluted our environment immensely. Biodegradation is the process of complete removal of toxic compounds from our environment to protect and safeguard our earth from pollution. A large number of microorganisms are being utilized for this purpose as they have proven nontoxic, eco-friendly and cost-effective and have gained more public acceptance than the conventional chemical methods. Although they have limited disadvantages the control and optimization of the biodegradation process is a complex system and have to consider many factors like the availability of a microbial population capable of degrading the pollutants, their growth conditions, the availability of nutrient factors including the pollutant of our concern and the environmental factors like temperature pH, presence of oxygen, etc. In the present research, an attempt has been made to study the various scenario of detoxification of o-cresol (methylated phenol) by a bacteria isolated from petroleum-contaminated soil. The isolated novel bacteria Pseudomonas monteilii SHY immobilized in an appropriate matrix showed increasing the removal efficiency of o-cresol from samples. When agar entrapped P. monteilii SHY cells were packed in the bed column and used, the removal efficiency of o-cresol was increased many folds. The process was applied to treat 2L of water from a river source and it could remove 700mg/L of o-cresol from the sample in 8 days and showed a removal efficiency of 99.42%.

Keywords: Pseudomonas monteilii SHY, immobilized beads, water treatment, Detoxification

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1 Introduction
Anthropogenic activities such as rapid industrialization and urbanization have spread their deadly organs in the form of air, water and land pollution to engulf our environment. Environmental preservation for sustainable development is one of the major concerns for a rapidly developing country like India. The main xenobiotic compounds include phenolic derivatives, halogenated benzenes, benzoates, polychlorinated biphenyls, chlorinated pesticides, etc. The cresols are
organic aromatic methylated phenolic compounds and are also known as hydroxytoluene, methyl phenol, methylhydroxybenzene, tricresol, etc. Cresol has 3 isomers which are ortho-Cresol(1,2-cresol), meta-cresol(1,3-cresol) and para-cresol(1,4-cresol) depending upon the position of the methyl group present. O-cresols are used in the production of herbicides, pesticides, dye intermediates, antiseptic, and antioxidants and directly as a valuable solvent. (Handbook of Commercial Catalysts: Heterogeneous Catalysts)¹. O-cresols after reacting with formaldehyde are widely used as Coatings, electronic insulation materials, adhesives and also for automotive applications. Even though they are used for many purposes their toxicity is of high concern because of their disastrous effect to flora and fauna² and it is in the EPA list since 1979. The o-cresol isomer which is more toxic than the other two isomers were selected for the present study. We examined the debasement of combinations of o-cresol, m-cresol, and p-cresol, by Pseudomonas putida disengaged from common sources, and the use of this corruption to the depuration and detoxification of engineered and modern wastewater. Biodegradation tests were acted in bunch and continuous-flow fixed-bed vigorous reactors. Biodegradation was assessed by cresol assurance utilizing micellar electrokinetic slender chromatography, UV spectrophotometry, and chemical oxygen demand (COD). Mineralization of cresols was surveyed by gas chromatography performed both toward the finish of the clump cycle and in the consistent stream reactor gushing. Microbial development was estimated by the plate tally technique. Checking electronic microscopy was utilized to notice bacterial cells adsorbed on polyvinyl chloride chambers in the reactor.

Objective of the study:

In the present research, an attempt has been made to study the various scenario of detoxification of o-cresol (methyalted phenol) by a bacterium isolated from petroleum-contaminated soil. The isolated novel bacteria Pseudomonas monteilii SHY immobilized in an appropriate matrix showed increasing the removal efficiency of o-cresol from samples.

MATERIALS AND METHODS

Microorganism used

The microorganism used for the study was a novel strain of Pseudomonas monteilii isolated from petroleum-contaminated soil was deposited in GeneBank (NCBI, USA) with Accession number: MF278026. The cells proved highly efficient in removing o-cresol in soil and water. Entrapping these cells in the agar matrix increased their removal efficiency many folds. This agar entrapped P. monteilii SHY cells were used in the present study.

Removal of o-cresol by the immobilized cells through a packed bed column reactor

A packed bed column reactor (PBCR) using agar - entrapped cells of P. monteilii SHY was designed for bioremediation of simulated o-cresol containing effluents. An experiment was performed in a glass column of height 50 cm, with a diameter of 3.2 cm was packed with immobilized cells up to 30 cm height under sterilized conditions. It was operated under optimal growth and immobilization conditions. The column was partially filled by the immobilized beads and the efficient bed heights from 5cm to 30cm were checked and selected for the study. The concentration of the substrate was 500mg/L. The flow rate of 5ml/hour was maintained. At the end of each batch cycle, the sample collected from the
column was estimated for residual o-cresol.

**Reusability of agar immobilized *P. monteilii* SHY cells by repeated batch cultivation**

The reusability of the agar immobilized cells was checked by repeated batch degradation experiments. The reactions were carried out in 30°C with 500mg/L of o-cresol. After each cycle of incubation (48 h/cycle), the spent solution in the packed bed column was decanted and the agar beads were washed with sterile water and a fresh solution of o-cresol was added. The process was repeated under identical conditions and the spent solution was analyzed for the residual of o-cresol by 4 AAP method.

**Detoxification of o-cresol from a water sample**

To check the efficiency of *P. monteilii* SHY in treating o-cresol contaminated water source, the water body called Kallai river which is used as a soaking yard for wood by many timber industries in and around the Mancave region of Kozhikode was selected. The water in the river is highly polluted with wood preservatives as the wood after adding preservatives containing o-cresol is dumped in the river body for prolonged storage before use.

The o-cresol removal efficiency was calculated using the formula:

\[
\text{% removal efficiency} = \left( \frac{\text{Initial concentration of o-cresol (mg/L)} - \text{Final concentration of o-cresol (mg/L)}}{\text{Initial concentration of o-cresol (mg/L)}} \right) \times 100
\]

The water for analysis (2 L) was collected in sterile bottles and a sample was transferred to the packed bed column set up having immobilized beads of *P. monteilii* SHY cells (50 gm cells having 0.78 mg protein / g of wet beads) and was operated in the optimized conditions formulated earlier. The eluted samples were centrifuged at 15000 rpm for 10 min at 4°C. Cell-free supernatants were used to estimate o-cresol (4-Aminoantipyrene method) and COD every 24hours.

**Determination of COD**

COD determines the number of organic pollutants found in surface water and in wastewater. The Chemical Oxygen Demand of the o-cresol solution before and after treatment with *P. monteilii* SHY determined to check the ability of the organism to decrease the pollution load according to IS 3025 (part 58), 2006. The samples were centrifuged at 15000 rpm for 10 min at 4°C. Cell-free supernatants were used to estimate o-cresol and COD. The GC-MS analysis of the sample was also done to check the removal efficiency of the organism. The COD was calculated using the formula:
Removal of o-cresol by the immobilized cells through PBCR

The packed bed column with agar immobilized beads of *P. monteilii* SHY cells proved to be highly efficient. Fig:1 The packed bed column set up in removing o-cresol from the medium provided with 700 mg/L of o-cresol. The removal efficiency is directly proportional to the bed height of the column. Fig: 2, has claimed that the removal efficiency of methanol and toluene was directly proportional to the column heights and has reported the same results. The packed bed column set up used for the treatment process is shown in Figure 1. The immobilized agar beads of *P. monteilii* SHY cells in the packed bed reactor were able to remove 99% of o-cresol (700mg/L to 7mg/l) by 7 days. Figure 3 shows the residual o-cresol concentration at different time duration.

The COD was calculated using the formula:

\[
\text{COD (mg O}_2/\text{L}) = (A - B) \times M \times 8000/ \text{vol of sample}
\]

Where, 
- \(A\) = volume of FAS used for blank (ml)
- \(B\) = ml FAS used for sample
- \(M\) = molarity of FAS

\(8000 = \text{milli equivalent weight of oxygen } (8) \times 1000 \text{ mL/L.}\)

Fig: 1 The packed bed column set up
Virender Kumar, \(^8\) have reported the increase in biodegradation efficiency of cyanide by *Serratia marcescens* RL2b immobilized in alginate beads in packed bed column. Phenol Degradation in a Packed Bed Reactor by immobilized cells of *P. aeruginosa* MTCC has been reported\(^9\). The degrading efficiency of the cells is increased in the immobilized stage when used in a packed column set up. The degradation efficiency of the cells was increased far better than in the shake flask. (Table 1). A progressive reduction in removal efficiency may be due to severe mass transfer limitations. Niladevi and Prema, \(^10\) have also employed the use of a packed column for phenol removing and found efficiency increase and the immobilized system maintained 50% of its efficiency after eight successive runs. The decrease in the degradation.
efficiency might be due to the substrate diffusion limitations in the reactor.\textsuperscript{11} This situation may also lead to cell death. The deformation and detachment of cells maybe the other reasons for the reduction in the degradation rate.

**Detoxification of o-cresol from a water sample**

The packed bed column set up used for river water analysis is shown in Figure 4. The treatment process could remove 99.4\% of the o-cresol present in the river water within 7 day. The effluent was highly contaminated and the initial concentration of o-cresol in the sample was 691.4 mg/L of o-cresol and it was reduced to 4 mg/L. (Figure5.)

Fig 4. PBCR set up for o-cresol removal from river water

**The residual o-cresol in the eluted river water samples**

Not much work has been carried out on the o-cresol degradation in a packed bed reactor. The only work reported so far is by Yasin Kaymaz in a continuous packed bed with *Pseudomonas putida* DSM 548 (pJP4). He compared the efficiency between Ca-alginate and pumice immobilized beads and Ca-alginate was found to be more efficient. The degradation rate was found to be reduced after second use and the degradation time required by the cells was 18hrs and 22hrs respectively for Ca-alginate beads and pumice beads (Table2)

<table>
<thead>
<tr>
<th>Days</th>
<th>Residual o-cresol (mg/L)</th>
<th>Removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>691.4</td>
<td>1.28</td>
</tr>
<tr>
<td>1</td>
<td>650</td>
<td>7.14</td>
</tr>
<tr>
<td>2</td>
<td>539.12</td>
<td>22.9</td>
</tr>
<tr>
<td>3</td>
<td>343.61</td>
<td>50.91</td>
</tr>
<tr>
<td>4</td>
<td>235.27</td>
<td>66.39</td>
</tr>
<tr>
<td>5</td>
<td>106.11</td>
<td>84.8</td>
</tr>
<tr>
<td>6</td>
<td>57.04</td>
<td>91.85</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>99.4</td>
</tr>
</tbody>
</table>

**Table 2. The residual o-cresol and the removal efficiency of river water in PBCR**

**Determination of COD**

The organism could reduce the chemical oxygen demand of the solution containing o-cresol to the acceptable limit. The treatment time for reducing the COD was directly proportional to the concentration of o-cresol. The initial COD was estimated before adding to the PBCR and a 94\% reduction in the COD of the sample was found after treatment in the column. Fig: 5. The chemical Oxygen Demand of the water sample was reduced from 1873 mg/L to 107 mg/L which is

under permissible limits. GC/MS analysis confirmed the removal of o-cresol from the water.

![COD reduction in river water](image1)

**Fig:5 COD reduction in river water**

**GC-MS ANALYSIS**
The continuous reduction in the o-cresol concentration can be observed from the chromatogram. This result provides the concrete evidence that the immobilized beads in the column bearing the *P. monteilii* SHY is highly capable of reducing the substrate concentration from the water sample. The compound o-cresol at 15.884 retention time shows the highest peak. GC – MS served as an important tool in confirming the absence of o-cresol in the degraded sample. No secondary metabolites were observed in GC analysis which is the same as observed by many.12,13 (Figure 6). Thus, based on the available information, we could report that cresols degrade rapidly in soils, possibly becoming incorporated into soil microorganisms, without leaving any secondary metabolites.

![GC-MS analysis of water samples collected at different time interval of river water treatment](image2)

**Figure 6: GC-MS analysis of water samples collected at different time interval of river water treatment**

**CONCLUSION**
The novel strain of *P. monteilii* SHY is a highly efficient organism which could remove o-cresol from the contaminated water bodies.
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