Phenol remediation by biofilm developed in sand soil media

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Abstract Laboratory scale sand columns were used in order to evaluate biodegradation of phenol, simulating the conditions which could be created in the non-saturated (vadose) zone by an accidental spill on the top of the soil, biologically remediated by a controlled recycled aqueous flow, enriched by the necessary nutrients. The parameters which have been studied as possible factors influencing the biodegradation process by fixed film biomass, included: (a) the effective size of the sand grain, coarse sand of 2.51 mm and medium sand of 0.81 mm operated in two different columns; (b) flow rate of the aqueous phase recycled through the columns, 0.090, 0.120 and 0.176 bed volumes/hr; (c) different initial loading of phenol applied on the top of the columns, 4, 5 and 6 grams. Daily monitoring of the experimental system included analyses of phenol, COD, turbidity, pH, ammonia, phosphate and suspended solids. Removal efficiencies of phenol were found to be high, close to 100%, within the first seven days following the exposure, indicating high rates of biodegradation. Measurements of volatile attached solids (VAS) connected to the sand, quantitatively indicating fixed film biomass, were higher at the top region and lower at the bottom, at a depth of about one metre: 44.5 and 28.1 mg VAS/gram of dry sand were obtained in the coarse sand column, 10.6 and 4.1 mg VAS/gram of dry sand in the medium sand column, respectively. The general yield coefficient was found to be 0.57 gram VAS/gram of phenol biodegraded. The results indicated that the medium sand size resulted in a slightly better performance, especially in cases of high flow rates, however, coarse sand showed quicker acclimation of microorganisms to biodegrade the phenol. Low rates of flow resulted in more effective biodegradation of phenol. High loading of phenol applied on the top of the columns inhibited, apparently, the microbial activity within the sand columns.

Keywords Sand soil; bioremediation; effective size; flow rate

Introduction Microbial growth plays an important role in the subsurface environment in cases of in situ bioremediation of soil sites contaminated by organic materials, usually of industrial origin. When a contaminant enters the soil, it can undergo a wide variety of physical, chemical and biological transformations within the soil matrix on both micro and macro scale. On a micro scale these transformations can result in the partitioning of the contaminant within the soil matrix. The contaminant may be distributed as a gas in the soil atmosphere, dissolved in pore water, or associated with soil particles, as well as in the form of free product. On a macro scale, the material can be transformed via biotic or abiotic processes and transported within the soil environment, entering either the atmosphere (volatilization/evaporation) or leaching into the groundwater.

The main processes involved in the removal of a contaminant, in a specific given case, are volatilization, sorption, chemical transformations and biodegradation. Spencer and Clai (1977) noted that volatilization of an organic contaminant from soil systems depended on the physico-chemical properties of the contaminant volatility, solubility and sorption), the soil environment (temperature, water content, bulk density and organic content), and the concentration of the contaminant present. Sorption also can influence the bio-availability and hence the susceptibility of the contaminants to biodegradation.

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The background of the present study refers to cases in which a spill of an organic matter occurs. The remediation technology would be based on recycling of local groundwater to the top of the soil surface so that it could enable biological activity of biomass to remove the contaminant until a final, maximal and best achievable stage. The study has focused on the biodegradation mechanism, therefore selecting appropriate soil media and contaminant sorption, volatilization and chemical reactions, were minimized. These requirements led to work with sand as the only type of soil and phenol as the only contaminant (Vincoli, 1997).

The concept of using biological treatment to remove phenol was first reported in the 1920s (Vipulanandan, 1994). Since then there have been many reports that discuss general design and operational guidelines for biological treatment of wastewater containing phenolic compounds. The existence of microbes capable of degrading phenolic compounds has been studied in laboratory experiments (Buitron et al., 1998, Karamanev and Samson, 1998, Edgehill, 1996, Essa et al., 1996, 1997; Yoong et al., 1997; Farooq et al., 1996; Nakhla and Al-Hazarin, 1993, and many others).

The objectives of the present work were to observe the influences exerted by: (a) the sand grain size; (b) the flow rate of recycled water; (c) the initial phenol loading on sand soil media, on the removal rates of phenol, simulating bioregeneration process, which would occur in the vadose zone.

**Methods and material**

The experimental system intended to simulate a sandy soil environment, accidentally contaminated by phenol and remediated by recycling groundwater through the contaminated zone. For this purpose a pilot plant was used, consisting of two identical PVC columns with internal diameter of 10.5 cm. At the lower outlet of each column, a 15 litre tank was placed in order to collect the effluent and to recirculate it to the top of the column by the use of a peristaltic pump (Figure 1). The columns were packed with clean, pre-washed sand containing 10.70 litres of medium grain effective size of 0.86 mm, and 11.04 litres of coarse grain effective size of 2.51 mm. The bed volume was defined as the volume occupied by sand grains and pores.

**Table 1** Operational conditions in the experimental work

<table>
<thead>
<tr>
<th>Run no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate (bed vol./hr)</td>
<td>0.135</td>
<td>0.135</td>
<td>0.135</td>
<td>0.090</td>
<td>0.120</td>
<td>0.176</td>
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<tr>
<td>Phenol loading (grams)</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<td>5</td>
<td>4</td>
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</table>
ambient temperature was around 25ºC. Measurements of dissolved oxygen in the recycled water stream enabled us to assume aerobic conditions during all the experiments.

Each run was characterized by different flow rates and by different phenol loading, as detailed in Table 1. The flow rate was kept constant during each run. Between runs, the columns were washed through with tap water and then let to drain before the next run started.

The tanks for liquid recycling each contained: 8 litres of tap water, 0.2 gram of NH₄Cl, and 0.04 gram of KH₂PO₄. The spill of phenol was simulated by adding a certain amount of phenol (as mentioned in Table 1) diluted in 100 ml water solution, on the top of each column, few seconds before the Masterflex peristaltic pumps started the liquid recycling.

Samples were collected from the recycled liquid and immediately characterized by analyses of phenol, total chemical oxygen demand (COD), ammonia nitrogen, phosphate, as well as pH and turbidity. Nutrients were added to the effluent in order to maintain appropriate C–N–P ratios. At the end of the experimental work, the columns were dismantled, five samples were collected from each column for the determination of volatile solids attached to the sand grains. All the analytical work was done following the procedures mentioned in the 15th edition of the Standard Methods for Examination of Water and Wastewater, 1995.

Results

The experimental work consisted of sequential exposures to phenol applied to the top of the sand found in the columns, and bioremediated by continuous recycling of aqueous solution enriched by nutrients, as described before. The experiments included three periods: (a) monitoring of the influences exerted by the sand grain effective size, keeping constant the flow rates of recirculated liquid and the initial loading of phenol applied on the top of the columns (runs 1-3); (b) three different flow rates operated in parallel on both sand columns, keeping the loading of phenol constant (runs 4-6); (c) three different phenol loadings, applied on both sand columns, keeping constant flow rates (runs 7-9).

The results in Figures 2A, B and C represent the phenol concentrations in the recycled liquid versus the cumulative bed volume number (CBVN) during the length of each run. CBVN is a non-dimensional parameter, which represents the ratio between the total cumulative volume of recycled liquid and the volume of the soil exposed to the bioremediation process.

The experimental runs 1, 2 and 3, were based on identical phenol loading and flow rates for both columns packed with either medium or coarse sand. During the first run, the phenol removal, probably by biodegradation, was somewhat faster for coarse sand, however, this tendency changed during run 3. From the results presented in Figure 2A, no major differences could be observed as being influenced by the effective size of the sand grains. The repeated exposures to phenol were characterized by lower time required for the phenol biodegradation. This could be explained by the assumption that the amount of acclimated biomass in the columns increased, as more contaminant had been biodegraded. As a result, the substantial reduction of phenol concentration in the recycled liquid occurred for CBVN > 50 in run 1, for CBVN > 27 in run 2, and for CBVN > 20 for run 3. While run 1 required about 70 bed volumes (22 days) to reduce the phenol concentration to less than 1 mg/L, in runs 2 and 3 only about 30 bed volumes (less than 10 days) were needed for the same purpose.

The experimental runs 4, 5 and 6 were based on identical phenol loading for both columns packed with either medium or coarse sand, however, each run was based on different rates of flow of the recycled liquid, 0.090, 0.120 and 0.176 bed volumes per hour (BV/hr), respectively. The results, summarized in Figure 2B, indicate that the lower flow rates enabled better biodegradation of the phenol, and achieved concentrations around 1 mg/L or less, after passing 15 bed volumes through the columns. In case of the high flow, less than 1 mg/L phenol was obtained only after 22 bed volumes with medium sand and 28 bed volumes with coarse sand.
The experimental runs 7, 8 and 9 were based on identical flow rates for both columns, however, the phenol loading applied on the top was 4, 5 and 6 grams, respectively. The results (Figure 2C) show that in cases of 4 and 5 grams of phenol applied, the concentration in the recycled liquid could be reduced to about 1 mg/L after 8 bed volumes (45 hours), while for 6 grams, the same reduction could be achieved only after 12 bed volumes (68 hours).

At the end of the experimental runs, the two sand columns were dismantled. A total of five samples were taken from each column along the bed depths and the contents were assayed for volatile attached solids (VAS) determination, as an indication of the fixed films. The results indicate a different biomass attachment to the sand. The highest amount of VAS in the column packed with medium sand was at a depth of 60 cm, 21 mg VAS/gram of dry sand, while in the column packed with coarse sand the highest VAS was found on the top, 45 mg/gram. While the medium sand appeared to concentrate most of the biomass distributed somehow uniformly along its depth, the coarse sand concentrated most of its biomass close to top and bottom. The amounts of VAS developed on coarse sand were higher than those developed on medium sand, except the results obtained at 95 cm depth, measured from the top of the column.

Discussion
The experimental work in this study aimed to compare the biodegradation of phenol achieved by attached biomass on two columns with sand characterized by different effective grain sizes, 0.86 mm (medium) and 2.51 mm (coarse). Although during run 1, with
“clean” sand, the acclimation time was shorter for the biomass developed on coarse sand, medium sand indicated during the following runs a slightly better performance. This advantage was more pronounced in conditions of increased flow rates of the recycled liquid.

Phenol loading affects its biodegradation rate. For a given bed volume, higher loading would require longer periods for bioremediation and this could be explained by considering the inhibitory character of phenol (Yoong et al., 1997).

The attached biomass in the columns appeared to be dependent on the grain size, in terms of total amount, as well as the distribution along the depth of the column. Farook et al. (1996) reported that a relatively uniform distribution of biomass was exhibited at phenol concentrations of 100 mg/L. Essa et al. (1996), worked with effective grain sizes of 0.2 to 0.4 mm and stated that due to larger surface area, fine sand could enable higher microbial densities as compared to coarse sand. The maximum biosolids density reported by Essa et al. was 9.2 mg VAS / gram sand of 0.4 mm size, much lower values than these measured in this study: 15–20 mg VAS / gram sand of 0.81 and 25–35 mg VAS / gram sand of 2.51 mm. The experimental results obtained in this study for coarse sand indicate higher amounts of attached biomass mainly concentrated at the top of the column. This could be a possible result of higher space volumes, different flow, shearing and transport conditions of the attached biosolids. The relatively uniform biomass distribution in the sand column, characterized by medium grain size, could explain its better performance under increased flow conditions. Recent studies by Simoni et al. (1998) refer to the different distribution of VAS within porous media and indicate that the travel distances of microorganisms might be due to the complex structure of the aquifer material, as well as to the heterogeneity in the adhesion properties within the bacterial population.

Previous works on phenol as the sole carbon source in biodegradation studies, relating to concentrations in the range 500–800 mg/L (Rosich et al., 1983; Nakhla and Al-Harrazin, 1993) reported yield coefficients of 0.53 mg VAS/mg phenol. The yield coefficient in the present work was 0.57 mg VAS/mg phenol. This value was measured at the end of all the experimental runs, over a period of about 200 days, therefore it might be affected by processes of biomass decay and stabilization.

Conclusions
Sand columns contaminated by concentrated solutions of phenol could be efficiently remediated by biosolids which developed as fixed films. In case of medium grain size (0.81 mm) the biosolids distribution was somehow uniform along the column depth, usually above 10 mg VAS/gram sand, with a maximum of 22 at a depth of 60 cm from the top. In the case of coarse sand (2.51 mm) most of the biosolids were accumulated in the upper zones of the column.

Coarse sand enabled quicker development of acclimated biomass, however, medium size sand showed better performance especially in case of high flow rates of the recycled liquid.

Low rates of flow of the recycled liquid resulted in more effective biodegradation of phenol.

High loading of phenol applied on the top of the column slowed down the microbial activity.

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References


