Treatment of wastewater containing high phenol concentrations using stabilisation ponds enriched with activated sludge

*Departamento de Fisiología y Farmacología; Universidad Autónoma de Aguascalientes, Av. Universidad 940, CP 20100, Aguascalientes, Ags., Mexico (E-mail: fjavelar@correo.uaa.mx)
**Departamento de Biotecnología y Bioingeniería, CINVESTAV-IPN, Av. IPN 2508, San Pedro Zacatenco, CP 07000, México DF, México (E-mail: fesparza@mail.cinvestav.mx; thalasso@mail.cinvestav.mx)

Abstract Treatment of wastewater containing high phenol concentrations (up to 4,000 mg/l, 1,600 kg/ha.d) in laboratory-scale stabilisation ponds enriched with activated sludge was studied. Phenol was biodegraded efficiently, even when fed as the sole carbon source. At influent concentrations of 1,000, 1,300, 1,600, 1,900, 2,500, 3,000 and 4,000 mg/l of phenol (loading rates of 400, 520, 640, 760, 1,000, 1,200 and 1,600 kg phenol/ha.d), the phenol removal efficiencies were 92, 89, 81, 81, 76, 65 and 22%, respectively. At 4,000 mg/l of phenol, the enriched ponds were significantly inhibited. The maximum phenol removal rate observed was 780 kg/ha.d, which is 7.7 times higher than the maximum value reported for attached-growth waste stabilisation ponds. All along the experiments, the enriched ponds showed removal rates 1.8–20.5 times higher than the values observed in control pond (not enriched). The results suggest that enrichment is an effective method to increase xenobiotic removal rates of stabilisation ponds.

Keywords Aerobes; algae; anaerobes; bioflocs; enriched ponds; phenol

Introduction
Waste stabilisation ponds are a cost-efficient method for the treatment of municipal and non-toxic industrial effluents. They are widely used in both developed and developing countries (Pearson, 1996; Zhao and Wang, 1996). This technology is characterised by a low active biomass concentration and low biodegradation rates, which imply large land requirements (Polprasert and Sookhanich, 1995). Several methods based on biofilm growth have been proposed to increase biomass concentration (Shin and Polprasert, 1987; Zhao and Wang, 1996). Recently, an enrichment procedure using activated sludge from a municipal wastewater treatment plant has been proposed to increase the biomass concentration and activity in stabilisation ponds (Avelar et al., 2001; Avelar et al., 2003). This paper presents the results obtained in enriched stabilisation ponds treating high phenol loading rates (up to 1,600 kg phenol/ha.d, 4,000 mg/l). The effects of conventional carbon source and biomass acclimatisation on phenol removal were also studied.

Methods
Four laboratory-scale ponds (831 volume, 0.8 m long, 0.37 m wide and 0.28 m deep) were built according to the original Eckenfelder design (Eckenfelder and Ford, 1970). Three of them were enriched using 211 of activated sludge (8 g VSS/l) obtained from the secondary settling tank of a municipal wastewater treatment plant. The working volume of the ponds was completed with synthetic wastewater (Avelar et al., 2003) to obtain a final biomass concentration of 1.8 g VSS/l. After enrichment, biomass was progressively acclimatised to high organic loading rates (1,000 kg COD/ha.d) using gelatine peptone and meat extract as the conventional carbon source (Avelar et al., 2001). The fourth pond
(control pond) was not enriched but was inoculated with 211 of the effluent of a municipal stabilisation pond. Except for the enrichment procedure, the control and the enriched ponds were operated identically. Throughout, the experiments, the hydraulic retention time was kept at 7.5 days and each pond was lighted by 690 lux during 12 h/day. After the acclimatisation, the conventional carbon source was progressively substituted by phenol. Throughout, the experiments, samples of the influent, effluent as well as from the top, medium depth and bottom of the ponds were taken. These samples were used to determine total and soluble COD (open reflux method), phenol concentration (4-aminoantipirine, chloroform extraction method), volatile suspended solids (VSS), heterotrophic aerobes, facultative anaerobes plate count and chlorophyll “a” concentration. Temperature, dissolved oxygen, pH, redox potential and conductivity were also frequently monitored. All measurements were made according to the Standard Methods for the Examination of Water and Wastewater (1995).

Results and discussion
During the first experiment, the total organic loading rate was kept constant (1,000 kg COD/ha.d, 2,500 mg COD/l), and the conventional carbon source was gradually substituted by phenol. Each substitution step was maintained during 30 days, and the influent phenol concentrations tested were 20, 150, 375, 660 and 1,005 mg/l. Table 1 shows the results obtained. The phenol removal rates observed in the enriched ponds were 1.8–2.6 times higher than the respective rates observed in the control pond.

During a second experiment, phenol as the sole carbon source was evaluated. With that purpose, in one experimental pond, the conventional carbon source was abruptly removed from the influent. In two more experimental ponds, the conventional carbon source was progressively reduced from 12 to 0.0% of the total organic loading rate, through five reduction steps of 30 days each. Figure 1 shows the results obtained. It was observed that after abrupt elimination of the conventional carbon source, the phenol removal efficiency showed an important decrease, from 65% to 48%. It was also observed that, on the contrary, when the conventional carbon source was progressively removed from the influent, the phenol removal rate increased and reached a removal efficiency of 92%. When phenol was the sole carbon source applied (1,000 mg/l), the phenol removal rate reached 370 kg/ha.d, which is 1.7 and 4.8 times higher than the values previously reported for enriched ponds (Avelar et al., 2003) and attached-growth waste stabilisation ponds (Polprasert and Sookhanich, 1995), respectively.

During a third experiment, phenol as the sole carbon source was fed at concentrations higher than 1,000 mg/l (400 kg/ha.d). Phenol influent concentration was progressively increased through six steps of 30 days each. The corresponding phenol loading rates applied were 520, 640, 760, 1,000, 1,200 and 1,600 kg/ha.d (1,300, 1,600, 1,900, 2,500, 3,000 and 4,000 mg/l, respectively). Figure 2 shows the results obtained. Up to an influent concentration of 1,600 mg/l, phenol removal efficiency was around 80%, which is comparable to those previously reported for enriched ponds (Avelar et al., 2003). At a higher influent concentration of 4,000 mg/l, phenol removal efficiency decreased to around 30%, which is lower than that previously reported for enriched ponds (Avelar et al., 2003). These results suggest that the enriched ponds have a higher phenol removal efficiency than the control pond, with a maximum phenol removal efficiency of 92%.

Table 1 Phenol removal observed in the enriched ponds and in the control pond (not enriched), during the gradual substitution of the conventional carbon source by phenol

<table>
<thead>
<tr>
<th>Step</th>
<th>Total loading rate (kg COD/ha.d)</th>
<th>Conventional carbon source loading rate (kg COD/ha.d)</th>
<th>Phenol loading rate (kg COD/ha.d)</th>
<th>Phenol removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Enriched ponds</td>
</tr>
<tr>
<td>1</td>
<td>983 ± 50.4</td>
<td>964 ± 49.2</td>
<td>19 ± 1.4</td>
<td>75 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>1000 ± 40.7</td>
<td>857 ± 30.9</td>
<td>143 ± 9.5</td>
<td>61 ± 4.0</td>
</tr>
<tr>
<td>3</td>
<td>1026 ± 25.4</td>
<td>669 ± 16.8</td>
<td>357 ± 9.5</td>
<td>62 ± 4.9</td>
</tr>
<tr>
<td>4</td>
<td>1000 ± 29.2</td>
<td>378 ± 28.9</td>
<td>626 ± 28.6</td>
<td>70 ± 4.3</td>
</tr>
<tr>
<td>5</td>
<td>1093 ± 18.8</td>
<td>136 ± 18.9</td>
<td>957 ± 19.0</td>
<td>65 ± 5.5</td>
</tr>
</tbody>
</table>

Downloaded from https://iwaponline.com/wst/article-pdf/51/12/257/477041/257.pdf by guest
phenol concentration of 3,000 mg/l (1,200 kg/ha.d), the enriched ponds did not show evidence of significant inhibition. This was not the case when 4,000 mg/l (1,600 kg/ha.d) of phenol was fed, since a clear inhibition was observed. By contrast, the control pond was almost completely inhibited at concentrations superior to 1,600 mg/l (640 kg/ha.d). The maximum phenol removal rate observed in the enriched ponds (780 kg/ha.d) was 7.7 times higher than the maximum value reported for attached-growth waste stabilisation ponds (Polprasert and Sookhanich, 1995). During this experiment, soluble COD analyses (data not shown) confirmed the phenol removal rates observed and suggest that phenol was completely mineralised. Control experiments done with sterilised biomass obtained from the enriched ponds, showed that the abiotic removal of phenol was insignificant (less than 2%). The formation of an active settled biomass bed could explain the results observed in the enriched ponds (Avelar et al., 2001). According to Avelar et al. (2003), this settled biomass contained more than 90% of the total microbial population and was about 100 times more active than the suspended biomass. The settled biomass was also significantly more resistant to the phenol inhibitory effects than the suspended biomass (Avelar et al., 2003).

The main biomass indicators were measured before and after phenol was added to the ponds (before experiment one and after experiment three). Before phenol feeding, the heterotrophic aerobes and facultative anaerobes plate counts were $1.1 \times 10^6$ and $2.1 \times 10^6$ UFC/ml, respectively. After phenol feeding, these indicators were $2.8 \times 10^6$ and $1.7 \times 10^7$ UFC/ml, for heterotrophic aerobes and facultative anaerobes, respectively.
By contrast, the chlorophyll “a” concentration showed a reduction, from $5.1 \pm 0.6$ to $3.1 \pm 0.8$ mg/l. These results suggest that algae population was more sensitive to phenol than bacterial population. Despite the presence of phenol, the biomass indicators were superior to common values reported for facultative ponds (Shin and Polprasert, 1987). Additional results showed that dissolved oxygen concentration was always below 0.01 mg/l, and the redox potential was $-247 \pm 43$ mV. However, the chlorophyll “a” concentrations and the heterotrophic aerobes plate counts were always above 3 mg/l and $1 \times 10^9$ UFC/ml, respectively. These data suggest anoxic, rather than strict anaerobic conditions (Pescod, 1996).

**Conclusions**

Stabilisation ponds enriched with activated sludge and progressively adapted to the pollutant to be treated were significantly more efficient than conventional ponds. The progressive substitution of conventional carbon source allowed the degradation of phenol as the sole carbon source at removal rates of up to 780 kg/ha.d (1,860 kg COD/ha.d). Moreover, it was surprisingly observed that the absence of peptone and meat extract, previously used as model conventional carbon source, favoured the phenol degradation process. The enrichment procedure could be therefore a potential suitable technique to improve ponds efficiency in the treatment of high loading rates of toxic compounds.

**Acknowledgements**

This study was supported by a SHIGO-CONACYT grant (19980206029).

**References**


