Degradation of di-2-ethylhexyl phthalate (DEHP) by indigenous isolate Acinetobacter sp.

J. Xu, X. Li, Q. Lu, R. A. de Toledo, H. Shim

Department of Civil and Environmental Engineering, Faculty of Science and Technology, University of Macau, Macau SAR, China
**BACKGROUND**

**Phthalate esters (PAEs)**

- Used as *plasticizers*
- Endocrine disrupting chemicals (EDCs)
- Physically (rather than chemically) bonded to the plastic matrix

![Chemical structures of different phthalates](image-url)
Di(2-ethylhexyl) phthalate (DEHP)

- One of the most cost effective and widely available general purpose plasticizers
- PVC, toys, and medical devices
- Dysfunction of endocrine, reproductive, and nervous systems
- Possible human *carcinogen* since 1987 (USEPA)

DEHP concentration in (waste)water in China (Wang et al., 2011)

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>industrial wastewater (μg/L)</th>
<th>well water (μg/L)</th>
<th>pond water (μg/L)</th>
<th>Standard drinking water (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEHP</td>
<td>42.4</td>
<td>14.2</td>
<td>135.7</td>
<td>8</td>
</tr>
</tbody>
</table>

BACKGROUND

Physicochemical processes

- Adsorption, Membrane filtration, Advanced oxidation processes (AOPs)
  - No mineralization
  - Generation of by-products
  - Not cost effective

Biological process

- Anaerobic and aerobic conditions
  - Environmentally friendly
  - Cost effective
  - Contaminant mineralization

No research about the effects of microelements (inhibitory/stimulatory) on PAEs biodegradation
OBJECTIVES

✓ To remove DEHP from artificially contaminated water using indigenous bacterial isolate, *Acinetobacter* sp.

✓ To optimize the DEHP biodegradation process

✓ To evaluate growth kinetics and biodegradation pathway for DEHP by the isolate

✓ To evaluate the effects of microelements (Fe^{3+} and Mn^{2+}) on DEHP biodegradation
MATERIALS AND METHODS

Microbial isolation

✓ Activated sludge samples from wastewater treatment plant (Macau SAR, China)

Enrichment

✓ Enriched in nutrient broth (3 g l⁻¹ beef extract + 5 g l⁻¹ peptone)
✓ Cultured in a basal salt medium (BSM) with increasing DEHP concentration (from 10 to 500 mg l⁻¹) as sole carbon source

Microbial identification

✓ 16S rRNA gene sequence analysis
✓ Sequences deposited in NCBI GenBank under the accession number KX_670538
MATERIALS AND METHODS

Experimental setup

Temperature, pH, and microelements

Inoculum (5 ml)
BSM solution (45 ml) spiked with 100 mg l⁻¹ DEHP

Temperatures (25°C, 30°C, 35°C)
pHs (3, 5, 7, 8, 9)
Microelements Fe³⁺ and Mn²⁺ (100, 500, 1,000 μg l⁻¹)

Kinetic studies:

Initial DEHP concentrations (10-500 mg l⁻¹) at pH 7.0±0.2 and 30°C.

✓ Treatments incubated in the dark at 150 rpm and 30°C, in replicates
✓ DEHP concentrations and OD₆₀₀ were determined in every 24 h for 5 days
✓ One-way analysis of variance (ANOVA) at the 95% confidence interval
MATERIALS AND METHODS

Analytical methods

**DEHP concentration: HPLC/DAD (Thermo Fisher Scientific, USA)**

- Column: Acclaim™ C18 (5 µm, 4.6 x 150 mm), temperature 45°C
- Mobile phase: acetonitrile:deionized water (9:1), flow rate 0.5 ml min⁻¹

- Linearity: 10 to 500 mg l⁻¹ (n=3), r² 0.9977
- Precision (100 mg l⁻¹):
  - Repeatability (n=6), 0.51%
  - Intermediate precision (d=6), 1.35%

- Accuracy (100 mg l⁻¹): 100.4±1.67% spike-recovery

**Biodegradation pathway: HPLC-MS (Thermo Fisher Scientific, USA)**

- Electrospray ionization (ESI) source
- Probe temperature: 300°C
- Polarity: positive

**Optical density: UV mini-1240 spectrophotometer (Shimadzu, Japan)**
RESULTS

Microorganism identification

Phylogenetic tree for the isolate Acinetobacter sp.
RESULTS

Effects of temperature and pH

There was no significant difference in DEHP biodegradation:

- $35^\circ C$ and $30^\circ C$ ($p=0.22$)
- pH 6-9 ($p=0.87$)

Neutral pH is also considered optimal for the growth of other *Acinetobacter* spp.
RESULTS

Biodegradation kinetics

Substrate inhibition kinetics:

\[ D = D_{\text{max}} \frac{s}{K_s + s + \frac{s^2}{K_i}} \]

\[ \mu = \mu_{\text{max}} \frac{s}{K_s + s + \frac{s^2}{K_i}} \]

Biodegradation kinetics parameters for the isolate *Acinetobacter* sp. grown on DEHP

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Degradation rate</th>
<th>Specific growth rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>(D_{\text{max}}) (mg/L•day)</td>
<td>124.8</td>
<td>-</td>
</tr>
<tr>
<td>(\mu_{\text{max}}) (day(^{-1}))</td>
<td>-</td>
<td>0.1192</td>
</tr>
<tr>
<td>(K_s) (mg/L)</td>
<td>272.3</td>
<td>137.6</td>
</tr>
<tr>
<td>(K_i) (mg/L)</td>
<td>720.5</td>
<td>850.3</td>
</tr>
</tbody>
</table>
RESULTS

DEHP biodegradation and cell growth

DEHP biodegradation (A) and cell growth (B) at different initial concentrations: (-■-) 500; (-●-) 400; (-▲-) 300; (-▼-) 200; and (-♦-) 100 mg/L
RESULTS

Effects of microelements

DEHP removal efficiencies after the addition of (a) Fe$^{3+}$ and (b) Mn$^{2+}$ at different concentrations
RESULTS

Intermediates identification by HPLC-MS

Mass spectra of DEHP (a), MEHP (b), β-carboxy-\textit{cis},\textit{cis}-muconic acid (c), 3-katoadipate (d), and di-ethyl hexanoic acid (e)
RESULTS

DEHP biodegradation pathway

Proposed DEHP biodegradation pathway for the isolate
CONCLUSIONS

✓ The optimal temperature for the biodegradation is considered 30°C and the neutral and alkaline conditions are shown favourable for DEHP degradation by *Acinetobacter* sp. SN13.

✓ High concentrations of DEHP (500 mg/L) were inhibitory to both biodegradation and cell growth.

✓ Ferric ion at 100-1,000 μg l⁻¹ showed the stimulatory effect on the DEHP biodegradation, while Mn²⁺ was stimulatory at the lower concentration (100 μg l⁻¹) but inhibitory at higher concentrations (500-1,000 μg l⁻¹).

✓ The biodegradation pathway for DEHP by the isolate is proposed with some metabolic products identified.

✓ The biological process could be further scaled up and applied to treat different types of wastewater, especially the ones containing high concentration levels of DEHP and other PAEs generated from the plastics industries.
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