Removal of Di-(2-ethylhexyl)phthalate (DEHP) from Water Using a LECA-*Pseudomonas putida* Biobarrier

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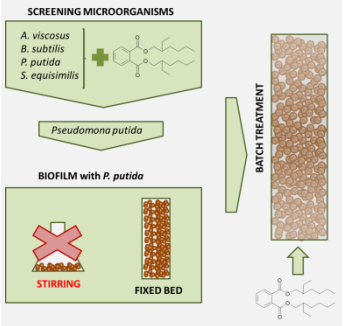


The removal and biodegradation of an organic toxic pollutant, di-(2-ethylhexyl) phthalate (DEHP), has been investigated. Initially, a screening of different degrading bacteria has been developed and *Pseudomonas putida* showed the highest degradation ability. This bacterium was immobilised in an inert support, light expanded clay aggregate (LECA). After the biofilm formation on the LECA, the degradation of DEHP was evaluated operating in a fixed bed reactor. In addition, several studies of DEHP adsorption on LECA were carried out in order to determine the mechanism of the degradation process that takes place. The degradation studies demonstrated that the developed system can be applied to DEHP removal and the degradation is due to adsorption process and the activity of *P. putida*.

Introduction

Phthalates are chemical compounds used in several industries to prepare different products like cosmetics, plastics, etc. Among these chemicals, bis(2-ethylhexyl) phthalate (DEHP) is the most common of the class of phthalate plasticizers, with a market share of almost 54% in 2010 [1].

These compounds can enter the environment through losses during manufacture or by leaching from final products. DEHP is considered a reproductive and developmental toxicant in humans and animals [2], moreover, it has been reasonably anticipated to be a carcinogen by the U.S. Department of Health and Human Services and classified as a priority pollutant by European Union.

Bioremediation has shown a huge potential for the degradation of organic pollutants such as phenols, dyes, polycyclic aromatic hydrocarbons, ... It is also known that a great variety of toxic chemicals can be removed from the environment by several chemical and physical treatments; the use of microorganisms (fungi or bacteria) is considered to be one alternative process to degradation of these toxic chemicals [3]. Moreover, the main advantage of bioremediation is its low cost in comparison with the other conventional process.

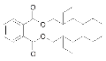
The aim of this work is to ascertain the potential of different bacteria for the degradation of DEHP. In order to evaluate its applicability as biobarrier, the bacteria were immobilised on the solid matrix such as LECA and the experiments were carried out in a fixed bed reactor.

Methods

Materials and reagents

DEHP was purchased from Acros Organics. The characteristics of the compound and the used concentration are described in Table 1. LECA was obtained from Siro Hidroton (Portugal). They were sieved and then washed with distilled water four times and dry at 60ºC for 24 hours. All the used chemicals were reagent grade.

Table 1. Pollutant properties and concentration

Pollutant	CAS	Structure	Conc. (mg/L)	Solubility (mg/L)
DEHP	117-81-7		0.45	0.5

Microorganisms and culture media

Arthobacter viscosus (AV), *Bacillus subtillis* (BS), *Pseudomona putida* (PP), *Streptococus equisimili* (SE) were purchased from the Spanish Type Culture Collection of the University of Valencia (CECT). Two different culture media were used: a rich media suggested by the CECT and a minimal culture media prepared according to Cobas et al [4] using glucose (2 g/L) as carbon source.

Screening of degrading bacteria

Batch experiments assays were developed in Erlenmeyer flasks (250 mL) with a working volume of 150 mL. The experiments were set up with a rich medium and DEHP concentration of 0.450 mg/L. Periodically, samples were taken and analysed for DEHP determination.

Biofilm formation

The biofilm was growth in the LECA using a rich culture media for 5 days. Two different set-ups were tested: growth in Erlenmeyer flask with stirring (150 mL of culture media, 1 g of LECA and 150 rpm) and growth in column with recirculation to favour the bacterial growth (Table 2).

Fixed-Bed bioreactor: degradation assays

These assays were developed in two fixed-bed bioreactor of 250 mL filled with LECA of different size. The characteristic of the bioreactor are shown in Table 2. The immobilised system (LECA-bacterium) was previously washed with distilled water to remove the free bacteria.

Table 2. Working conditions in column immobilisation

	COLUMN A	COLUMN B
LECA size (cm)	< 0.8	> 1.2
LECA amount (g)	83.789	65.719
Total volume (mL)	250	250
Working volume (mL)	95	105
Temperature (ºC)	20	20

DEHP with a concentration of 0.450 mg/L was added to a minimal medium initially and the system worked in reflux. Periodically, samples were taken and analysed for pollutant determination.

DEHP *determination*

DEHP was quantified using gas chromatography with an Agilent GC 6850 gas chromatograph equipped with a HP5 MS capillary column (30 m x 250 µm x 0.25 mm), operating with hydrogen as carrier gas, and coupled to an Agilent MD 5975 mass spectrometer (MS). The GC injector was operated in splitless. 1 µL of the organic aliquots were injected using an autosampler. The GC oven was programmed to hold to 75ºC for 1 min, and then raise the temperature at 15ºC/min to 280ºC, which was held for 5 min.

The samples for DEHP determination were previously extracted with dichloromethane according to EPA 3510 and at the end the eluent was exchanged to hexane.

Results

Screening of DEHP degrading bacteria

Initially, four bacteria (AV, BS, PP, SE) have been evaluated in order to determinate their ability to degrade DEHP.

The obtained results (Table 3) showed that AV was not able to use the studied pollutant in its metabolic pathway. SE showed a low degradation level and BS and PP reached a removal value close to 44% and 85%, respectively.

Table 3. Screening of DEHP degrading bacteria

AV	BS	PP	SE
✗	✓✓	✓✓✓	✓

The degradation of the DEHP was confirmed by GC-MS. Different degradation intermediates as: phthalic acid, ethyl-2-methyl butyl ester; 1-hexanol, 4-methyl; octane were determined.

As consequence of the previous results and due to the higher performance of PP, this bacterium was selected for the next studies.

Bacteria immobilisation

The development of a biobarrier requires the immobilisation of the bacterium in a solid matrix. For this reason, an innert support, LECA was selected to immobilise the bacterium. LECA consists of small, lightweight particles of burnt clay with small cavities.

Previously to the immobilisation, the innert support has been evaluated as adsorbent. LECA shows a DEHP removal of approximately 10%. This result allows to state that LECA not only support the bacterium but also acts as adsorbent.

Two different approaches were tested so as to create the biofilm in the surface of LECA and immobilise the bacterium: moderate stirring of the bacteria and support and biofilm formation in a static column.

Degradation of DEHP in fixed-bed bioreactor

Different degradation assays were developed working with the PP immobilised in static conditions. Bacterium was supported in two different particles size (Figure 1).

Figure 1. Picture of the columns with immobilised PP in LECA particles with different sizes.

The batch degradation studies were developed during 7 days. At time 4 hours the DEHP

degradation reached a value of 60.36% and 69.57% for columns A and B, respectively. When the experiments were ended after 7 days the removal increased up a value of 82% for both columns. Moreover, the size of LECA particles has shown no effect in the degradation of DEHP.

The obtained results are in agreement with those found in the literature [5]. Chang et al [5] reports that working with free bacterium *Corynebacterium sp* at 20ºC the total removal of the DEHP was not possible after 7 days.

Conclusions

BS, PP and SE have shown to be able of degrading DEHP. Among them, PP achieved the highest degradation levels.

The immobilisation of PP on LECA only showed to be feasible in the columns and this system was able to reduce the pollutant concentration more than 60% after 4 hours.

The obtained results demonstrate that the developed system can be applied to DEHP removal and more studies are required in order to evaluate the performance of the system working in a continuous way.

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