Biochemical Engineering Journal 93 (2015) 289–293

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Short communication

Coupling extraction and enzyme catalysis for the removal of anthracene present in polluted soils

A. Arca-Ramos*, G. Eibes, G. Feijoo, J.M. Lema, M.T. Moreira

Dept. of Chemical Engineering, Institute of Technology, University of Santiago de Compostela, Rua Lope Gomez de Marzoa, 15782 Santiago de Compostela, Spain

ARTICLE INFO

Article history:
Received 20 March 2014
Received in revised form 15 October 2014
Accepted 22 October 2014
Available online 28 October 2014

Keywords:
Enzyme biocatalysis
Multiphase bioreactor
Mass transfer
Vegetable oil
Anthracene

ABSTRACT

In this study, a novel system for removing anthracene from polluted soils using biodegradable vegetable oil was investigated. The process consisted of the extraction of anthracene from the soil (1004 mg/kg) by pomace olive oil followed by its degradation in a surfactant-assisted two phase partitioning bioreactor (TPPB), operated with a laccase-mediator system. The main outcomes of this study showed high extraction efficiency for both fresh and regenerated pomace olive oil (higher than 84%) and almost complete removal of anthracene in the TPPB after 48 h. Finally, the feasibility of reusing both the aqueous and organic phases of the TPPB in successive batches of anthracene degradation in the bioreactor was also demonstrated.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are highly persistent pollutants due to their hydrophobic nature and low water solubility, which hinders natural biodegradation [1]. An interesting possibility for their removal from soils has relied on the use of vegetable oils (Table 1) [2–7]. The residual oil remaining in the soil after the extraction is biodegradable and mineralization is expected to occur by endogenous microbial activity [8,9]. In addition, the presence of low concentration of vegetable oils in soil has been demonstrated to facilitate the biological degradation of PAHs present in soils, by enhancing their bioaccessibility to endogenous microorganisms [10].

After extraction, the regeneration of the solvent by enzymatic catalysis may constitute an interesting approach to physical separation alternatives, specifically the system based on a two-phase partitioning bioreactor (TPPB) with oxidative enzymes [11]. In this sense, the suitability of fresh pomace olive oil to act as a non-aqueous phase liquid in TPPB to remove anthracene has already been demonstrated in previous research [11]. In the present study, a novel process for removing anthracene from soil based on its initial extraction by pomace olive oil followed by the enzymatic degradation of the dissolved anthracene in a TPPB is proposed (Fig. 1). The reuse of both the regenerated pomace olive oil for successive extraction processes and the aqueous and organic phases of the TPPB in subsequent batches of enzymatic oxidation are additional objectives of the study.

2. Materials and methods

2.1. Chemical reagents and enzyme

Anthracene (99%), Triton X-100 (≥98%) and 1-hydroxybenzotriazole (HBT) (99%) were purchased from Janssen Chimica, Merck and Fluka, respectively. In addition, anthraquinone (97%), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) (≥98%), organic solvents (HPLC grade) and commercial laccase from *Trametes versicolor* (≥ 10 U/mg) were purchased from Sigma-Aldrich. The pomace olive oil utilized in the study was edible commercial oil.

2.2. Soil preparation

The soil sample selected was taken from a B horizon of a Cambisol soil. The excavated soil, taken at a depth of 60–70 cm, was homogenized, sieved at 2 mm and air-dried at 25 °C. The main physico-chemical characteristics of the soil are provided in Table 2. The soil was spiked with anthracene prepared in an acetone solution for a final concentration of anthracene in soil of 1004 mg/kg (i.e. 5633 μmol/kg).

* Corresponding author. Tel.: +34 881816773; fax: +34 881816702.
E-mail address: adriana.arca@rai.usc.es (A. Arca-Ramos).

http://dx.doi.org/10.1016/j.bej.2014.10.015
1369-703X/

Table 1
Studies on the application of vegetable oil in batch extraction of PAHs from contaminated soils.

Reference	Oil type	PAH	Spiked or field soil	Oil:soil (v:w)	PAH concentration (mg/kg)	% PAH removed
[5]	Sunflower	Mixture	Field	1:1 or 2:1	5453	81–100
[7]	Sunflower	Mixture	Field	1:1	1255	90
			Field	2:1	1255	97
[6]	Soybean	Anthracene	Spiked	1:1	50	72–75
				or	200	74–73
				2:1		
[8]	Soybean	Phenanthrene	Spiked	1:1	200	68
					1000	56
		Fluoranthene	Spiked	1:1	200	69
					1000	59
	Palm kernel	Phenanthrene	Spiked	1:1	200	68
					1000	55
		Fluoranthene	Spiked	1:1	200	70
					1000	61
This work	Fresh pomace olive	Anthracene	Spiked	1:1	1000	84
	Regenerated pomace olive	Anthracene	Spiked	1:1	1000	84

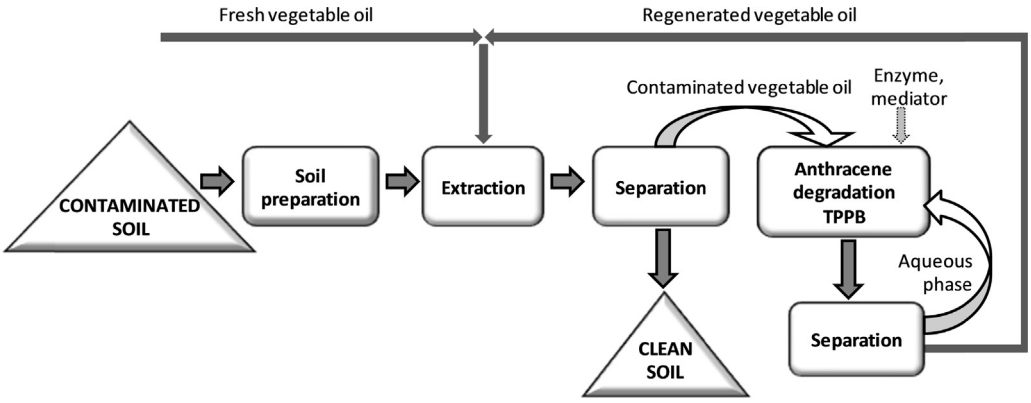

Fig. 1. Proposed scheme for the remediation of soil contaminated with anthracene using vegetable oil as extraction agent.

2.3. Extraction with pomace olive oil

Soil extraction of anthracene by pomace olive oil was performed in Teflon bottles at room temperature (24 ± 2 °C). Fresh or regenerated oil was poured into the bottles containing the soil (36.0 g and 8.5 g for the experiments with fresh and regenerated oil, respectively) with an oil-to-soil ratio of 1:1 (v:w) (Table 3). The bottles were sealed and mechanically shaken at 180 rpm on a linear shaker for 24 h, a time period deemed sufficiently long to reach equilibrium extraction for three ring PAHs [5]. Thereafter, the content was centrifuged at 4000 rpm for 15 min to separate the oil from the solid phase. An aliquot of 40 μL of the oil was added to a final volume of 4 mL of acetonitrile and mixed in a vortex for 5 min to extract the anthracene from the oil. A sample of 1 mL of acetonitrile was analyzed by HPLC for anthracene measurement. These experiments were done in duplicate. Additionally, an analogous experiment using acetonitrile as the solvent instead of vegetable oil was conducted in parallel for comparison purposes.

Table 2
Physicochemical characteristics of the soil sample.

Sand (%)	84.2
Silt (%)	13.6
Clay (%)	2.2
pH (H_2O)	5.12
pH (KCl)	3.99
Total N (g/kg)	1.1
Total C (g/kg)	3.3
% Organic matter	–
% Organic carbon	–
Ca^{2+} (cmol(+)/kg)	0.09
Mg^{2+} (cmol(+)/kg)	0.04
Na^{+} (cmol(+)/kg)	0.41
K^{+} (cmol(+)/kg)	0.11
Al^{3+} (cmol(+)/kg)	1.03
Cation exchange capacity (cmol(+)/kg)	1.2
Surface area (m^2/g)	10.1

2.4. Enzyme activity and anthracene analysis

Laccase activity was spectrophotometrically determined. The concentrations of anthracene and anthraquinone, which was the main product of anthracene oxidation by laccase in the TPPB, were determined by high performance liquid chromatography (HPLC). The equipment and methodologies used were described elsewhere [12].

2.5. Regeneration of pomace olive oil in a TPPB

The degradation of anthracene extracted in pomace olive oil was performed at 30 °C and 250 rpm for 48 h in a TPPB consisting of a conventional stirred tank reactor (BIOSTAT® Q reactor, B. Braun-Biotech International). The extract containing the polluted vegetable oil accounted for 10% of the total reaction volume. The aqueous phase was comprised of 1200 U/L laccase, 0.1 M sodium acetate (pH 5), 1 mM HBT and 1% Triton X-100 (v:v). Operational conditions included aeration (periodic pulses 0.2 L/min of air for 1 min every 15 min) and pulses of HBT (1 mM at 8 h and 24 h) to the reaction medium [11].

The reusability of both aqueous and organic phases in the TPPB was assessed in three consecutive cycles. The first cycle in the TPPB comprised 10% (v:v) of polluted pomace olive oil in a total volume