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Preformance of an Electrobiochemical Slurry Reactor for the Treatment of a Soil Contaminated with Lindane

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Abstract: *The purpose of this research was to evaluate the biodegradation of lindane with simultaneous electricity generation in an electrobiochemical slurry reactor (EBCR). The EBCR was inoculated with a sulfate reducing inoculum acclimated to lindane, further characterized, and batch operated for 30 day at room temperature. No external carbon source and supplementation with a stock solution of sucrose: sodium acetate: lactate was performed in experiments with soil concentrations 66% and 33%, respectively. Electrochemical impedance characterization of the EBCR (concentration of soil was 66%) showed that the equivalent circuit had a high anodic resistance R_1=2064Ω, cathodic resistance R_3 = 192 Ω; and electrolyte/membrane resistance R_2 = 7Ω, totaling a high overall internal resistance R_{int} of 2263 Ù. During the batch operation, the EBCR showed a 30% lindane removal efficiency along with a maximum volumetric power of 165 mW m^{-3}. The organic matter removal was very high (72% as soluble COD, NOM) whereas the coulombic efficiency was low (5.4%). In the experiment where the concentration of soil was 66% both cell characteristics and performance significantly improved. The internal resistance as determined by polarization curve was 102 Ω when the two-electrode sets were connected in parallel. During the batch operation, the EBCR showed a 78% lindane removal, a maximum power of 634 mW m^{-3}, the organic matter removal was 76%, and coulombic efficiency of 15%. Therefore, it can be concluded that the EBCR exhibited a high lindane removal capability and holds promise for bioremediation of soils with the bonus of electricity generation.*

Keywords: Electrobiochemical slurry reactor, lindane, soil remediation, sulphate reducing

1. INTRODUCTION

The widespread use of pesticides has lead to pollution of soil, water bodies and aquifers, and atmosphere [1-7]. The γ-hexachlorocyclohexane (γ-HCH; also called lindane) is a highly halogenated organic pesticide that has been used worldwide, particularly in Mexico, in spite of its banning in first world countries [8-9]. Lindane has been used for crop protection and prevention of vector-borne diseases for many decades [10-13]. Negative impacts of lindane on the environment and human health have been reported worldwide [14]. Due to their hydrophobicity, lindane is tightly bound to the organic matter and clay of soils. This, in turn, decreases their bioavailability. Lindane is introduced into the envi-

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ABBREVIATIONS AND ACRONYMS

1,2, 3-TCB	1,2,3-trichlorobenzene
1,3-DCB	1,3-dichlorobenzene
1,4-DCB	1,4-dichlorobenzene
BOD	Biochemical oxygen demand
CB	Chlorobenzene
COD	Chemical oxygen demand
EBCR	Electrobiochemical slurry reactor
E_{EBCR}	Voltage
$\hat{E}_{harvested}$	Energy per tonne of soil associated to 30 days of treatment in an EBCR
$\hat{E}_{mixing}$	Energy per tonne of soil required for mixing during 30 days of treatment
EIS	Electrochemical impedance spectroscopy
GC-MS	Gas chromatography coupled to mass spectrometry
HCH	Hexachlorocyclohexane
I_{EBQR}	Current intensity
Max	Maximum
MFC	Microbial Fuel Cell
M-SR	Methanogenic-sulfate reducing
NC	No supplementation with carbon source
ND	Not detected
NOM	Natural organic matter
NR	Not reported
P_{An}	Surface area power density
P_{ave}	average power
PCCH	Pentachlorocyclohexene
P_{EBCR}	Power delivered
P_V	Volumetric power
R_{int}	Internal resistance
SB	Slurry bioreactors
SMFC	Soil microbial fuel cell technology
SR	Sulphate reducing
TCCH	Tetrachlocyclohexene
THCH	Technical grade hexachlorocyclohexane
UASB	Upflow anaerobic sludge blanket
VSS	Volatile suspended solids
V_t	Working volumen
Greek characters	
η_{coul}	Coulombic efficiency
η_{COD}	Removal efficiency of organic matter as chemical oxygen demand
$\eta_{Lindane}$	Removal efficiency of lindane
$\eta_{Sulphate}$	Removal efficiency of sulphate

ronment mainly via diffuse sources (agricultural use and runoff), but also from point sources like production sites and pesticide spills [15]. In a large set of countries (Austria, Brazil, China, Czech Republic, France, Germany, Hungary, India, Italy, Japan, Macedonia, Mexico, Nigeria, Poland, Romania, Slovakia, South Africa, Spain, Switzerland, Turkey, The Netherlands, UK, USA, and former USSR) between 4 and 7 million tonnes of wastes of toxic, persistent and bioaccumulative lindane residues have been produced and discarded during 60 years of lindane production [16-18]. The amounts of lindane wastes and number of countries with lindane problems increase if wastes and contaminated sites from lindane application are considered [17,19-20].

It is commonly recognized that mass transfer of HCH from soil to liquid phase is the limiting process in biodegradation processes used for soil bioremediation [21-22]. Bioavailability of HCH in polluted soils could be increased by using slurry bioreactors (SB). SB is an ad situ soil bioremediation technology that allows the adjustment and optimization of several process variables such as mixing and water addition, nutrient supplementation, addition of surfactants and solvents to increase pollutant desorption from soil, temperature and pH control, bioaugmentation (seeding the bioreactor with microbial strains or consortia acclimated or specialized in pollutant degradation), etc., with the purpose to increase mass transfer, foster biodegradation and decrease treatment time [23-28]. On the other hand, microbial fuel cells (MFC) constitute a promising technology for the biodegradation of several organic substrates and wastes such as glucose, acetate, xylose, cysteine, cellulose, leachates from solid substrate fermentation of municipal wastes [28-29], and other organic pollutants with simultaneous power generation [30-40]. In MFC the microorganisms oxidize different substrates at the anode producing protons and electrons, which flow through an external circuit to the cathode that is in contact with oxygen, in this part the protons are used in the reduction of oxygen producing water [32,36, 39].

Recently, it has been proposed that soil microbial fuel cell (SMFC) technology could be applied to enhance the removal of organic matter, phenol, and petroleum hydrocarbons in contaminated soil and simultaneous electricity output [41-43]. The purpose of this research was to study the biodegradation of lindane with simultaneous electricity generation using an electrobiochemical slurry reactor (EBCR) for the remediation of a heavy soil polluted with lindane.

2. MATERIALS AND METHODS

2.1. Chemicals

The HCH (97% purity) was purchased from Sigma-Aldrich. Lindane is a moderately lipophilic, organo-chlorinated substance characterized by a high partition coefficient octanol-water Kow ≈ 4*103, with low solubility in water, approx. 7 mg/L at 20 ºC, and slightly polar due to the strong electronegative effects of chlorine atoms bound to the aliphatic ring. Chlorobenzene (CB), dichlorobenzene isomers (1,2-DCB, 1,3-DCB) and 1,2,4 trichlorobenzene (99–99.9% purity) , hexane and acetone were of analytical grade.

2.2. Lindane and metabolite analysis

Lindane was analyzed by Headspace-Solid Phase Microextraction-Gas Chromatography- Electron Capture Detector. The procedure for the extraction of HCH residues in the soil slurry reactor

was performed according by [44-45]. The intermediate metabolites in the experiment 1 were analysed in an Agilent Technologies GC/MS with an autosampler Gerstel (MPS-2 Twister), the oven temperature were programed as follows: hold time 40 °C, 2 min; ramp rate at 3 °C/min to 180 °C; ramp rate at 8 °C/min to 270 °C. The injection volume was 1µl via a split-less injection at 280 °C. Helium was used as a carrier at a flow rate of 1.0 ml/min. The intermediate metabolites in the experiment 2 were analyzed in a Perkin Elmer gas chromatograph equipped with an electron capture detector. Selected samples of EBCR (experiment 2) were analyzed in a Perkin Elmer GC-MS, the oven temperature were programed as follows: hold time 40 °C, 6 min; ramp rate at 3 °C/min to 180 °C; ramp rate at 10 °C/min to 300 °C. The injection volume was 1 µl via a split-less injection at 250 °C. Helium was used as a carrier at a flow rate of 1.0 ml/min.

The soil pH was determined in a slurry soil/deionized water 1:2 (w/w) [22], soil texture was measured by the hydrometer method, biochemical oxygen demand (BOD) was estimated according to the [46] (Method 507) and organic matter content was estimated by the method of oxidation with $K_2Cr_2O_7$ [47]. In sulphate reducing seed reactor (Table 1) were determined: pH, sulphate, organic matter content such as COD and biomass according to the Standard Methods [46] (methods 423, 426C, 508, and 209E respectively; Standard methods, 1981). The alkalinity and alpha ratio were determined according to [48].

2.3. Soil

The soil used in this research was an agricultural soil of Cambisol type from San Miguel Tequixtepec, Oaxaca. The main physicchemical characteristics of our mineral agricultural soil are: pH, 7.2 ± 0.06; organic matter, 8.12 ± 0.09%; soluble COD, 5100 ± 436 mg COD/kg dry soil; soluble BOD 3725 ± 353 mg BOD5/kg dry soil; clay content, 42.3 ± 0.8%; sand content , 37.5 ± 2.7%; silt content, 21.2 ± 3.3%, Its texture corresponded to a clayish soil, whereas its hydraulic conductivity was low. The model soil was contaminated with a dose of 100 mg lindane/kg dry soil.

2.4. Electrobiochemical slurry reactor

EBCR consisted of a Plexiglass cylinder approximately 6 cm in diameter and 8 cm in height (308 mL capacity), fitted with two anodes and two cathodes. The anodes were graphite discs (5cm D x 0.5 cm) whereas the cathodes were of Toray carbon cloth, the cathodes were in contact with atmospheric air (Fig. 1). The electrodes were separated by a cation exchange membrane (Nafion 117, coated with 0.5 mg cm^{-2} platinum catalyst, Pt 10wt%/C-ETEK) and was inoculated with a sulfate reducing inoculum acclimated to lindane [41].

Table 1. Performance of the sulphate reducing seed reactor used for inoculation of electrobiochemical slurry reactor

Parameter	Value
$\eta_{Lindane}$ (%)	76.4 ± 15.2[a]
pH	7.55 ± 0.15[a]
η_{COD} (%)	52.9 ± 11.5[a]
Biomass concentration (mgVSS/L)	1470 ± 380[a]
Factor α	0.17 ± 0.47[a]
$\eta_{Sulphate}$ (%)	76.8 ± 14.3[a]
Lindand sorbed onto biomass (mg lindane/g VSS)	0.465

Notes: [a] standard deviation with respect to time; $\eta_{Lindane}$: Removal efficiency of lindane; $\eta_{Sulphate}$: Removal efficiency of sulphate; VSS: Volatile suspended solids; η_{COD}: Removal efficiency of organic matter as chemical oxygen demand

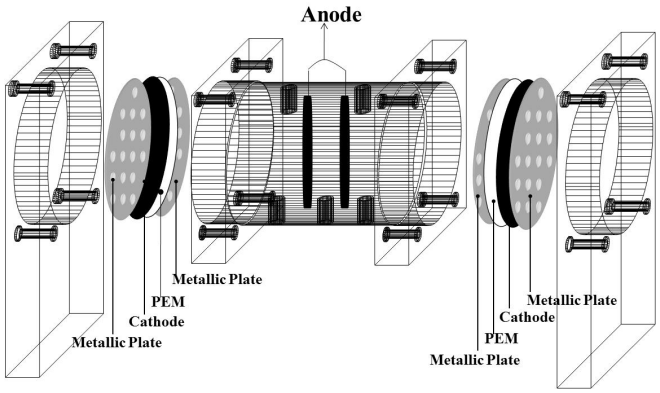

Figure 1. Schematic diagrams of electrobiochemical slurry reactor.

2.5. Experimental design

2.5.1. Experiment based on a concentration of 66% soil

The EBCR was batch operated for 30 day at room temperature. The concentration of soil was 66% w/v. No external carbon source was supplemented; the substrate was the soluble natural organic matter of the soil (NOM). Measurements of the power output were performed using a Multimeter ESCORT 3146A.

2.5.2. Experiment based on a concentration of 33% soil

The EBCR was also batch-operated for 30 day at room temperature. The concentration of soil was 33% w/v. The EBCR was fed a solution stock of sucrose: sodium acetate: lactate to give a final concentration of 2 g COD/L in the EBCR at 15 y 25 d. The contents of the EBCR was mixed by bubbling with nitrogen gas once a day for the first 15 days, afterwards gentle mixing was performed in an orbital shaker at 100 rpm. Measurements of the power output were performed using a Multimeter ESCORT 3146A. The process controls were *(i)* and EBCR operated under open-circuit with live inoculum and soil, and *(ii)* sterile conventional slurry bioreactors as the biotic control and abiotic control, respectively.

2.6. Determination of internal resistence of the electrobiochemical slurry reactor

2.6.1. Electrochemical impedance spectroscopy in the experiment 1

The internal resistance (Rint) of EBCR was calculated as a function of cell voltage using electrochemical impedance spectroscopy (EIS). The electrochemical impedance spectra were recorded over a frequency range of 1 mHz to 100 kHz [38, 40, 49], equivalent circuit models were fitted to the data using the program of ZView2.

2.6.2. Polarization curve method in the experiment 2

The internal resistance of was determined using the polarization curve method, by varying the external resistance (100-105 Ω) according to procedures suggested by [32, 36, 39, 50], this was carried out 0 and 7 d of operation.

3. RESULTS AND DISCUSSION

3.1. Experiment based on a concentration of 66% soil

3.1.1. Determination of internal resistance

Internal resistance is an important factor in the characterization of a MFC since low values tend to result in high values power output. On the other hand according to Jacobi's theorem of maximum power delivered by an electromotive force, an MFC fitted with an external resistance equal to its internal resistance will give its maximum power output [36, 38, 39, 49, 51].

The equivalent circuit obtained from the Nyquist plot (Fig. 2) had an anodic resistance R1=2064 Ω, cathodic resistance R3 = 192

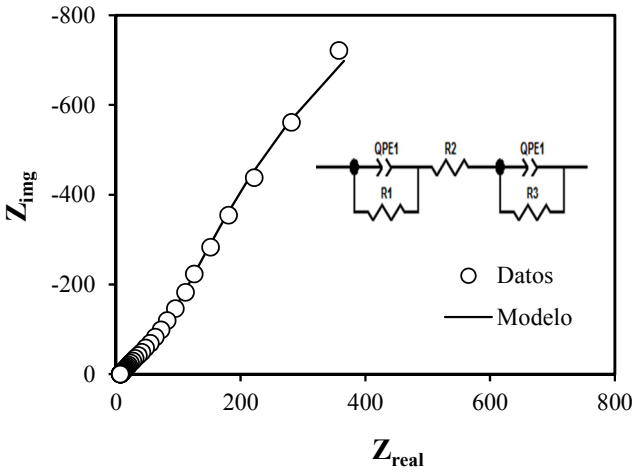

Figure 2. Nyquist plot and equivalent circuit of the electrobiochemcial slurry reactor in the Experiment 1 with 66% soil concentration .

Table 2. Use of soil microbial fuel cell for generating electricity and/or bioremediation pollutants

Reactor configuration	Soil characteristics and pollutant	Electrode system	Efficiencies - COD (%) - Coulombic (%)	- Voltage - Maximum power - Maximum volumetric power - Pollutant removal	Ref.
Cylinder: - D[a]: 2.2 cm - h[b]: 10 cm - ER[c] : 10kΩ - Cation exchange membrane	- OC[d]:11.1% - Texture: Silt loam	- Anode: Carbon cloth (8 x 1 cm), 16 cm^2 of surface area - Cathode: Carbon cloth (8 × 1-cm) coated on one side with 20% platinum (0.5 mg/cm^2) - Cation exchange membrane (CMI-7000, Membranes International, Inc.)	- NR[e] - NR	- 22 mV - 0.03 mW/m^2 - 1.27 mW/m^3 - NA	[48] [52]
- A U-tube air-cathode soil MFC system, inserting a hollow membrane electrode assembly into a rectangle box - ER: 1000Ω	- Texture: Silt loam - Total petroleum hydrocarbon: 28.3 g/kg of soil	- Anode: Carbon mesh - Cathode: Carbon mesh, 0.1 mg/cm^2Pt - Anodes and cathodes were connected in parallel	- NR - NR	- 155 mV - 0.85 mW/m^2 - 15.2 %	[39] [43]
- PVC tube (20cm length ×10cm diameter) containing 250 mL of waterlogged paddy soil. The paddy soil was covered with 3.0 cm of water - ER: 100Ω	- Waterlogged soil - SCOD (mg/L): 430 - TCOD (mg/L): 35 500 - TOM (%, dry soil): 3.17 - Phenol : 80mg/L	- Anode: A layer of carbon felt (15.0cm ×12.5cm × 0.5cm) - Cathode: GORE-TEX cloth (15.0cm × 12.5cm), coated with Ni-based paint (7.0g) and Pt/C solution mixed with Nafion (0.094g)	- NR - 3.7	- 150 mV - 29.45 mW/m^2 - 0.56 mW/m^3 - 90.1%	[38] [42]
- Plexiglass columns (4-L volume, 12cm × 35cm, d × h) with 1600g wet sediment and 1L overlying water. - ER: 100Ω	- Sediment - Phenanthrene: 10 mg/kg dry sediment - Pyrene 5mg/kg dry sediment	- Anode: Two stainless steel cylinders (80mesh x 1mm thickness) - Cathode: A stainless steel cylinder (9.6 cm × 4cm, d × h) - Not applicable	- NR - NR	- 16.8 mV - 0.14 mW/m^2 - $1.08x10^{-3}$ mW/m^3 - Phenanthrene: 99% - Pyrene: 95%	[53] [57]
- PET container,1L volume - ER: NR	- Agricultural soil	- Anode: Circular carbon cloth, total geometric area was 81.07 cm^2 - Cathode: Carbon felt , diameter of about 8.8 cm, thickness of 1.27cm, 1.91 cm wide, carbon cloth strips woven into the top - Not reported	- NR - NR	- NR - 42.49 mW/m^2	[62] [66]
- Plexiglass cylinder 6 cm in diameter and 8 cm in height, volume308mL - ER: 560Ω	- Agricultural soil with high contents of organic matter (8%) and clay - Lindane: 100 mg/kg dry soil	- Anodes: Graphite discs (5cm D x 0.5 cm) - Cathode: Toray carbon cloth (7cm D) - Cation exchange membrane (Nafion 117, coated with 0.5 mg/cm2 platinum catalyst, Pt 10wt%/C-ETEK)	76 15	- 330 mV - 25 mW/m^2 - 634 mW/m^3 - 78% - Potencia: 4.3 MJ/ tonne soil during 30 days of operation	*

Notes: [a] cell diameter; [b] cell height or length; [c]ER: External resistance, [d]OC: Organic content, [e]NR: Not reported. *This study.

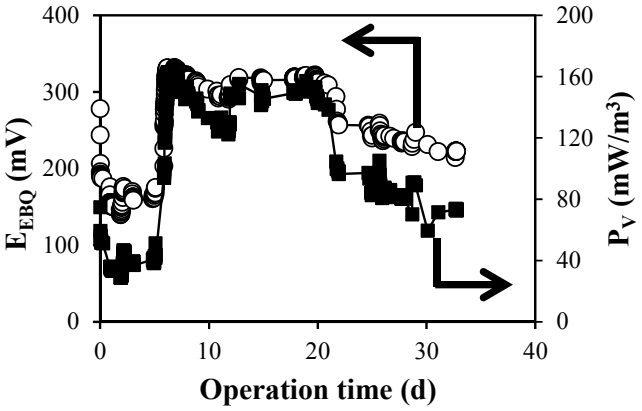

Figure 3. Electricity generation by an electrobiochemical slurry reactor during batch operation for 30 d in the Experiment 1 with 66% soil concentration .

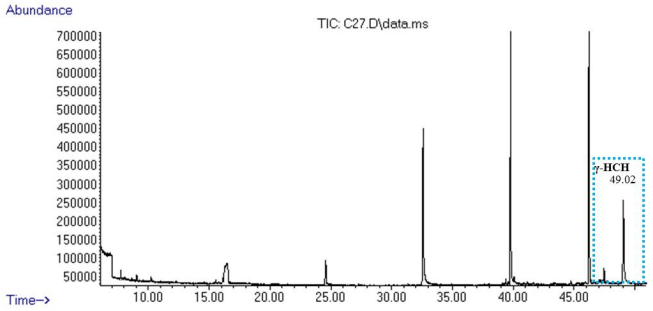

Figure 4. GC-MS detection of lindane and intermediate metabolites in electrobiochemical slurry reactor at the end of operation (30 d) in the Experiment 1 with 66% soil concentration. (The peaks a 16.50, 24.59, 32.86, 39.77 and 46.23 min retention time are Octamethyl-cyclotetrasiloxane, Decamethyl-cyclopentasiloxane, Dodecamethyl cyclohexasiloxane, Tetradecamethyl cycloheptasilox-ane and hexadecamethyl cyclooctasiloxane, respectively, presumably from the column phase).

Ω; and electrolyte/membrane resistance R2 = 7 Ω; so the total internal resistance was 2263 Ω. Compared with other soil microbial cells our value is lower than 10 kΩ reported by Ringelberg *et al*. [52]. They worked with a cylinder (2.2 cm x 10 cm, D x h) as the reactor [52], a non-contaminated silt loam soil with organic matter content of 11.1%, whereas the anode was carbon cloth with 16 cm^2 of surface area and the cathode was carbon cloth coated on one side with 0.5 mg Pt cm^{-2} (Table 2). On the other hand, our internal resistance was higher than that reported by [43] and [42]. The former worked with a U-tube air-cathode soil MFC and a silt loam soil, the anodes and the cathodes both were made of carbon mesh and were connected in parallel. The cathode was coated with 0.1 mg cm^{-2} Pt.

Table 3. Average performance of the electrobiochemical slurry reactor in Experiment 1 with 66% soil concentration.

Parameter	Value
$h_{lindane}$ (%)	30.25 ± 6.33
$P_{An\text{-}max}$ (mWm^{-2})	6.62
$P_{V\text{-}max}$ (mWm^{-3})	165.31
$E_{EBCR\text{-}max}$ (V)	0.33
$I_{EBCR\text{-}max}$ (mA)	0.15
$P_{EBCR\text{-}max}$ (mW)	0.05
$P_{An\text{-}ave}$ (mWm^{-2})	4.12 ± 1.35
$P_{V\text{-}ave}$ (mWm^{-3})	103 ± 34
$E_{EBCR\text{-}ave}$ (V)	0.26 ± 0.07
$I_{EBCR\text{-}ave}$ (mA)	0.12 ± 0.03
$P_{EBCR\text{-}ave}$ (mW)	0.03 ± 0.01
η_{COD} (%)	72.36 ± 15
η_{SO4} (%)	22.07 ± 0.01

Notes: $\eta_{Lindane,}$ lindane removal efficiency; P_{An}, surface area power density; P_V, volumetric power; E_{EBCR}, voltage; I_{EBQR}, current intensity; P_{EBCR}, power delivered; $\eta_{COD,}$ organic matter removal efficiency as COD, $h_{Sulphate,}$ sulphate removal efficiency. Subindices: max, maximum; ave, average.

They [43] reported an internal resistance of 1000 Ù; their soil was contaminated with 28.33 g total petroleum hydrocarbon/kg of soil. They also observed a pollutant removal of 15 % in 25 days of batch operation. On the other hand Huan *et al*. [42] determined an internal resistance of 100 Ω in a system to phenol loaded with paddy soil and supplemented with phenol (80 mg/L). The paddy soil was covered with 3.0 cm of water; the anode was a layer of carbon felt (15.0 cm ×12.5 cm × 0.5 cm) and the cathode was a GORE-TEX cloth (15.0 cm × 12.5 cm), coated with Ni-based paint (7.0 g) and Pt/C solution mixed with Nafion (0.094 g). They observed phenol removal of 90.1% in 10 days of operation. This relatively high result could be ascribed to (i) that phenol is not strongly sorbed on to soils [53], and (ii) it phenol can be degraded by a great variety of microorganisms and its toxicity is relatively low [54-55]. In contrast, lindane is known to be very recalcitrant, toxic, and hydrophobic [10, 22].

3.1.2. Perfomance of the electrobiochemical slurry reactor

The ECBR reached a voltage output of approximately 330 mV at 7 days (Fig. 3, Table 3), whereas the power density normalized by the anode surface was 6.6 mW m^{-2} and the volumetric power was 165 mW m$^{-3.}$ The voltage remained constant until day 20, afterwards a lower value 240 mV was registered. The organic matter removal was very high (72% as soluble COD, NOM) whereas the coulombic efficiency was low (5.4%).

3.1.3. Lindane removal and intermediate metabolites

The EBCR showed 30% lindane removal efficiency at the end of 30 d batch operation. Huang *et al*. [42] observed a phenol removal *ca.* 90% in 10 days in a soil microbial fuel cell. Our results were relatively lower, although it has to be considered that the log of octanol water partition coefficient of phenol is 1.46 whereas that of lindane is 3.62, that is, lindane is less bioavailable, more toxic, and much less soluble than phenol. Also, a great variety of bacteria has

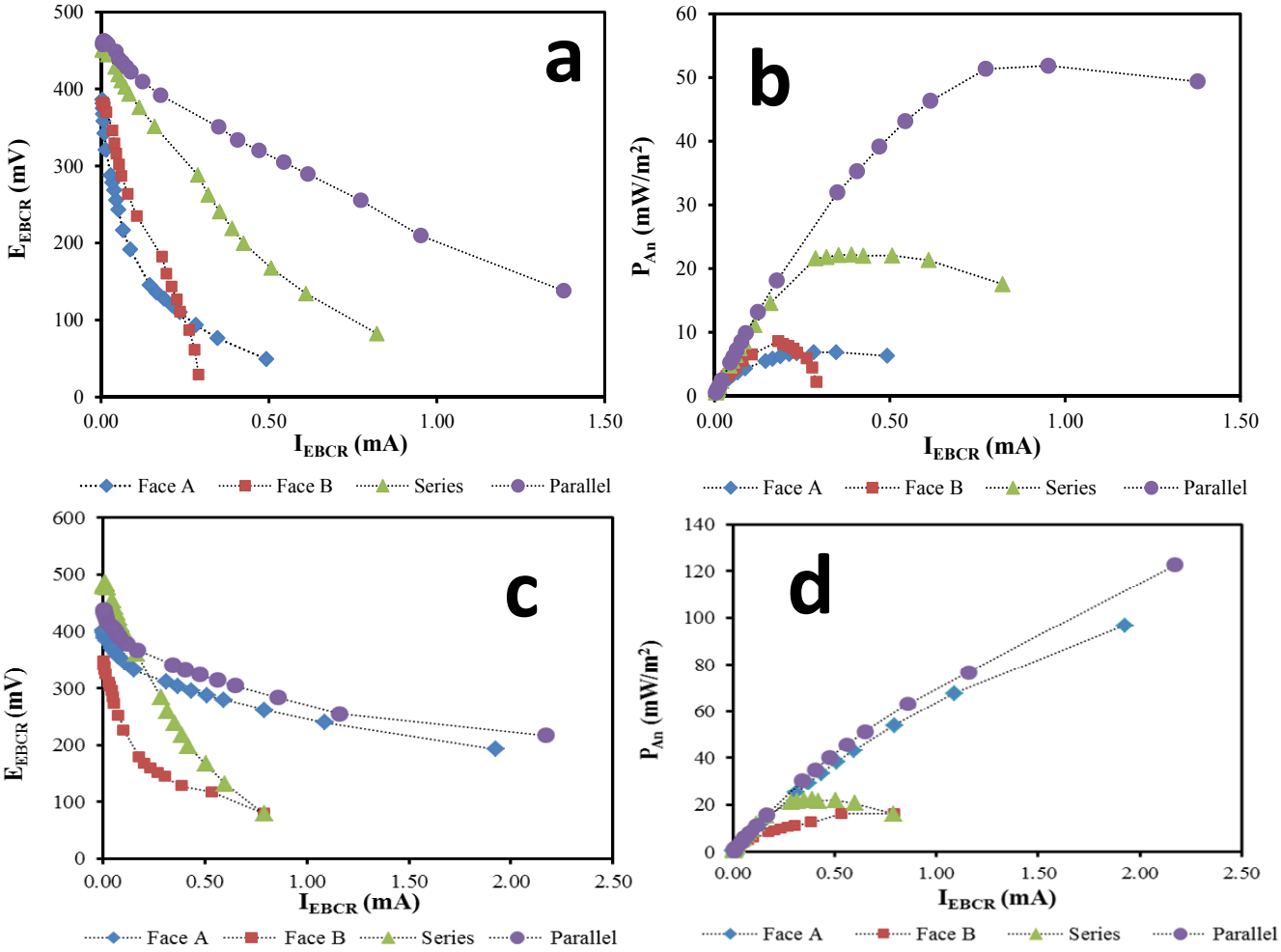

Figure 5. Characterization of the electrobiochemical slurry reactor in the Experiment 2 with 33% soil concentration at 0 d (a) Polarization curves, (b) Power densities; at 7d: (c) Polarization curves, (d) power densities. Keys: light blue rhombus (♦): Face A of the EBCR; brown square (■): Face B of the EBCR; green triangle (▲): faces A and B were connected in series; blue circle (●): faces A and B were connected in parallel.

been reported to use phenol as carbon and energy source, whereas lindane is less biodegradable [10, 13, 22, 54, 55]. At the end of incubation, metabolites from lindane degradation/transformation were not detected by GC-MS (Fig. 4).

3.2. Experiment based on a concentration of 33% soil

3.2.1. Characterization of the electrobiochemical slurry reactor

The polarization curves and the power variation with current intensity of the EBCR at time 0 days are shown in Figure 5a and 5b respectively. The values obtained from the polarization curves method were 2046, 1288, 897 and 255 Ω for face A, face B, connection in series and parallel, respectively (Table 4). The maximum volumetric power was obtained when the connection was in parallel (739 mW m^{-3}) followed by the face B, connection in series and face A with 421, 340 and 86 mW m^{-3} respectively.

After 7 days of operation another reactor characterization was carried out. The internal resistances decreased very much compared to those of the first characterization. Their values were approximately of 140, 339, 442, 102 Ω for face A, face B, connection in series and connection in parallel, respectively (Figures 5c and 5d, Table 4). The maximum volumetric power was obtained for parallel connection (1531 mW m^{-3}, Table 4, Fig. 5); it was twice the volumetric power obtained with characterization at 0 days. The improved characteristics might be a consequence of the increased microbial activity resulting from enrichment of the biofilm on the anode [56]. The internal resistance was smaller than the value of 10 kΩ obtained by Ringelberg and coworkers [52]. They [52] worked with a cylinder (2.2 cm × 10 cm, D × h) as the reactor, a non-contaminated silt loam soil with organic matter content of 11.1%, whereas the anode was carbon cloth with 16 cm^2 of surface area and the cathode was carbon cloth coated on one side with 0.5 mg Pt cm^{-2} (Table 2) [52]. Furthermore Wang *et al.* [43] reported values

Table 4. Values of several variables of electrobiochemical slurry reactor characterization at 0 and 7 day of operation.

Parameter	Face A		Face B		Series		Parallel	
Time (days)	0	7	0	7	0	7	0	7
R_{int} (Ω)	2046	140	1288	339	897	442	255	102
$P_{An\text{-}max}$ (mWm^{-2})	6.88	96.60	33.72	16.32	13.60	13.93	29.57	61.27
$P_{V\text{-}max}$ (mWm^{-3})	86	1207	421	204	340	348	739	1531
$I_{EBCR\text{-}max}$ (mA)	0.49	1.93	1.14	0.79	1.02	1.03	1.50	2.17
$E_{EBCR\text{-}max}$ (V)	0.39	0.41	0.38	0.35	0.45	0.49	0.46	0.44
$P_{EBCR\text{-}max}$ (mW)	0.03	0.37	0.13	0.06	0.10	0.11	0.23	0.47
$P_{An\text{-}ave}$ (mWm^{-2})	2.72	19.91	6.13	5.88	5.08	4.95	0.87	12.12
$P_{V\text{-}ave}$ (mWm^{-3})	34	249	76	73	127	124	22	303
$I_{EBCR\text{-}ave}$ (mA)	0.09	0.30	0.15	0.15	0.20	0.19	0.02	0.33
$E_{EBCR\text{-}ave}$ (V)	0.22	0.34	0.26	0.25	0.33	0.34	0.37	0.38
$P_{EBCR\text{-}ave}$ (mW)	0.01	0.08	0.02	0.02	0.04	0.03	0.07	0.09

Notes: R_{int}: internal resistance; P_{An}, surface area power density; P_V, volumetric power; E_{EBCR}, voltage; I_{EBCR}, current intensity; P_{EBCR}, power delivered. Subindices: max, maximum; ave, average

of 1000 Ω. They worked with a U-tube air-cathode soil MFC and a silt loam soil, the anodes and the cathodes both were made of carbon mesh and were connected in parallel. The cathode they used was coated with 0.1 mg cm^{-2} Pt [43]. On the other hand the internal resistance obtained in our work when the connection was in parallel was similar to the low internal resistances of 100 Ω reported by Huan *et al*. [42]. They [42] worked in a system containing phenol loaded with paddy soil and supplemented with phenol (80 mg/L) [42]. This paddy soil was covered with 3.0 cm of water; the anode was a layer of carbon felt (15.0 cm ×12.5 cm × 0.5 cm) and the cathode was a GORE-TEX cloth (15.0 cm × 12.5 cm), coated with Ni-based paint (7.0 g) and Pt/C solution mixed with Nafion (0.094 g).

3.2.2. Performance of the electrobiochemical slurry reactor

Table 5. Average performance of electrobiochemical slurry reactor in the Experiment 2 with 33% soil concentration.

Parameter	Value
$\eta_{Lindane}$ (%)	78.25
R_{int} (Ω)	560
$P_{An\text{-}max}$ (mWm^{-2})	25
$P_{V\text{-}max}$ (mWm^{-3})	634
$E_{EBCR\text{-}max}$ (V)	0.33
$I_{EBCR\text{-}max}$ (mA)	0.59
$P_{EBCR\text{-}max}$ (mW)	0.20
η_{Coul} (%)	15.17
η_{COD} (%)	76.35

Notes: $\eta_{Lindane}$: Removal efficiency of lindane; R_{int}: internal resistance; P_{An}, surface area power density; P_V, volumetric power; E_{EBCR}, voltage; I_{EBQR}, current intensity; P_{EBCR}, power delivered; η_{Coul} (%):Coulombic efficiency. η_{COD}, organic matter removal efficiency as COD; . Subindices: max, máximum; ave, average.

Figure 6 shows the time course of voltage generation of the device when the anodes and cathodes of the EBCR were connected in parallel whereas results of the average performance are exhibited in Table 6. The voltage with the EBCR in open circuit conditions (at the early 20 h) was approximately 530 mV (phase I). The voltage remained stable when the cell was operated with an external resistance of 120 Ω (first hours of phase II); however the voltage decreased to less than 200 mV afterwards. So, open circuit conditions were re-established in phase III where an expected increase of voltage occurred. Subsequently, in phase IV, the cell was operated with an external resistance of 220 Ω and a drastic voltage decrease was observed. Again, open circuit conditions were re-established in phase V. Phase VI was run with an external resistance of 560 Ω. Approximately at day 8 the cell contents was mixed by bubbling nitrogen gas, once a day for 10 minutes each (by drop pneumatic agitation). It was found that cell voltage first increased to a maxi

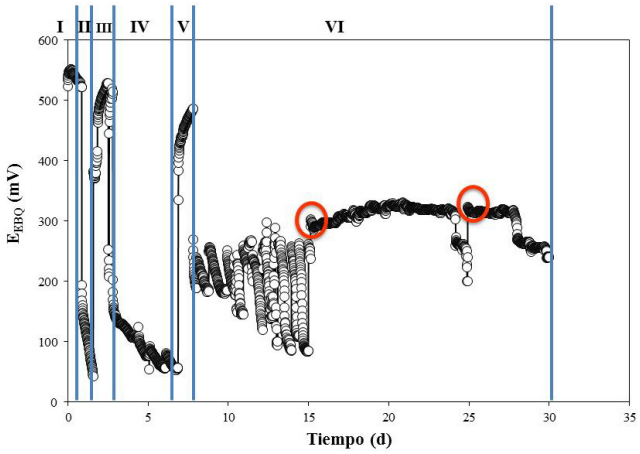

Figure 6. Electricity generation in electrobiochemical slurry reactor during batch operation for 30 d in the Experiment 2 with 33% soil concentration. The addition of substrate is indicated by the red circles. Phase I, open circuit; phase II, closed circuit with external resistance 120Ω; phase III, open circuit; phase IV, external resistance 220Ω; phase V, open circuit; phase VI, external resistance 560Ω.

Table 6. Use of slurry reactor for bioremediation of lindane contaminated soils

Microorganism	External sources of carbon and energy/ Electron acceptors	Initial concentration of HCH	Matrix	Experimental conditions	Intermediate metabolites	Removal (%) and removal rate	Ref.
Lindane acclimated inocula (500 mg VSS/L)	Sucrose/Sulphate	100mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm	PCCH 1,2,4-TCB; 1,2,3-TCB; CB, B	- 88% in 30 days - 2.93 mg/kg*d	[19] [22]
Lindane acclimated inocula (500 mg VSS/L)	NC/Sulphate	100mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm	NR	- 82 % in 30 days - 2.73 mg/kg*d	[19] [22]
Lindane acclimated inocula (500 mg VSS/L)	Sucrose / Sulphate	100mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm Triphasic reactor: 20% v/v silicone oil	CB 1,2 DC 1,DCB 1,2,4-TCB	- 84% in 30 days - 2.8 mg/kg*d	[16] [20]
Lindane acclimated inocula (500 mg VSS/L)	NC / Sulphate	100mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm Triphasic reactor: 20% v/v silicone oil	NR	- 78% in 30 days - 2.6 mg/kg*d	[16] [20]
Lindane acclimated inocula (500 mg VSS/L)	Sucrose/Carbon dioxide	100 mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm	CB 1,2-DCB	- 47% in 30days - 1.57 mg/kg*d	[19] [22]
Lindane acclimated inocula	NC/Carbon dioxide	100mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm	CB 1,2-DCB	- 41 % in 30days - 1.37 mg/kg*d	[19] [22]
Lindane acclimated inocula (500 mg VSS/L)	Sucrose/Carbon dioxide	100mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm Triphasic reactor: 20% v/v silicone oil	NR	- 33% in 30 days - 1.1 mg/kg*d	[16] [20]
Lindane acclimated inocula (500 mg VSS/L)	NC/Carbon dioxide	100mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm Triphasic reactor: 20% v/v silicone oil	NR	- 22% in 30 days - 0.73 mg/kg*d	[16] [20]
Lindane acclimated inocula (500 mg VSS/L)	-Sucrose -Simultaneous electron Carbon dioxide-sulphate	100mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm	NR	- 16% in 30 days - 0.53 mg/kg*d	[19] [22]
Lindane acclimated inocula (500 mg VSS/L)	-NC -Simultaneous electron Carbon dioxide-sulphate	100mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm	NR	- 34 % in 30 days - 1.37 mg/kg*d	[19] [22]
Lindane acclimated inocula (500 mg VSS/L)	-Sucrose -Simultaneous electron Carbon dioxide-sulphate	100mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm Triphasic reactor: 20% v/v silicone oil	NR	-94 % in 30 days - 2.17 mg/kg*d	[16] [20]
Lindane acclimated inocula (500 mg VSS/L)	-NC -Simultaneous electron Carbon dioxide-sulphate	100mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm Triphasic reactor: 20% v/v silicone oil	NR	-90% in 30days - 2.17	[16] [20]

Lindane acclimated inocula (500 mg VSS/L)	- Sucrose - Sequential M-SR	100 mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm	CB 1,2-DCB	- 66% in 30 days - 2.2 mg/kg*d	[60] [64]
Lindane acclimated inocula (500 mg VSS/L)	- NC - Sequential M-SR	100 mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm	PCCH 1,2,4-TCB	- 98% in 30 days - 3.3 mg/kg*d	[59] [63]
Lindane acclimated inocula (500 mg VSS/L)	- Sucrose - Sequential M-SR	100 mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm Triphasic reactor: 20% v/v	NR	-54% in 30 days -1.8 mg/kg*d	[60] [64]
Lindane acclimated inocula (500 mg VSS/L)	- NC - Sequential M-SR	100 mg/kg	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm Triphasic reactor: 20% v/v	NR	- 93% in 30 days - 3.1mg/kg*d	[59] [63]
Lindane and parathion acclimated inocula (500 mg VSS/L)	- NC - Nitrate	100 mg/kg Lindane 100 mg/kg parathion	Soil slurry (clayish soil with 8% organic matter)	pH 7 Vt: 100 mL 120 rpm	NR	- 30% in 30 days, for lindane - 1 mg/kg*d - 40% in 30 days for parathion -1.3 mg/kg*d	[61] [65]

Notes: HCH: gamma hexachlorocyclohexane; 1,2,3.TCB:1,2,3-trichorobenzene,1,2,4-TCB:1,2,4-trichlorobenzene; 1,2-DCB:1,2-dichlorobenzene; 1,3-DCB:1,3-dichlorobenzene; CB: Chlorobenzene; COD: chemical oxygen demand; M-SR: methanogenic-sulfate reducing; NC: no supplementation with carbon source; ND: Not detected; NR: Not reported; PCCH: Pentachlorocyclohexene; UASB: upflow anaerobic sludge blanket; Vt: working volumen; VSS: Volatile Suspended Solids; TCCH: Tetrachlocyclohexene; THCH: technical grade hexachlorocyclohexane

mum 350 mV and later on, it significantly decreased after each mixing episode (down to between 100 to 200 mV, Fig. 6, days 8 to 15). Due to pneumatic and hydraulic difficulties of such a mixing, starting at day 15 the cell content was continuously mixed in an orbital shaker at low speed (100 rpm.) Interestingly voltage output recovered and was stabilized around 300 mV.

The change of type of mixing was implemented with supplementation of substrate 2 g/L (sucrose: sodium acetate: lactate) that was used as the fuel in the EBCR at 15 d. Electricity generation began to increase and reached a voltage output of approximately 303 mV (Fig. 6). In this period, the power density normalized to anode area was 21.3 mW m^{-2} and the average volumetric power was 531 mW m^{-3}. At approximately 20 days of operation, the cell reached a maximum voltage output of 329 mV and volumetric power of 629 mW m^{-3} (Table 5); the voltage remained constant until day 24. Afterwards, it decreased again to a value of 260 mV. On day 25 the EBCR was fed with 2 g COD/L of substrate and reached a voltage of 321 mV, the EBCR voltages decreased below 280 mV at 28 day.

The maximum voltage output of the EBCR (330 mV) and maximum power (25 mW m^{-2}) were higher than those reported by Wang *et al*. [43] (155 mV and maximum power 0.85 mW m^{-2}) for a cell loaded with soil polluted with total petroleum hydrocarbons (Table 2). Our results also compared very favorably to [57] who reported a voltage as low as 17 mV in the treatment of a sediment contaminated with phenanthrene and pyrene (Table 2).

On the other hand, Huang *et al*. [42] registered a power density slightly superior (*ca.* 30 mW m^{-2}) and a lower voltage (150 mV) in the treatment of a waterlogged soil polluted with phenol (Table 2).

The EBCR exhibits a bonus besides soil remediation: the bioelectrity generation. The latter was estimated with Eq 1 below

$$\hat{E}_{harvested}\ (\text{MJ/tonne soil}) = P_{ave}\ (\text{W})*(30\ \text{d}*24\ \text{h/d}*3600\ \text{s/h})/0.0001\ \text{tonne soil} \quad (1)$$

$\hat{E}_{harvested}$ is the energy produced in MJ/tonne soil, where P_{ave} is the average power in 30d of operation, 24 h/d and 3600 s/h are conversion factors for converting time from days to seconds; 0.0001 tonne soil is soil mass in the lab scale electrobiochemical slurry reactor.

The $\hat{E}_{harvested}$ was 4.3 MJ/tonne soil during 30 days of operation. This energy partially offsets the power required for mixing of both the EBCR and typical SBs. For instance, power requirements for mixing are determined empirically and can be estimated from manufacturer´s equipment specifications. Typical power requirements for complete mixing are in the range 20 to 50 kW/1000 m^3 for moderately thick suspensions [58]; a mid-point value of 35 kW/1000m^3 was chosen.

Performing the calculations with similar assumptions to those of Eq. 1, the energy required for mixing during the 30 d batch would be given by Eq 2 below

$$\hat{E}_{mixing} = 35\ (\text{W/m}^3)*(0.33\ \text{tonne soil/m}^3)*(30\ \text{d}*24\ \text{h/d}*3600\ \text{s/h}) = 29.9\ \text{MJ/tonne soil} \quad (2)$$

That is, the EBCR allows for a bioelectricity harvest that could represent *ca.* 14% of the energy required for mixing.

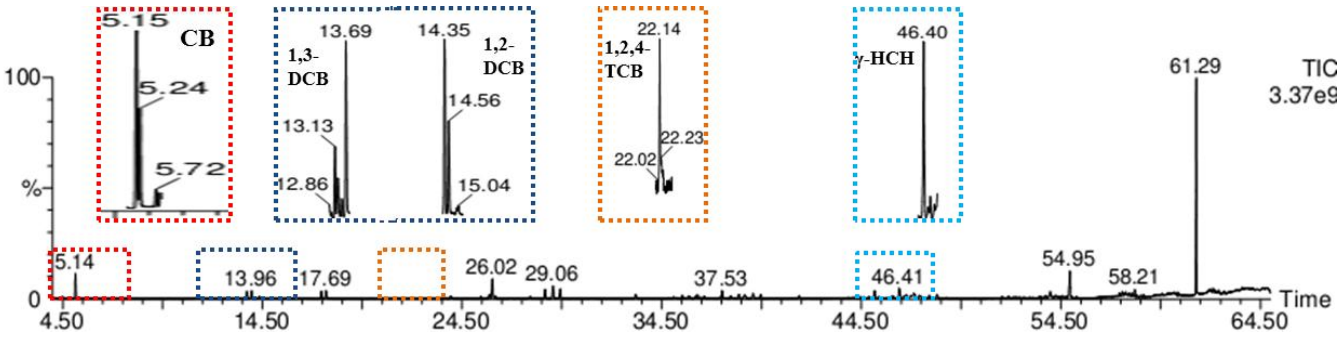

Figure 7. GC-MS detection of intermediate metabolites in electrobiochemical slurry reactor at the end of operation (30 d) in the Experiment 2 with 33% soil concentration.

3.2.3. Lindane removal and intermediate metabolites

Lindane removal achieved in the EBCR was 78%, whereas the removals of the biotic (live) control and abiotic control slurry reactors were 80 and 3%, respectively. Main metabolites due to lindane degradation in the EBCR were detected by analysis by GC/MS in the EBCR: 1,2,3-trichlorobenzene (1,2,3 TCB), 1,3-dichlorobenzene (1,3-DCB), 1,2-dichlorobenzene (1,2-DCB), and chlorobenzene (CB) (Figure 7).

Lindane removals observed in our EBCR compared very favorable with lindane removals reported for standard slurry bioreactors in the literature. Some experiments with SB inoculated with *Pandorea* sp., with a presumably anaerobic operation of 9 weeks duration have been reported [59]. Initial lindane concentration was 100 mg kg^{-1}; they found removals of 59.6% γ-HCH. Quintero *et al*. [13] treated a sandy soil polluted with a mixture of isomers α, β, γ and δ-HCH (100 mg/kg each) in anaerobic SB. Starch was supplemented at 2 g L^{-1} every 3 days. High removals of nearly 100 % for α and γ isomers of HCH and 65 to 70% for β and δ HCH were found. On other hand, the bioremediation of a heavy soil polluted with 100 mg lindane kg^{-1} in full sulfate reducing slurry bioreactors was reported [22]. Removal was 88% whereas the detected metabolites after 30 d operation were PCCH; 1,2,4-TCB; 1,2,3-TCB; CB, and benzene. They [22] have previously demonstrated that in soil slurry reactor with electron acceptor carbon dioxide the removal lindane was approximately between 41-47% (Table 6). The soil SB was operated with similar operational conditions (soil physico-chemical characteristics, pH, temperature, agitation rate, soil loading rate), except that in our research we used an electrobiochemical slurry reactor technology. In other studies of our Group, we have carried out experiments with SB in sulphate-reducing conditions; they reported removals of 78% γ-HCH in 30 days that was the same removal amount observed in our experiments using soil concentration of 66% [20].

The degradation of HCH isomers in slurry reactors in anaerobic conditions was reported [13]. They found traces of diverse intermediate metabolites, such as pentachlorocyclohexane (PCCH), 1,2-dichlorobenzene (1,2-DCB), 1,3-dichlorobenzene (1,2-DCB) and clorobenzene (CB). The low concentrations of the metabolites indicated that intermediate compounds were not accumulated and they proceed to their further degradation to CB, the end product in the degradation mechanism. It has been observed the total depletion of α and HCH in a polluted soil after 3 days anaerobic incubation [44] observed total depletion of α and HCH in a polluted soil after 3 days anaerobic incubation; they used an initial lindane concentration of 100 mg kg^{-1} soil, bioaugmentation with a high concentration of methanogenic anaerobic sludge (8 g VSS L^{-1} in the bioreactor), starch (2 g COD/L) as electron donor, and semi-continouous operation. During the degradation, traces of diverse intermediate and end-products compounds were detected similar to those found in their other work (PCCH, TCCH, 1,2, 3-TCB, 1,3-DCB, and CB.

It was found that lindane could be dechlorinated by mainly sulfate-reducing bacteria with generation of monochlorobenzene and benzene as main intermediates [60]. Similarly, we detected chlorobenzene at 30 days of operation of the ECBR loaded with a sulfate-reducing inoculum. On the other hand in our Group of work [34], reported that clones found in the sulfate-reducing consortium were *Clostridia*, *δ-Proteobacteria* (electrochemically active bacteria) and *Firmicutes,* where *Clostridia* are recognized to be electrochemical active bacteria.

The high lindane removals obtained in our work in only 30 d of EBCR operation are very promising: it achieves similar lindane removals as in conventional slurry bioreactors with the additional bonus of bioelectricity generation. Thus, EBCR emerges as a fast and attractive technology for pesticide degradation and soil remediation. Indeed, it has been reported the recalcitrance (persistence) of organo-chlorinated pesticides in soils, with half lives of the order of 2 to 5 years [1, 61]. In particular, lindane has an average half-life of 2.6 years in soils, depending on the physico-chemical characteristics of soils (texture, organic matter, depth, etc.) as well as environmental conditions [62].

4. CONCLUSION

-The bioremediation of lindane in soil could be achieved in an EBCR with similar removals to those reported in anaerobic slurry bioreactors loaded with lindane-acclimated sulphate-reducing inoculum, other conventional slurry bioreactors as well as other bioremediation technologies.

-Mixing and the supplementation with organic substrate seemed to significantly improve the EBCR performance, both the efficiency of the removal of lindane and the production of electricity significantly increased.

-The EBCR not only provided effective bioremediation of a toxic, recalcitrant organo-chloinated pesticide, but also supplied (as

bioelectricity) ca. 14% of the energy required for mixing the device.

-We detected intermediate metabolites typical of anaerobic degradation pathways of lindane that were similar to those reported in previous research in conventional anaerobic slurry bioreactors.

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