Production of Electricity in an Electrobiochemical Slurry Reactor used for the Bioremediation of Pesticide Contaminated Soil

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ABSTRACT

It is recognized that mass transfer of lindane from soil to liquid phase is the limiting process in biodegradation processes used for soil bioremediation. Surfactants are used due to their ability to increase water solubility and mass transfer. On the other hand, electrobiochemical slurry reactor (EBCR) constitutes a promising technology that could remove organic matter, phenol, petroleum hydrocarbons, and pesticides in contaminated soils with simultaneous electricity output. The aims of this research were *(i)* to evaluate the desorption of lindane from soil with Tween 80 at different concentrations; *(ii)* to determine the power output and removal of lindane in an electrobiochemical slurry reactor; and (*iii*) to characterize the dominant microorganisms in the electrobiochemical slurry reactor using 16S rRNA and denaturing gradient gel electrophoresis. The EBCR consisted of a Plexiglass cylinder approximately 6 cm in diameter and 8 cm in length, fitted with two anodes and two cathodes.

Addition of 2000 mg L^{-1} Tween 80 removed 9.61% of lindane in *in vitro* desorption experiments. The internal resistance of the EBCR determined by polarization curve was 820 Ω; a moderate volumetric power was recorded (374 mW m^{-3}) along with a potential of 600 mV when the two-electrode sets were connected in parallel. During the batch operation, the EBCR showed a 56% lindane removal whereas the reduction in the abiotic control was 3%. Unexpectedly the removal efficiency of lindane ($\eta_{lindane}$) in the EBCR was lower than that in an EBCR operated without surfactant in a previous experiment. This could be ascribed to the increased degradable organic matter supply related to Tween in our EBCR that possibly shifted microbial metabolism from lindane degradation to degradable matter uptake. An average volumetric power of 685 mW m^{-3} and average voltage of 420 mV were achieved. Results from the DGGE analysis and further sequencing, indicate the presence of *Trichococcus palustris* strain DSM 9172 (99% sequence identity). This bacterium has been reported in autotrophic biocathodes of other bioelectrochemical systems. Finally, the energy output obtained in our EBCR allows for the recovery of 20% of the power required for mixing, thus paving the way to sustainable bioremediation of soils

Keywords: Electrobiochemical slurry reactor; lindane; Tween 80

1. Introduction

Agriculture is an essential activity of modern societies. While agricultural products provide most of the food consumed by the world population, they also impose great pressures on global natural resources. Reliance on pesticides and fertilizers because of intense agricultural practices could also contribute to environmental degradation [1, 2]. Lindane (1, 2, 3, 4, 5, 6 - hexachlorocyclohexane, γ-HCH) is a moderately lipophilic, organochlorinated substance characterized by a high partition coefficient octanol-water $K_{ow} \approx 4*10^3$ with a low solubility in water, approx. 7 mg L^{-1} at 20ºC, and slight polarity due to the strong electronegative effects of chlorine atoms bound to the aliphatic ring [3, 4, 5]. The γ-HCH has been used in important agricultural applications as on fruits, vegetables crops, and forestry for control of leaf-eating insects, and it is used as a fungicidal seed treatment worldwide [6, 7, 8]. It is a Persistent Organic Pollutant (POP), potent carcinogen and teratogen and classified by the World Health Organization (WHO) as 'moderately hazardous' (human oral LDo: 840 mg kg^{-1}). Paknikar *et al*. [9], have been reported potential adverse health effects include neurological problems and immunosuppression in human and liver cancer in rats and mice [10]. The use of lindane has been restricted since the 1970s and banned later on because of their toxicity, but the problem of residues of lindane remains because of the high persistence and inter-conversion of lindane in soil [8, 10, 11]. Under natural conditions, the high adsorption of the hydrophobic compounds in the soil solid phase (mainly in the organic matter) and its restricted availability for the biological action of endogenous or exogenous microorganisms limits the extent and rate of degradation. The size of particles and aggregates, and soil structure, also contribute to making the desorption rate (mass transfer rate) the limiting stage in their degradation [12]. One possibility to favor the mobility of the pollutants is their transfer from the soil to the liquid phase; this possibility would enhance the pollutant bioavailability. Bioavailability is considered a dynamic process, determined by the rate of substrate-mass transfer to microbial cells relative to their intrinsic catabolic activity

One alternative to facilitate lindane desorption from the soil, and thus to increase lindane bioavailability in to use *ex-situ* technology such as soil slurry reactors. This technology consist of treating mixtures of contaminated soil and water in controlled stirred reactors [13].The advantages of the slurry reactor is the feasibility of controlling operating parameters such as mixing (facilitates aeration and enhances the rate of chemical exchange between soil particles), controlling pH, temperature, providing nutrients and other specialized amendments such as surfactants [14-16].

The addition of surfactants to increase the mass-transfer rate of PAHs in soils has received attention [17]. Surfactants consist of organic molecules with a hydrophobic and a hydrophilic part and can interact with polar as well as nonpolar surfaces. At low concentrations, surfactants exist solely as monomers; above a critical aqueous concentration, which is specific for each surfactant monomer, called critical micellar concentration (CMC), the surfactant monomers are aggregated in solution to form entities made up of a hydrophobic core and a hydrophilic shell [18]. These micelles result in increased pseudo-water-solubilities of pollutants, thereby increasing the concentration gradient and mass transfer rates [11, 17,18].

On the other hand, microbial fuel cells (MFC) constitute a promising technology for the biodegradation of several organic substrates such as glucose, acetate, xylose, cysteine, cellulose, leachates from solid substrate fermentation of municipal wastes and waterwastes, and other organic pollutants with simultaneous power generation [19- 31]. Recently, it has been proposed that soil microbial fuel cell (SMFC) technology could be applied to enhance the removal of organic matter, lindane, phenol, and petroleum hydrocarbons in contaminated soil, while at the same time allowing electric energy generation [32-34]. The aims of this research were *(i)* to evaluate the desorption of lindane from soil with Tween 80 at different concentrations; *(ii)* to determine the power output and removal of lindane in an

electrobiochemical slurry reactor; and (*iii*) to characterize the dominant microorganisms in the electrobiochemical slurry reactor using 16S rRNA and denaturing gradient gel electrophoresis; and (iv) to characterize the microorganisms in the electrobiochemical slurry reactor using the Ion Torrent Personal Genome Machine. We used a difficult to remediate soil. Since it had high contents of organic matter and clay.

2. Experimental

2.1 Desorption tests. Desorption assays were carried out in serum bottles at 100 rpm for 6 days in order to evaluate the effect of surfactant on lindane desorption. In each serum bottles was loaded with 5 g of sterilized soil polluted with lindane (100 mg kg^{-1} dry soil), 25 mL of a solution Tween 80 at different concentrations (0, 75, 500, 2000, 5000 mg L^{-1}). Sodium azide (300 mg L^{-1}), was added as a microbiological inhibitor, and $CaCl_2$ (0.01M). The supernatant solution was analysed for lindane concentration.

2.2 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 (Figure 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 [32].

The EBCR was batch-operated for 30 day at room temperature. The concentration of soil was 33% w/v and of Tween 80 was 2000 mg L^{-1}. The EBCR was fed a solution stock of sucrose: sodium acetate: lactate to give a final concentration of 2 g COD L^{-1} in the EBCR at 0 y 15 d. The contents of the EBCR were performed in an orbital shaker at 100 rpm. Measurements of the power output were performed using a Multimeter ESCORT 3146A. The process control was EBCR operated under open-circuit with live inoculum and soil.

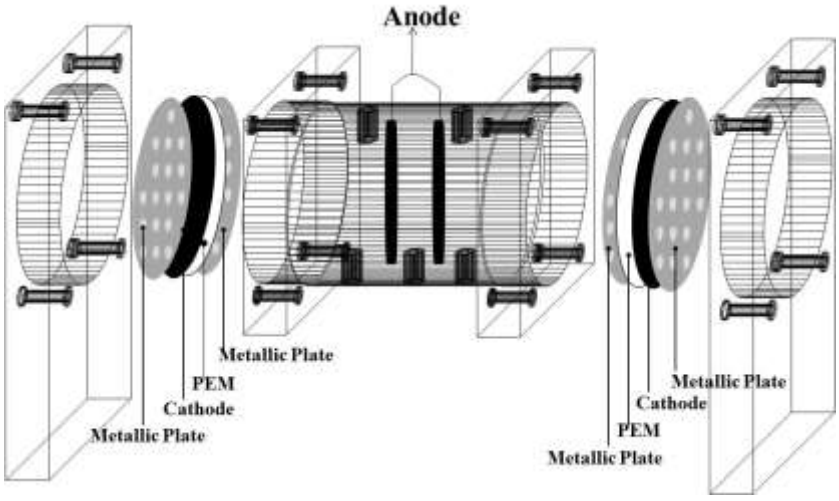

Fig 1. Schematic diagram of electrobiochemical slurry reactor.

2.3 Determination of internal resistence of the electrobiochemical slurry reactor. The internal resistance was determined using the polarization curve method, by varying the external resistance (100- 100 000 Ω) according to procedures outlined by elsewhere [23, 27, 29, 30], this was carried out 0 y 7 d of operation.

*2.4 Chemicals***.** The γ-HCH isomer (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^{-1} at 20ºC, and slightly polar due to the strong electronegative effects of chlorine atoms bound to the aliphatic ring. Hexane and acetone were of analytical grade. Tween 80 was purchased from Sigma-Aldrich.

2.5 Soil. An agricultural soil with high contents of organic matter and clay, was sieved through a 20 mesh, and sterilized by tyndallisation. The soil was contaminated with 100 mg lindane kg^{-1}. Soil pH was determined in a slurry soil/deionized water 1:2 (w/w) [3], soil texture was measured by the hydrometer method, soluble BOD and soluble COD were determined in soil water extracts, according to the Standard Methods [35]. The main characteristics of the soil used in this work were: soil type, Cambisol; pH, 7.2; organic matter (%), 8.1 ± 0.1; soluble COD (mg COD kg^{-1}), 5100 ± 436; soluble BOD (mg BOD_5 kg^{-1}), 3725 ± 350; clay content (%), 42.3 ± 0.8; sand (%), 36.5 ± 2.7; silt (%), 21.2 ± 3.3. The texture was clayish and the hydraulic conductivity was low.

2.6 PCR-DGGE and Sequencing of 16S rDNA. A sample of 200 µl from the electrobiochemical slurry reactor (0, 15, 22, 30 days of treatment) were used to extract DNA using the PowerSoil® DNA Isolation kit (MoBio). Whole-community DNA from each sample was subjected to PCR amplification using of specific primers for the Eubacterial. The Eubacterial specific primers were CGO465F-GC (CTCCTACG GGAGGCAGCAGCGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCCCGGGGGG) and CGO465R (CTACCAGGGTATCTAATCCTG) targeting a region of 332-775 bp of *E. coli* 16S rRNA gen (gen 16S rDNA GenBank:J01859.1). The PCR mixture consisted of Buffer (1X), $MgCl_2$ (2 mM), dNTP´s (0.2 mM), Taq DNA polymerase (0.02U μL^{-1}), forward primer CGO465F-GC (0.2µM), reverse primer CGO465R (0.2µM), and template DNA (100 ng). Amplification was performed in a TECHNE (TC-312) thermocycler with cycling as follows: an initial denaturation step at 95°C for 5 min, followed by 25 cycles of 95°C for 45 s, 55 °C for 30 s, and 72°C for 30 s, and finally an extension step at 72°C for 10 min.
Denatured gradient gel electrophoresis (DGGE) was performed with a DCode™ universal mutation detection system (Bio-Rad®) according to the manufacturer's instruction. PCR amplicons were loaded onto 8% polyacrylamide gel with a denaturing gradient range from 25 to 70% consisting of urea and formamide. The gel was stained with SYBR® Green and DNA bands were observed by a Gel-Doc™ image analyzer (Bio-Rad) under UV illumination. After that denaturing gradient gel electrophoresis (DGGE), a representative band (when the EBCR exhibited the maximum voltage) was excised, cloned (Vector PCR® 2.1 TOPO®), sequenced (ABI PRISM 310, Applied Biosystems), sequence data were analyzed with BioEdit software and compared with sequences in GenBank (http://www.ncbi.nlm.nih.gov).

2.6.1 Richness estimation. Microbial richness variation between days of operation was estimated by the Jaccard similarity index IJ and Poggi's discrete divergence index Δ_{Poggi} [36, 37]:

$$IJ = \frac{n_{AB}}{(n_A + n_B - n_{AB})} \quad (1)$$

Where : IJ= Jaccard similarity index, n_{AB} = number of bands that are present both in lane A and lane B, nA = total number of bands in lane A, nB = total number of bands in lane B.

$$\Delta_{Poggi} = \frac{(n'_A + n'_B)}{n_A + n_B} \quad (2)$$

Where : Δ_{Poggi}= Poggi´s discrete divergence index , n'_A number of bands of lane A absent in lane B, n'_B = number of bands of lane B absent in lane A, nA = total number of bands in lane A, nB = total number of bands in lane B.

The IJ ranges from 0 to 1 where 1 occurs when the community profiles are completely similar and 0 when the profiles are completely different; the Δ_{Poggi} varies between 0 and 1, where 0 indicates similar communities and 1 indicates completely different profiles (the same bands and number of bands). Both indices can be used for comparing richness of two different communities, or comparing the richness of a community with respect to time. The divergence index Δ_{Poggi} allows for the calculation of the turnover ratio of richness of a community with respect to time $\dot{\Delta}_{Poggi}$ that gives more insight on the richness stability [35, 36]. In effect, $\dot{\Delta}_{Poggi}$ is defined by Equation (3) below as the derivative of Δ_{Poggi} with respect to time.

$$\dot{\Delta}_{Poggi} = {}^{d(\Delta_{Poggi})}/_{dt} \quad (3)$$

The units of $\dot{\Delta}_{Poggi}$ are (1/time), i.e., (1/day or day^{-1}).

Thus, a low value of $\dot{\Delta}_{Poggi}$ indicates that the microbial community is relatively stable with respect to time. For instance, analysis of a microbial community in attached-growth bioreactors with this tool has shown very low values of $\dot{\Delta}_{Poggi}$. On the other hand, higher values of $\dot{\Delta}_{Poggi}$ are related to significant dynamic changes on community composition.

2.7 Metagenome sequencing using the Ion Torrent (PGM) Platform. Total genomic DNA of the collected samples was extracted using PowerSoil® DNA Isolation kit (MoBio) according to the manufacturer's instructions. The extracted DNA was amplified with the primers targeting V3 region of the 16S rDNA**.** The forward primer was V3-341F containing a 12bp Golay barcode [38] and adapters for massive sequencing, and antisense V3-518R containing A and Truncated P1 adapters [39]. The PCR mixture consisted of Buffer (1X), $MgCl_2$ (2 mM), dNTP´s (0.2 mM), Taq DNA polymerase (0.025U μL^{-1}), forward primer V3-341F (0.2µM), reverse primer V3-518R (0.2µM), and template DNA (10 ng). Amplification was performed in a GeneAmp PCR System 2700 Thermocycler (Applied Biosystems). The PCR amplification was performed with an initial denaturation (95°C for 5 min) followed by 30 cycles of denaturation (94°C for 15 s), annealing (62°C for 15 s) and extension (72°C for 15 s) and final extension (72°C for 10 min). The PCR products were purified by electrophoretic separation on 2% agarose gel and using Wizard SV Gen PCR Clean-Up System (Promega). The concentration of PCR amplicons were

measured by NanoDrop spectrophotometer (ThermoScientific). After that the samples were sent out for the barcoded libraries preparation and sequencing on an Ion Torrent PGM with 316 chip using the Ion Sequencing 200 bp kit (Life Technologies) according to the standard protocol (Ion Xpress_ Plus gDNA and Amplicon Library Preparation, Life technologies).

After sequencing, sequence reads were filtered by the PGM software to remove low quality and polyclonal sequences. During this process sequences matching the 3'-adapter were automatically trimmed and filtered. Sequenced data-Microbiota analysis Ion torrent PGM software, Torrent_Suite v 4.0.2 was used to demultiplex the sequenced data based on their barcodes and poor quality reads were eliminated from the datasets, i.e. quality score <20, containing homopolymers >6, length <200 nt, and containing errors in primers and barcodes. Filtered datas were exported as Fastq files. Demultiplexed sequencing data were analized using QIIME version 1.8.0 software pipeline [40]. Fastq files were converted into Fasta files, and all the demultiplexed files were concatenated into a single fasta file. Closed reference Operational taxonomic units (OTU) were determined at 97% similarity level with UCLUST Algorithm [41].

Chimeras were detected and removed from the datasets using the Chimera Slayer [42]. Sequence alignments were done against the Greengenes core set [43]. Statistical analysis of the OTU table Microbial diversity had been assessed through both alpha and beta diversity. Using rarefied OTU tables, alpha diversities were calculated using various matrices like Shannon, PD whole tree, chao1, observed species. The beta diversity analysis was calculated using UniFrac analysis [44], by phylogenetic tree computed with FastTree and a rarefiled biom table as inputs. Abundance of the bacterial groups at different taxonomic levels (phylum, order, and genus) was separately explored with a Principal Component Analysis (PCA) and Unweighted Pair Group Method with Arithmetic mean (UPGMA) Clustering.

2.7.1 Calculations of ecological indices. Shannon-Weaver diversity index has been a popular index in the ecological literature [45-46]. Shannon-Weaver index is defined as:

$$H' = -\sum_{i=1}^{S}(p_i \log p_i) \quad (4)$$

Where:

p_i= is the proportion of characters belonging to the i type of letter in the string of interest; yet, in ecology, p_i is often the proportion of individuals belonging to the i species in the dataset of interest, S is the total number of species.

Pielous evenness index is a measure of a biodiversity which quantifies how equal the community is numerically [46-47]. This index is defined as:

$$J' = \frac{H'}{\log S} \quad (5)$$

where:

H´ = is the Shannon-Weaver index
S = is the total number of species
J´ = is constrained between 0 and 1, 1 indicates máximum evenness or the equitative distribution of species.

It is worth emphasizing that the log in Equations (4) and (5) should be on base 2, since the ShannoneWeaver index was originated on the grounds of the theory of information that uses binary system of numbers. Yet, in the literature there is a variety of bases. Most researchers have chosen base 10, others have selected base e (natural logarithms) for the calculation of H´. Because of this, we distinguish and report here the índices H´2, H´e, and H´10 to indicate that the base of the logarithm is 2, e, and 10, respectively. The base of log S in the denominator of the Pielou index should be consistent with base of the log used in the calculation ShannoneWeaver index. Furthermore and interestingly, Pielou index is invariant with respect to the base of the logarithm, as long as the base of logs in the numerator and denominator are the same [45].

2.8 Lindane and metabolite analysis. The procedure for the extraction and determination of HCH and intermediate metabolites in the electrobiochemical reactor were performed according by Quintero *et al.* [48]. The lindane was analyzed in a Perkin Elmer gas chromatograph equipped with an electron capture detector. The intermediate metabolites were analyzed in a Perkin Elmer GC/MS [32].

3. Results and discussion

3.1 Desorption tests. Figure 2 shows the lindane desorbed at different initial concentrations of Tween 80 in the water phase. The maximum desorption effect was observed at a dose of 2000 mg L^{-1} of Tween 80, 9 mg of lindane removed kg^{-1} of dry soil. Because of this, we applied an initial concentration of 2000 mg L^{-1} of Tween 80 in our EBCR.

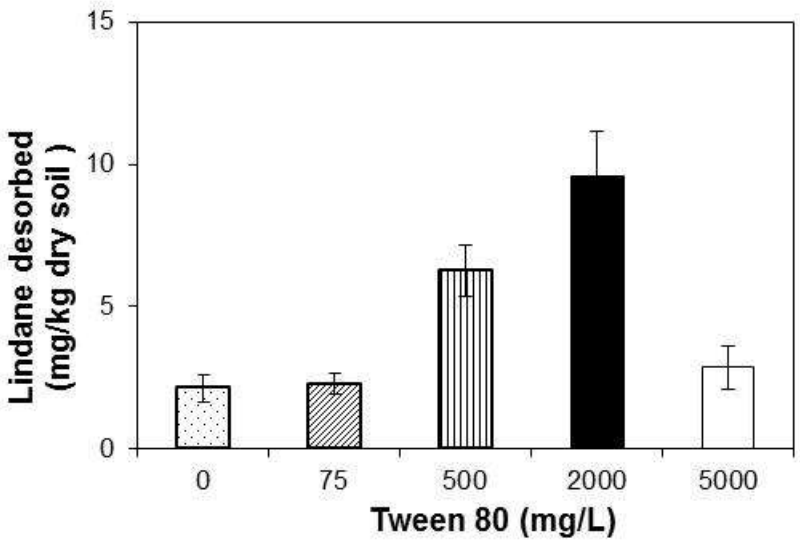

Fig 2. Lindane desorbed from the soil to the water phase. The initial concentration of lindane in soil was 100 mg kg^{-1} of dry soil.

3.2 Characterization of the electrobiochemical slurry reactor. The values obtained of internal resistance from the polarization curves method were, 5660, 6022, 12419 y 3845 Ω for face A, face B, connection in series and parallel, respectively, at time 0 day (Table 1). The maximum volumetric power was obtained when the connection was in parallel (60 mW m^{-3}) followed by connection in series, face A, and face B with 38, 12 and 11 mW m^{-3} respectively. After 7 days of operation another electrochemical characterization was carried out. The internal resistances decreased very much compared to those of the first characterization. Their values were approximately o 4652, 3867, 1041, 804 Ω for face A, face B, connection in series and connection in parallel, respectively (Table 1). The maximum volumetric power was obtained for parallel connection (373 mW m^{-3}, Table 1); it was sixfold the volumetric power obtained with characterization at 0 days.

Table 1. Values of several variables of electrobiochemical slurry reactor characterization at 0 and 7 day.

Parameter	**Face A**		**Face B**		**Series**		**Parallel**	
Time (days)	**0**	**7**	**0**	**7**	**0**	**7**	**0**	**7**
R_{int} (Ω)	5660	4652	6022	3687	12419	1041	3845	804
$P_{An\text{-}max}$ (mW m^{-2})	0.95	12.33	0.86	8.01	3.04	29.58	4.84	29.90
$P_{V\text{-}max}$ (mW m^{-3})	11.86	154	10.72	100	38.08	369	60.55	373
$I_{EBCR\text{-}max}$ (mA)	0.11	0.67	0.08	0.55	0.08	0.72	0.11	0.94
$E_{EBCR\text{-}max}$ (V)	0.31	0.57	0.32	0.58	0.50	0.60	0.54	0.59
$P_{EBCR\text{-}max}$ (mW)	0.003	0.05	0.001	0.03	0.01	0.11	0.02	0.12
$P_{An\text{-}ave}$ (mW m^{-2})	0.37	4.54	0.39	3.93	1.04	9.98	1.72	9.92
$P_{V\text{-}ave}$ (mW m^{-3})	4.57	56.82	4.86	49.20	13.01	124.79	21.46	123.96
$I_{EBCR\text{-}ave}$ (mA)	0.02	0.11	0.02	0.09	0.03	0.18	0.05	0.18
$E_{EBCR\text{-}ave}$ (V)	0.13	0.30	0.13	0.30	0.21	0.38	0.25	0.39
$P_{EBCR\text{-}ave}$ (mW)	0.001	0.02	0.001	0.02	0.004	0.04	0.007	0.04

Notes: 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. Subindices: max, maximum; ave, average.

The improved characteristics might be a consequence of the increased microbial activity resulting from enrichment of the biofilm on the anode [49].

Figure 3 shows the time course of the potential delivered by the device when the anodes and cathodes of the EBCR were connected in parallel; results of the average performance are exhibited in Table 2. The voltage with the EBCR in open circuit conditions (at the early 11 h) was approximately 666 mV (phase I). The voltage remained stable when the cell was operated with an external resistance of 3300 Ω, average 318 mV (first nineteen of phase II); however the voltage decreased to less than 160 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 820 Ω, the voltage remained stable (341 mV). The EBCR at 16 d was supplemented with 2 g L^{-1} substrate (sucrose: sodium acetate: lactate) and Tween 80 (2 000 mg L^{-1}) that was used as the fuel. The electricity generation began to increase and reached a voltage output of approximately 488 mV (Figure 3).

The maximum voltage output of the EBCR (580 mV) and maximum power (108 mW m^{-2}) were higher than those reported by Wang *et al*. [34] (155 mV and maximum surface power 0.85 mW m^{-2}) for a cell loaded with soil polluted with total petroleum hydrocarbons. Our results also compared very favorably to

those observed by Yan *et al*. [50] who reported a voltage as low as 17 mV in the treatment of sediment contaminated with phenanthrene and pyrene. On the other hand, Huang *et al*. [33] registered a power density *ca.* 30 mW m^{-2} and a lower voltage (150 mV) in the treatment of a waterlogged soil polluted with phenol.

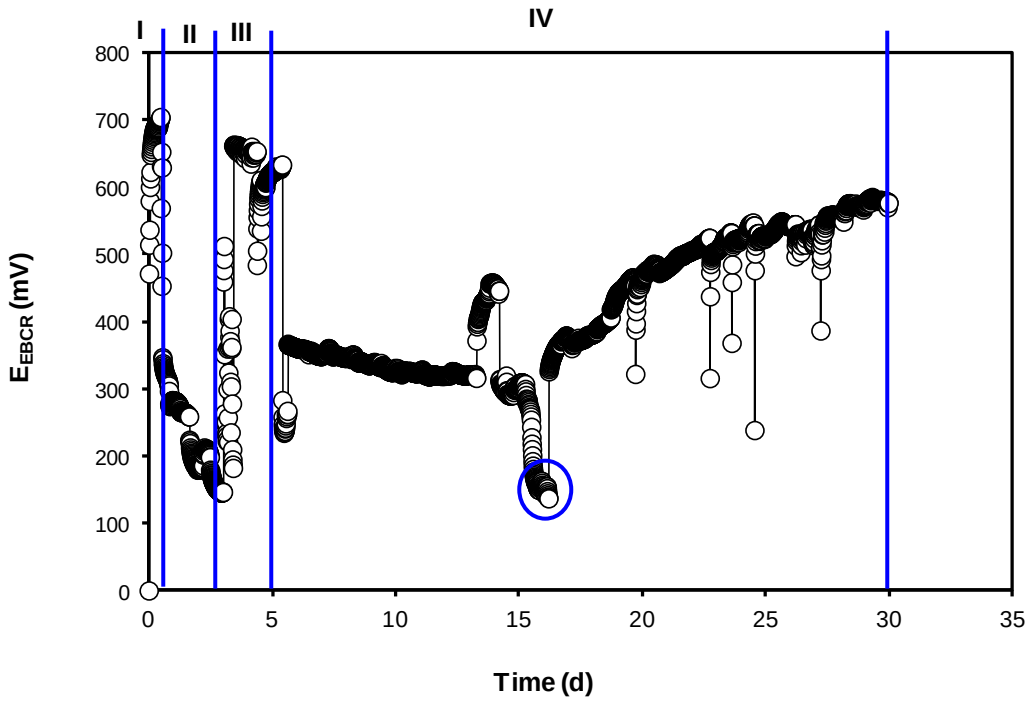

Fig 3. Electricity generation in electrobiochemical slurry reactor during batch operation for 30 d. The addition of substrate is indicated by the blue . Phase I, open circuit; phase II, closed circuit with external resistance 3300Ω; phase III, open circuit; phase IV, external resistance 820Ω.

Our EBCR exhibits a bonus besides soil remediation: the bioelectricity generation. The latter was estimated with Equation 1 below

$$\hat{E}_{harvested}\ (\text{MJ/tonne soil}) = P_{ave}\ (\text{W})*(30\ \text{d}*24\ \text{h/d}*3600\ \text{s/h})/(0.0001\ \text{tonne soil} * 1000000\ \text{J/MJ}) \quad (6)$$

where $\hat{E}_{harvested}$ is the energy produced in MJ tonne $soil^{-1}$, P_{ave} is the average power in 30 d of operation, 24 h d^{-1} and 3600 s h^{-1} are conversion factors for converting time from days to seconds; 0.0001 tonne soil is soil mass in the lab scale EBCR.

The $\hat{E}_{harvested}$ was estimated at 5.9 MJ $tonne^{-1}$ soil during 30 days of operation. This energy can partially offset 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. Indeed, typical power requirements for complete mixing are in the range 20 to 50 kW/1000 m^3 for moderately thick suspensions [51]; a mid-point value of 35 kW/1000m^3 was chosen. Performing the

calculations with similar assumptions to those of Equation 6, the energy required for mixing during the 30 d batch would be given by Equation 7 below

$\hat{E}_{mixing}$ = 35 (W/m^3)*(0.33 tonne soil/m^3)*(30 d*24 h/d*3600 s/h) = 29.9 MJ/tonne soil (7)

That is, the EBCR allows for a bioelectricity harvest that could represent *ca.* 20 % of the energy required for mixing.

Table 2. Average performance of electrobiochemical slurry reactor

Parameter	
$P_{An\text{-}max}$ (mW m^{-2})	108
$P_{V\text{-}max}$ (mW m^{-3})	1357
$_{EBCR\text{-}max}$ (V)	0.58
$I_{EBCR\text{-}max}$ (mA)	0.71
$P_{EBCR\text{-}max}$ (mW)	0.42
$P_{An\text{-}ave}$ (mW m^{-2})	54 ± 30
$P_{V\text{-}ave}$ (mW m^{-3})	685± 377
$E_{EBCR\text{-}ave}$ (V)	0.42± 0.12
$I_{EBCR\text{-}ave}$ (mA)	0.47 ± 0.17
$P_{EBCR\text{-}ave}$ (mW)	0.19 ± 0.02

Notes: P_{An}, surface area power density; P_V, volumetric power; E_{EBCR}, voltage; I_{EBCR}, current intensity; P_{EBCR}, power delivered. Subindices: max, máximum; ave, average. Standard desviation is with respect to time

3.3 Lindane removal and intermediate metabolites. Lindane removal achieved in the EBCR was 56 %, whereas the removals of the biotic (live) control and abiotic control EBCRs were 72 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), 1,4 dichlorobenzene (1,4-DCB), 1,2-dichlorobenzene (1,2-DCB), and chlorobenzene (CB) (Figure 4). Lindane removals observed in our EBCR compared similarly with lindane removals reported for standard slurry bioreactors in the literature. Okeke *et al*. [52] carried out experiments with SB inoculated with *Pandorea* sp., with a presumably anaerobic operation of 9 weeks duration. Initial lindane concentration was 100 mg kg^{-1}; they found removals of 59.6% γ-HCH.

Unexpectedly the $\eta_{lindane}$ in EBCR was lower than that in EBCR operated without surfactant, 78% [32]. This could be ascribed to the influence of increased degradable organic matter supply in the experiment. Quintero *et al*. [12] treated a sandy soil polluted with a mixture of isomers α, β, γ and δ-HCH (100 mg kg^{-1} 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, Robles-Gonzalez *et al*. [3] studied the bioremediation of a heavy soil polluted with 100 mg lindane kg^{-1} in full sulfate reducing SB. 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 also demonstrated that in methanogenic SBs the removal of lindane was between 41-47%. The SB was operated with similar soil operational conditions. In other studies of our Group, Varo-Arguello *et al*. [4] carried out experiments with SB in sulphate-reducing conditions; they reported removals of 78% γ-HCH in 30 days.

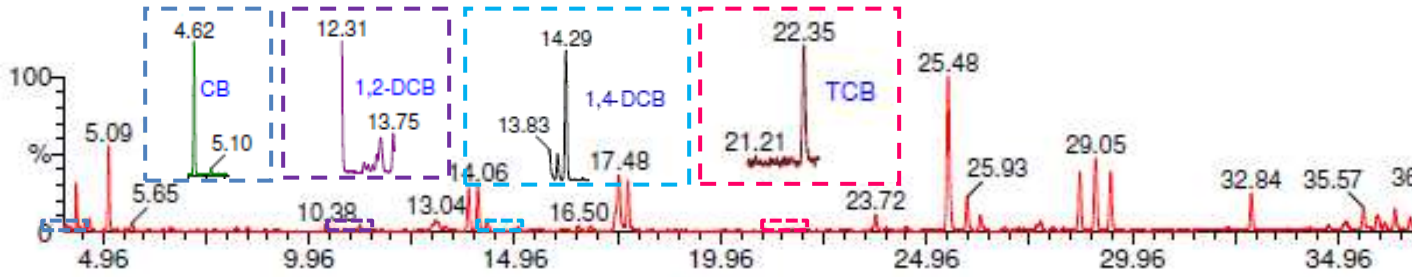

Fig 4. GC-MS detection of intermediate metabolites in electrobiochemical slurry reactor at the end of operation (30 d)

3.4. Microbial consortia characterization

3.4.1. Resuls of DGGE analysis

Results from the DGGE analysis (Figure 5) and further sequencing, indicate the presence of *Trichococcus palustris* (99% sequence identity, Band 11). This bacterium has been reported in autotrophic biocathodes of other bioelectrochemical systems [53]. *Trichococcus* (Firmicutes) also has been isolated of Arctic tundra soil and was identified as facultatively anaerobic bacterium [54].

On the other hand Freeman *et al*. [55] found *Trichococcus flocculiformis* as the dominant clone in the microflora of a sulfidogenic lab scale expanded granular sludge blanket bioreactor (mesophilic conditions, V = 2.9 l) fed with a simulated semiconductor manufacturing wastewater containing 425 mg L^{-1} isopropanol, 1,370 mg L^{-1} citric acid, and 615 mg L^{-1} polyethylene glicol, and divalent copper (Cu(II) . They further characterized this microbe as a fermentative organism in the low G-C Gram positive bacteria, originally isolated from anoxic digestor sludge [56]. *T. flocculiformis* could grow on citrate [57] and might have contributed to the effective removal of this substrate in the bioreactor.

In the case of DGGE analysis (Figure 5); twenty-four initial bands were found at the beginning of the experiment; this bands were constants at the 15 and 22 days of operation, at the end of experiment (30 days of operation) 9 bands dissappeared. On the other hand, at 22 days of operation the band 1 and 11 were more intense, when the EBCR exhibited the maximum voltage. The richness of eubacterial community was relatively stable during the 15 and 22 days of operation time, as reflected by high IJ values (0.71 and 0.77 at15d and 22d, respectively, Table 3) or alternatively, by low divergence index values (0.16 and 0.12 at15d and 22d, respectively). On the other hand, $\dot{\Delta}_{Poggi}$ (0.005) at 22 days of operation shown that the microbial community is relatively stable with respect to time.

Table 3. Summary of variation of richness of eubacterial communities in the bioreactor. The left number is the Jaccard index of similarity, the medium number is the divergence index, the right number is the $\dot{\Delta}_{Poggi}$

Operation time	15d	22d	30d
	Jaccard index/divergence index/$\dot{\Delta}_{Poggi}$	Jaccard index/divergence index/$\dot{\Delta}_{Poggi}$	Jaccard index/divergence index/$\dot{\Delta}_{Poggi}$

0d	0.71/0.16/0.01	0.77/0.12/0.005	0.39/0.43/0.01
15d		0.71/0.16/0.02	0.50/0.33/0.02
22d			0.44/0.38/0.04

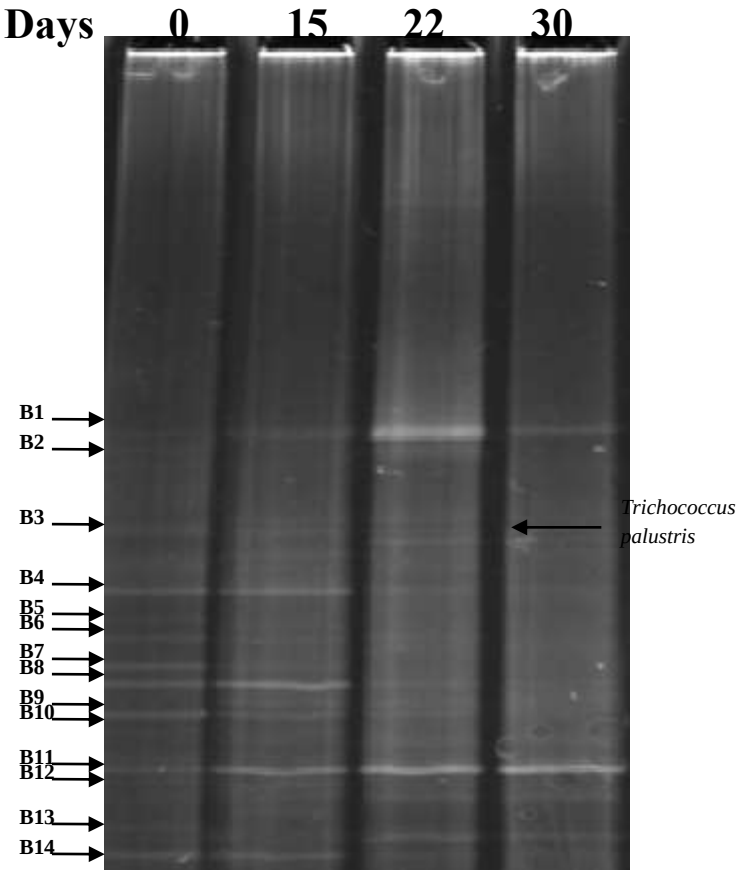

Fig 5. DGGE gels for microbial community monitoring for DGGE gels. eubacterial community. Bj: eubacterial bands

3.4.2. Results from massive semiconductor sequencing

Figure 6. shows the composition of bacterial population obtained from sulphate inoculum and EBCR at different days of operation. The more representative phylum were actinobacteria, bacteroidetes, firmicutes, proteobacteria and synergistetes.

Kim *et al*. [58] performed a phylogenetic analysis of microbial samples that revealed a diverse bacterial community consisting of Proteobacteria, Firmicutes, Bacteroidetes and Spirochaetes. The authors worked with a single MFC with air cathode membrane electrode assembly combined into duplicate two-module longitudinal tubular reactors. The reactor was inoculated with anaerobic digester sludge.

In our work, the most dominant genera belonged to *Arcobacter*, 40.8%, and *trichococcus* (41.6%) at 22 days of operation (Table 6), when the EBCR exhibited the maximum voltage. *Arcobacter* was reported to

harbor in electrodes of MFC, and rapidly generates a strong electronegative potential as a pure culture when it is supplied with acetate [59]. *Thrichococcus* has been reported in autotrophic biocathodes of other bioelectrochemical systems [53]. *Trichococcus* was identified as facultatively anaerobic bacteria by Kim *et al*. [54].

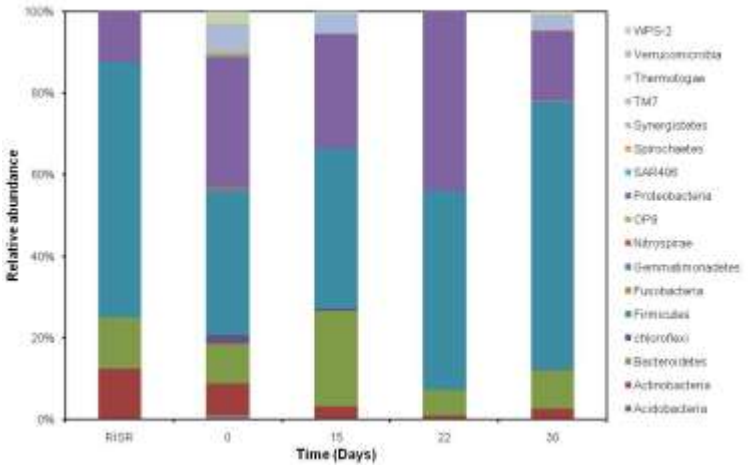

Fig 6. Phylum level identification by massive semiconductor sequencing of microbial community in our electrobiochemical slurry reactor.

3.4.3. Analysis of ecological indices

Shannon-Weaver index and Pielou´s indices for the sulphate reducing inoculum were 1.54 and 0.38 respectively (Table 5). Ortega-Martínez *et al.*[46] characterized a sulphate reducing inoculum, they reported 1.84 and 0.66 for Shannon-Weaver index and Pielou´s index respectively. In the case of the electrobiochemical slurry reactor , Shannon-Weaver index was 2.42, 2.00, 1.33, and 1.39 (Table 5) for 0, 15 , 22, and 30 days of operation respectively. The Pielou´s index for electrobiochemical slurry reactor was 0.59, 0.49, 0.32, and 0.39 (Table 5) for 0, 15, 22, and 30 days of operation respectively. The above results concluded that the different distributions of species were identified in the electrobiochemical slurry reactor at diferents days of operation.

Table 5. Ecological indices of the electrobiochemical slurry reactor based on phylum

Sample	**Shannon-Weaver index based on phylum**			**Pielou´s index based on phylum**
	H'_e [a]	**H'_{10} [b]**	**H'_2 [c]**	**J´**
SR-In [d]	1.07	0.46	1.54	0.38
0d [e]	1.68	0.73	2.42	0.59
15d [e]	1.39	0.60	2.00	0.49
22d [e]	0.92	0.40	1.33	0.32
30d [e]	1.10	0.48	1.59	0.39

Notes: [a], calculated with logarithm base $_e$; [b], calculated with logarithm base $_{10}$; [c], calculated with logarithm base $_2$; [d], sulphate reducing inoculum; [e]. operation time

Table 6. Genus level identification of all the sequences (Only relative abundances of identified Genus higher than 1 % are listed and all the other sequences are included in "others")

Genus/Time (Days)	SR In	0	15	22	30
Actinobacteria Acidimicrobiia Acidimicrobiales	0	1.3	0.6	0.2	0.3
Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae __N09	0	1.2	0.6	0.2	0.9
Actinobacteria Actinobacteria Actinomycetales Nocardioidaceae Propionicimonas	6.2	0	0.1	0	0.1
Actinobacteria Actinobacteria Actinomycetales Propionibacteriaceae	6.2	0.2	0	0	0.2
Bacteroidetes Bacteroidia Bacteroidales	0	4.2	17.6	0.3	1.7
Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae	0	0.9	3.5	5.4	4.9
Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella	6.2	2.1	0.4	0	0.8
Bacteroidetes Bacteroidia Bacteroidales Rikenellaceae Blvii28	6.2	0.1	0.5	0	0
Firmicutes Bacilli Bacillales Bacillaceae Bacillus	6.2	2	1.9	0.6	0.8
Firmicutes Bacilli Lactobacillales Carnobacteriaceae Trichococcus	6.2	1.9	7.4	41.6	45
Firmicutes Bacilli Lactobacillales Lactobacillaceae Lactobacillus	0	1.6	0	0	0.5
Firmicutes Bacilli Lactobacillales Streptococcaceae Lactococcus	6.2	0.2	0	0	0.1
Firmicutes Clostridia Clostridiales	12.5	0.8	3.2	0.3	1.4
Firmicutes Clostridia Clostridiales Clostridiaceae	0	1.3	0.6	0.6	1
Firmicutes Clostridia Clostridiales Clostridiaceae Clostridium	0	8.8	3.9	1.1	3.9
Firmicutes Clostridia Clostridiales Lachnospiraceae	6.2	1.9	6.9	0.1	0.7
Firmicutes Clostridia Clostridiales Lachnospiraceae Anaerostipes	6.2	0	0	0	0
Firmicutes Clostridia Clostridiales Ruminococcaceae	0	1.8	2.9	0.6	1.6
Firmicutes Clostridia Clostridiales Ruminococcaceae Ethanoligenens	0	1.8	0	0	0.3
Firmicutes Clostridia Clostridiales Ruminococcaceae Faecalibacterium	12.5	1.5	0.5	0	0.6
Firmicutes Clostridia Clostridiales Ruminococcaceae Oscillospira	6.2	0.4	0.2	0.2	0.4
Firmicutes Clostridia Clostridiales [Mogibacteriaceae] Anaerovorax	0	0.2	1.6	0.3	0.8
Firmicutes Clostridia Clostridiales [Tissierellaceae] Sedimentibacter	0	0.1	3.5	1	1.2
Proteobacteria Alphaproteobacteria Sphingomonadales Sphingomonadaceae	0	0.2	1.1	0	0
Proteobacteria Alphaproteobacteria Sphingomonadales Sphingomonadaceae Sphingomonas	0	0.1	1.8	0	0
Proteobacteria Deltaproteobacteria Desulfovibrionales Desulfovibrionaceae Desulfovibrio	0	2	0.6	0.1	0.7
Proteobacteria Deltaproteobacteria Desulfuromonadales Pelobacteraceae	0	0.1	1.3	0.1	0.2
Proteobacteria Epsilonproteobacteria Campylobacterales Campylobacteraceae Arcobacter	0	0.2	1	40.8	9.7
Proteobacteria Epsilonproteobacteria Campylobacterales Helicobacteraceae	0	2.3	6.2	0.8	0.5
Proteobacteria Gammaproteobacteria Enterobacteriales Enterobacteriaceae	6.2	6.1	3.6	0.1	0.8
Proteobacteria Gammaproteobacteria Enterobacteriales Enterobacteriaceae Enterobacter	0	1.5	2.1	0	0.2
Proteobacteria Gammaproteobacteria Pasteurellales Pasteurellaceae Haemophilus	6.2	0	0	0	0
Proteobacteria Gammaproteobacteria Thiotrichales Piscirickettsiaceae Thioalkalimicrobium	0	2.1	0	0	0.1
Synergistetes Synergistia Synergistales Dethiosulfovibrionaceae HA73	0	1.7	1.9	0	1.8
Synergistetes Synergistia Synergistales Thermovirgaceae	0	4.7	2.4	0	0.1
Thermotogae Thermotogae Thermotogales Thermotogaceae Kosmotoga	0	2.8	0.5	0	0.7
Others	0.6	41.9	21.6	5.6	18

CONCLUSION

- A dose of 2000 mg L^{-1} Tween 80 exhibited the best desorption of lindane from soil to water phase, although its absolute value was low-to-moderate
- 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, methanogenic inoculum, as well as other conventional slurry bioreactors.
- The EBCR not only provided bioremediation of a toxic, recalcitrant organo-chlorinated pesticide, but also supplied (as bioelectricity) *ca.* 20 % of the energy required for mixing the device.
- The results of bacterial community analysis of the electrobiochemical slurry reactor, the phylum more representative were actinobaceria, bacteroidetes, firmicutes, proteobacteria, and synergistetes.

Thus, the EBCR is a significant step towards the green/sustainable remediation of contaminated soils.

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Abbreviations and Acronyms

sCOD	soluble 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
HCH	Hexachlorocyclohexane
I_{EBQR}	Current intensity
Max	Maximum
MFC	Microbial Fuel Cell
NOM	Natural organic matter
P_{An}	Surface area power density
P_{ave}	average power
P_{EBCR}	Power delivered
P_V	Volumetric power
R_{int}	Internal resistance
SMFC	Soil microbial fuel cell technology
SB	Slurry reactors
SR	Sulphate reducing

Greek characters

η_{coul}	Coulombic efficiency
η_{COD}	Removal efficiency of organic matter as chemical oxygen demand
$\eta_{Lindane}$	Removal efficiency of lindane