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# A Microbial Fuel Cell Equipped with a Denitrifying Biocathode Effectively Degrades the Toxic Carbon Tetrachloride

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**ABSTRACT**

The aim of this work was to design and operate a MFC equipped with a denitrifying biocathode (MFC-BIO-DN) in the perspective of carbon tetrachloride reduction from polluted effluents.
The MFC-BIO-DN consisted of two plexiglass cubic chambers of 3 cm filled with small graphite cubes (3 mm side length). The anode and cathode compartments were separated by a proton exchange membrane (Nafion 117). The anode chamber was loaded with 12 mL of enriched inoculum, E-In that was cultured in an acetate-ferric citrate medium. The cathode chamber was seeded with a mixed culture of denitrifying bacteria. A synthetic wastewater containing potassium buffer salts, $Na_2CO_3$, and $KNO_3$ was fed to the cathode chamber. The CT concentration within the cathode of the MFC-DN was 10 mg/L.
In the characterization experiment, the internal resistance $R_{int}$ of MFC –DN was 1450 Ω with a maximum cell voltage of 230 mV. The values of $P_v$ and $P_S$ of the MFC were 772 mW/m$^3$ and 24 mW/m$^2$, respectively, where $P_S$ is the power density expressed on the basis of projected surface area of membrane. During the batch operation the average voltage of the MFC-DN was 132 mV, whereas average $P_v$ and $P_S$ were 11 620 mW/m$^3$ and 35 mW/m$^2$. A nearly 100% CT depletion in the cathodic liquor was observed in the first 12 h of operation, which was equivalent to a removal rate higher than 20 mg CT/(L.d). Abiotic removal of CT by sorption to the electrode graphite cubes (anodic and cahtodic) was negligible as determined by headspace GC-FID. We can conclude that a MFC-DN shows promise for the bioelectrochemical remediation of waters polluted with CT.

*Keywords: Biocathode, Carbon Tetrachloride, Microbial Fuel Cell*

## 1. Introduction

Chlorinated hydrocarbons such as carbon tetrachloride and chloroform have been widely used in large quantities for different industrial and domestic purpose [1]. Carbon tetrachloride (CT) is a volatile chlorinated solvent, which has been used widely over decades as an industrial degreasing agent, as a pesticide, for dry cleaning and in fire extinguishers [2]. It is toxic and predicted to be carcinogenic, with deleterious effects on stratospheric ozone. As a consequence, commercial production and use of has been progressively restricted. Its use as pesticide and grain fumigant was banned in 1986 [3]. Currently, CT is still produced, but only as a intermediate in the production of other chemical compounds. With an estimated half-life for abiotic hydrolysis of 7000 years in water at 20°, CT is highly persistent in the environment compared with other halogenated aliphatic compounds [4]. The low water solubility of CT leads to its accumulation in subsurface aquifers as a poorly bioavailable, dense non-aqueous-phase liquid, which only dissolves very slowly into groundwater [5]. Degradation or transformation of CT, is the other major source of toxicity of the compound, as some dechlorination pathways generate toxic intermediates and products. This mainly seems to be due to

intracellular CT transformation of reactive radicals that, by promoting nonspecific oxidation, can detrimentally affect and inactivate key cellular components [6].

CT has been shown susceptible to degradation in anaerobic environments by both biotic and abiotic mechanisms. Much of the research on the microbial transformation of CT has focused on the roles of methanogenic, sulphate-reducing, and nitrate-reducing bacteria [7]. Denitrifying conditions do not produce any secondary pollutant, and denitrifying bacteria grow much faster and can a wider range of substrate, including some reductive dechlorination products [8]. There is a need to remove low-level CT concentrations from contaminated drinking water supplies, as well as a high-level CT concentration from superfund sites and industrial wastewaters, because of the risks posed by CT to human health and the environment [9]. While a number of bioreactors have been developed for CT reduction [10], reduction using a biologically active cathode (biocathode) within a microbial fuel cell (MFC) is a novel and potentially cost-effective approach. Biocathodes harness the capacity of specific microorganims to accept electrons from a solid surface (cathode) [11]. High-rate oxygen reduction without a platinum catalyst and denitrification have been accomplished using biocathodes [12-13], as well as reduction of chlorinated groundwater pollutants [11,14]. An advantage of this process is the opportunity for electrical energy recovery from the treatment process [15].

Therefore, the objective of this work was to design and operated a MFC equipped with a denitrifying biocathode (MFC-BIO-DN) in the perspective of carbon tetrachloride reduction from polluted effluents.

## 2. Materials and methods

### *2.1 Microbial fuel cell equipped with a denitrifying biocathode.*

The MFC fitted with a denitrifying biocathode consisted of two plexiglass cubic chambers of 3 cm (Fig. 1). Each electrode compartment was filled with small graphite cubes (3 mm x 3mm) and they had a 27 mL geometric volume, net volume of 15 mL discounting the volume of the electrode material. External contact to the electrodes was made via graphite rods inserted into the anode and cathode chambers.

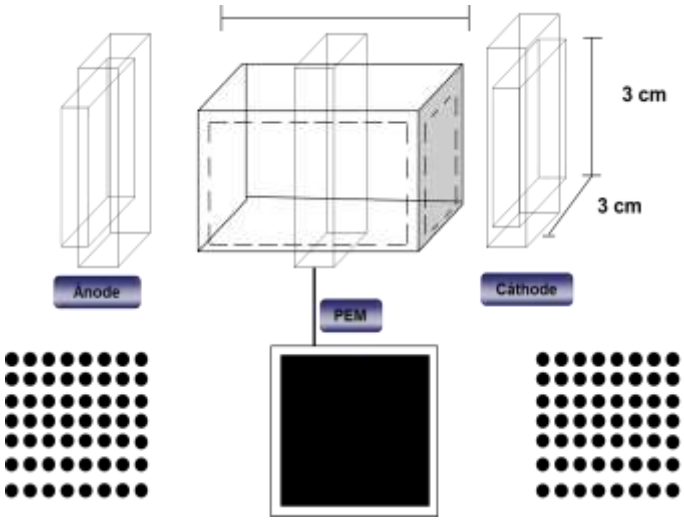


Fig. 1. Isometric view of the lab scale microbial fuel cell equipped with biocathode MFC-BIO.

The anode and cathode compartments were separated by a proton exchange membrane (Nafion 117). The anode chamber was loaded with 12 mL of biocatalyst (E-In), whereas the cathodic chamber received a denitrifying inoculum previously acclimated to carbon tetrachloride. Initial COD and biomass concentrations in the anode liquor of the cell were *ca.* 950 mg $O_2$/L and 800 mg VSS/L, respectively. The cathode chamber was seeded with a mixed culture of denitrifying bacteria sampled from a denitrifying lab-scale reactor acclimated to 6 mg/L CT. The synthetic wastewater fed to the cathodic chamber contained (in g/L) 6.8 of $K_2HPO_4$. 8.7 of $KH_2PO_4$, 2.0 of $Na_2CO_3$, finally 6.0 of $KNO_3$. The initial CT concentration within the cathode of the MFC-BIO-DN was 10 mg/L.

*2.2 Enriched inocula*

The E-In for the anodic chamber of the MFC-BIO-DN was obtained with serial transfers. A sediment sample was suspended in nitrogen filled pressure tubes containing media with ferric citrate (55 mM) as electron acceptor and sodium acetate (2 M) as electron donor. The tubes were incubated at 30°C for 7 days in the dark. The enrichment procedure was repeated 3 times [16].

The bioreactor with E-In had an operation volume of 1.5 L was operated at 35°C in a constant temperature room. The bioreactor was fed at a flow rate of 75 mL/d an influent whose composition was (in g/L): Sodium acetate (2.0), $NaHCO_3$ (1.8), $Na_2CO_3$ (0.5), $Na_2SeO_4$ (0.1).

The biocatalyst used in the cathodic chamber was sampled from a denitrifying complete mix bioreactor that was acclimated to 6 mg/L carbon tetrachloride in the influent [17].

*2.3 Analyses*

Polarization curves. The main conditions in both chambers of the MFC-BIO used in the characterization studies are shown in Table 1. The internal resistance of cells was determined using the polarization curve method, by varying the external resistance ($R_{ext}$) and monitoring both the voltage and the current intensity, according to procedures suggested by [14,18]. MFC was batch-operated for 8 h at room temperature. The circuit of the MFCs were fitted with an external variable resistance. In this regard, we carried out the polarization curve of the MFC, relating mathematically the cell voltage ($E_{MFC}$) and current intensity ($I_{MFC}$) against the $R_{ext}$ value, forwards and backwards regarding the $R_{ext}$ values. *Ab initio*, the MFCs were operated at open circuit for 1 h. Afterwards, the $R_{ext}$ was varied from 100 Ω to 100 KΩ and backwards. After this, the cell was set to open circuit conditions for 1 h in order to check the adequacy of the procedure (values of initial and final open circuit voltages should be close). The voltage was measured and recorded with a multimeter. The current was calculated by the Ohm's law [19].

Analytical methods and calculations. The COD and VSS of the liquor of iron-reducing seed bioreactor and cell were determined according to the Standard Methods [20]. The current intensity ($I_{MFC}$), the power ($P_{MFC}$) and the power density ($P_S$) were determined according to the [19].

The power per unit volume or volumetric power ($P_V$) was calculated as follows:

$$P_V = \frac{E^2_{MFC}}{V_{MFC} \cdot R_{ext}} \quad (1)$$

where: $R_{ext}$ = the external resistance, $E_{MFC}$ = the voltage, and $V_{MFC}$ = the cell volume.

Table 1. Conditions for the characterization experiments by polarization curve method

| | Anode | Cathode |
|---|---|---|
| **Biocatalyst** | Enriched inoculum (iron-reducing bacteria) | Denitrifying inoculum |
| **Substrate** | 2 M sodium acetate | None |
| **Toxic compound** | None | $CCl_4$ (10 mg/L) |
| **Electron acceptor** | None | Nitrate |

*2.4 Determination of Fe(III) reduction*

It is known that the ferric ion ($Fe^{3+}$) can serve as an exogenous electron acceptor during microbial respiration (dissimilatory Fe(III) reduction) [21-24]. Ferric ion-respiring microorganisms are diverse and some of them have a biotechnological interest because of their potential role in electricity production in microbial fuel cells (MFCs) where the terminal acceptor of the electrons during anaerobic respiration is not a ferric ion or different metal but the anode [22]. We used ferric citrate in order to enrich the metal-reducers from the initial consortium. Fe(III) reduction activity was determined using a previous method [25]. A half mL of culture was aseptically sampled with a syringe and mixed with 1 mL HCl solution (0.5 N). This mixture was reacted for 15 min. at room temperature, and then centrifuged for 5 min. The mixture of supernatant (0.1 mL) and ferrozine solution (1mL, 1 g/L in 50 mM HEPES buffer) was reacted for 15 min before measurement of optical density at 562 nm using a spectrophotometer. The fresh medium was used as a control sample instead of culture. Ferrous ethylenediammonium sulfate tetrahydrate ($C_2H_{10}N_2O_4SFeSO_44H_2O$) was used to make a standard Fe(II) solution [23].

An enrichment factor ε was defined as follows:

$$\varepsilon \text{ (mM/d)} = ([Fe^{+2}]_{final} - [Fe^{+2}]_{initial})/t_{lag} \quad (2)$$

where $[Fe^{+2}]_{final}$ is the final concentration of Fe (II) in a given transfer, $[Fe^{+2}]_{initial}$ is the initial concentration of Fe (II) in the

transfer (both concentrations in mM), and $t_{lag}$ is the lag time (in d) of Fe(II) appearance in the transfer. Please note that in spite that $\varepsilon$ seems to have units of concentration rate mM/d, it is not a true rate because the denominator is the lag time of the given culture [26].

*2.4 Determination of carbon tetrachloride*

Carbon tetrachloride concentration in the influent and liquors was determined by gas chromatography in /(GC-FID Perkin Elmer 9000)/ flame ionization analysis of a 0.5 mL sample taken from the 60 mL headspace of the serum bottles, as previous described [27-28].

## 3. Results and discussion

*3.1 Cell characterization*

In the characterization experiment, the $R_{int}$ of MFC-BIO was 1450 Ω. The values of $R_{int}$ was calculated as the slopes of the sets of aligned points of the corresponding polarization curve. The maximum cell voltage was of 230 mV (Table 2, Fig. 2). This relatively high value of $R_{int}$ is typical of two-chamber MFCs (Lefebvre *et al.* 2008). The maximum values of $P_v$ and $P_S$ of the MFC were 772 mW/m$^3$ and 24 mW/m$^2$, respectively, where $P_S$ is the power density expressed on the basis of projected surface area of membrane.

Our results seem to be in agreement with other works that report high values of $R_{int}$. For instance Lefebvre *et al.*, 2008 [17] designed a type of two-chambered microbial fuel cell wherein an autotrophic denitrifying biofilm. This two-chambered MFC consisted of two acrylic cubic chambers separated with a PEM made of Nafion. On each side of the PEM, two electrodes consisting of non-wet-proof carbon paper. The volume of each chamber was 125 cm$^3$. Two-chambered MFC equipped with a biocathode generated a $R_{int}$ of 5000 Ω. The maximum values of $P_v$ and $P_S$ of the two-chambered MFC were 190 mW/m$^3$ and 9.7 mW/m$^2$, respectively.

In our work the characteristics parameters of MFC-BIO were higher than two-chambered MFC, this could be related to the electrodes materials. MFC-BIO electrodes were small graphite cubes, this cubes filled each compartment of MFC. On the other hand, the two-chambered MFC was equipped with non-wet-proof carbon paper.

*3.2 Enrichment of inoculum.*

Fig. 3 shows the results of the serial transfers procedure for inoculum enrichment. A concentration of 12 mM $Fe^{+2}$ was achieved on day 4 in the first stage inoculation, whereas this concentration in the other stages is achieved on the second day or shorter.

Table 2 shows $Fe^{+2}$ initial and final concentration in serial transfers procedure. We could achieve a 43.7 mM $Fe^{+2}$ final concentration in the last transfer, this value is more than twice what was obtained in the final inoculation stage. Increase of the enrichment factor in subsequent transfers (Table 3) was an evidence that the enrichment procedure was successful.

Our results were similar to those reported by Hyun *et al*. (1999) [16] who isolated a Fe(III)-reducer from the natural environment. Soil samples were collected from various paddy fields and enriched with ferric citrate as a source of Fe(III) under anaerobic conditions. The final enriched culture showed the highest Fe(III)-reduction activity. Bacterial growth was coupled with oxidation of lactate and pyruvate to Fe(III)-reduction, final concentration $Fe^{+2}$ was 19.23 mM and 23.19, respectively, their results were obtained after 4 days of incubation.

Table 2. Values of selected parameters in the characterization studies of the MFC-BIO[a].

| Parameter | MFC-BIO |
|---|---|
| $R_{int}$[b] (Ω) | 1453 ± 250 |
| $P_s$[c] (mW/m$^2$) | 24 ± 2 |
| $P_{V\text{-}max}$[d] (mW/m$^2$) | 772 ± 120 |
| $E_{MFC\text{-}max}$[e] (V) | 0.230 ± 0.08 |
| $I_{MFC.max}$[f] (mA) | 0.141 ± 0.05 |
| $P_{MFC.max}$[g] (mW) | 0.013 ± 0.003 |

Notes: [a]Microbial fuel cell equipped with a biocathode, [b]Internal resistance; [c]Maximun power density expressed on the basis of projected surface area of membrane; [d]Maximum volumetric power; [e]Maximum voltage; [f]Maximum current intensity, [g]Maximum power output.

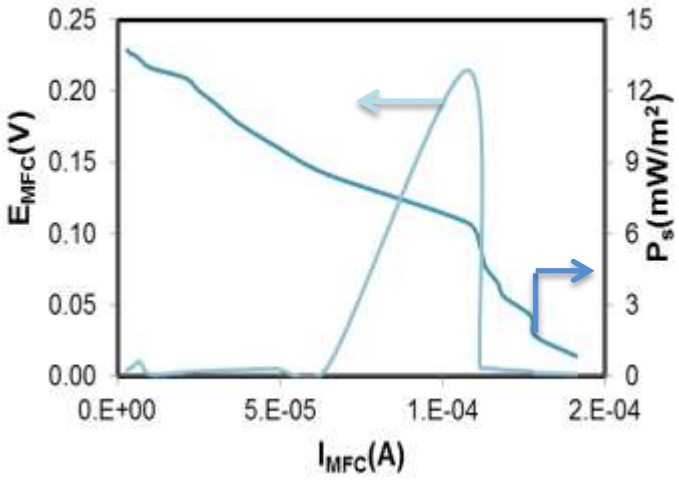


Fig. 2. Electric potential variation in the variable resistance procedure for cell characterization and power with respect to cell current intensity.

Table 3. Results of the enrichment procedure in the serial transfers: Concentration of $Fe^{+2}$ during serial transfer procedure

| Stage | Initial $Fe^{+2}$ (mM) | Final $Fe^{+2}$ (mM) | ε (mM/d) |
|---|---|---|---|
| **Inoculation** | 2.4 ± 0.01 | 19.0 ± 0.8 | 5.5 |
| **First transfer** | 4.3 ± 1.61 | 35.4 ± 3.6 | 31.1 |
| **Second transfer** | 4.3 ± 0.16 | 36.3 ± 3.1 | 45.7 |
| **Third transfer** | 4.3 ± 0.10 | 43.7 ± 2.3 | 56.3 |

*3.3 Batch operation of the cell with denitrifying biocathode and 10 mg/L carbon tetrachloride in the cathodic chamber.*

For the batch operation, the MFC-BIO was connected to an external resistance of 100 Ω because when connected to 1500 Ω the cell potential drastically decreased to 32 mV. During the batch operation the average voltage was 132 mV, whereas average $P_v$ and $P_S$ were 11 620 mW/m$^3$ and 35 mW/m$^2$ (Fig. 4, Table 3).

A 92% CT depletion in the cathodic liquor was observed in the first 12 h of the run. This corresponded to a volumetric removal rate higher than 18 mg CT/(L.d). Abiotic removal of CT by sorption to the electrode graphite cubes in the cathodic chamber was negligible as determined by headspace GC-FID. We can conclude that a MFC fitted with a denitrifying biocathode holds promise for the bioelectrochemical remediation of effluents contaminated with CT.

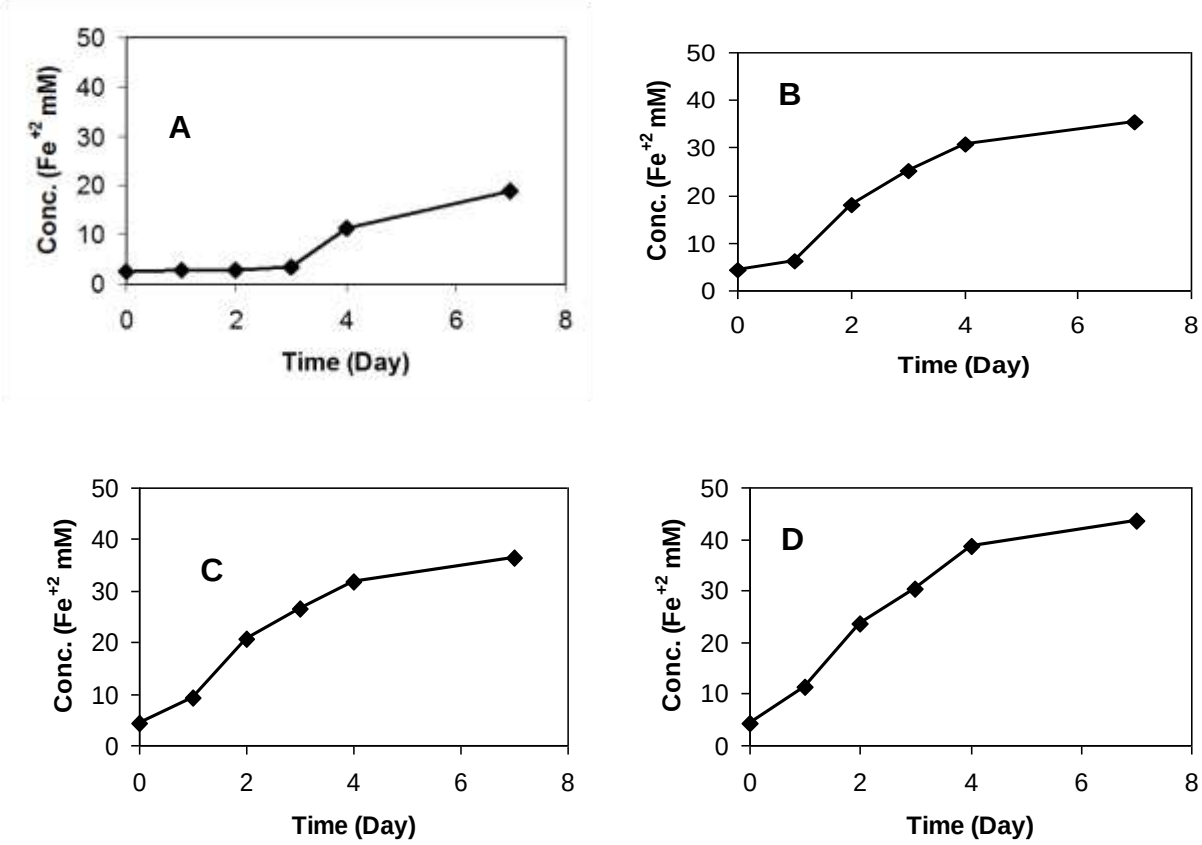


Fig. 3. Serial transfers procedure. (A) Inoculation, (B) First Transfer, (C) Second Transfer, and (D) Third Transfer.

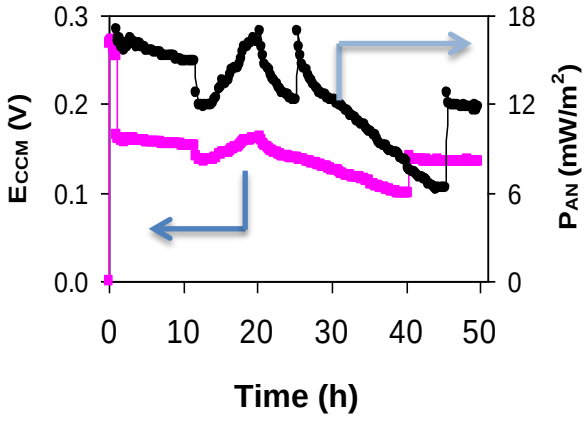


Fig. 4. Time course of cell potential and power density during the batch operation of the MFC-BIO-DN with carbon tetrachloride in the cathodic chamber. An external resistance of 100 ohms was used.

Table 3. Average results from the batch run of the MFC-BIO-DN with carbon tetrachloride in the cathodic chamber.

| Parameter | MFC-BIO[a] |
|---|---|
| $R_{ext}$ [b] (Ω) | 100 |
| $P_s$ [c] (mW/m$^2$) | 35 ± 1.4 |
| $P_{V\text{-}max}$ [d] (mW/m$^2$) | 11620 ± 136.5 |
| $E_{MFC\text{-}max}$ [e] (V) | 0.132 ± 0.0008 |
| $I_{MFC.max}$ [f] (mA) | 1.32 ± 0.008 |
| $\eta_{CCl4}$ [g] (%) | 92 |
| $\eta_{NO3.}$ [h] (%) | 90 |
| $\eta_{COD.}$ [i] (%) | 87 |
| $\eta_{Coul}$ [j] (%) | 24 |

Notes: [a]Microbial fuel cell equipped with a biocathode, [b]External resistance; [c]Maximun power density expressed on the basis of projected surface area of membrane; [d]Maximum volumetric power; [e]Maximum voltage; [f]Maximum current intensity, [g]Removal efficiency of $CCl_4$, [h]Removal efficiency of $NO_3^-$, [i]Chemical oxygen demand removal efficiency; [j] Coulombic efficiency .

## 4. Conclusion

In the characterization studies performed with the variable resistance method, the $R_{int}$ of MFC-BIO was 1450 Ω with a maximum cell voltage of 230 mV. The values of $P_v$ and $P_S$ of the MFC-BIO were 772 mW/m$^3$ and 24 mW/m$^2$, respectively.

During the batch operation the average voltage was 132 mV, whereas average $P_v$ and $P_S$ were 11 620 mW/m$^3$ and 35 mW/m$^2$. A 92% CT removal (from an initial 10 mg CT/L) in the cathodic liquor was observed in the first 12 h of the run, which was equivalent to a removal rate higher than 18 mg CT/(L.d). Also, a high removal of nitrate was achieved in the cathodic chamber, as high as 90%.

We can conclude that a MFC fitted with a denitrifying biocathode holds promise for the bioelectrochemical bioremediation of effluents contaminated with CT.

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**Notation**

| | |
|---|---|
| CT | carbon tetrachloride |
| E-In | enriched inoculum |
| MFC | microbial fuel cell |
| MFC-BIO-DN | microbial fuel cell equipped with a denitrifying biocathode |
| $P_S$ | power density per unit area of electrode |
| $P_v$ | volumetric power |
| $R_{ext}$ | external resistance |
| $R_{int}$ | internal resistance |

*Greek characters*

| | |
|---|---|
| ε | enrichment factor |
| $\eta_{CCl4}$ | carbon tetrachloride removal efficiency |

| | |
|---|---|
| $\eta_{coul}$ | coulombic efficiency |
| $\eta_{NO3}$ | nitrate removal efficiency; $\eta_{COD}$, organic matter removal efficiency, as COD; |

*Subindices*

| | |
|---|---|
| ave | average |
| CCM | microbial fuel cell |
| max | maximum |
| MFC | microbial fuel cell |