



Bioelectrochemical BTEX removal at different voltages: assessment of the degradation and characterization of the microbial communities



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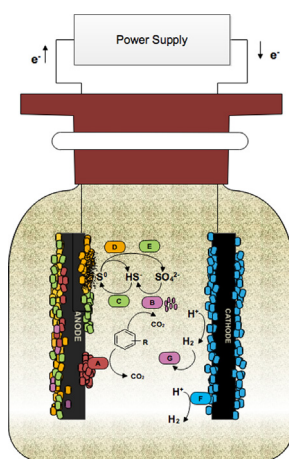
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HIGHLIGHTS

- BTEX removal was stimulated in bioelectrochemical systems at different voltages.
- The best performances were observed when 0.8 V were applied between the electrodes.
- The family *Desulfobulbaceae* was enriched on the anodes.
- The family *Desulfomicrobiaceae* dominated the communities on the cathodes.
- Sulfur and hydrogen cycling may have influenced the performances of the reactors.

GRAPHICAL ABSTRACT



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ABSTRACT

BTEX compounds (Benzene, Toluene, Ethylbenzene and Xylenes) are toxic hydrocarbons that can be found in groundwater due to accidental spills. Bioelectrochemical systems (BES) are an innovative technology to stimulate the anaerobic degradation of hydrocarbons. In this work, single chamber BESs were used to assess the degradation of a BTEX mixture at different applied voltages (0.8 V, 1.0 V, 1.2 V) between the electrodes. Hydrocarbon degradation was linked to current production and to sulfate reduction, at all the tested potentials. The highest current densities (about 200 mA/m² with a maximum peak at 480 mA/m²) were observed when 0.8 V were applied. The application of an external voltage increased the removal of toluene, *m*-xylene and *p*-xylene. The highest removal rate constants at 0.8 V were: 0.4 ± 0.1 days⁻¹, 0.34 ± 0.09 days⁻¹ and 0.16 ± 0.02 days⁻¹, respectively.

At the end of the experiment, the microbial communities were characterized by high throughput sequencing of the 16S rRNA gene. Microorganisms belonging to the families *Desulfobulbaceae*, *Desulfuromonadaceae* and *Geobacteraceae* were enriched on the anodes suggesting that both direct electron

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transfer and sulfur cycling occurred. The cathodic communities were dominated by the family *Desulfomicrobiaceae* that may be involved in hydrogen production.

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1. Introduction

Due to accidental spills BTEX compounds (Benzene, Toluene, Ethylbenzene and Xylenes) can contaminate soil and water, raising concern because they are recalcitrant, toxic for human health and relatively mobile in water [1].

Chemical, physical and biological strategies (bioremediation) are currently used for the remediation of BTEX contaminated environments [2]. Bioremediation relies on the ability of different groups of microorganisms to degrade organic contaminants which are used as a source of energy and carbon [3]. The goal of the biological technologies is to overcome the limitations to the microbial activity and to stimulate the biodegradation of the contaminants [3]. This can be achieved by supplying oxygen or other water soluble electron acceptors but it can be, in some cases, expensive and technically difficult [4,5]. Indeed, oxygen is rapidly depleted in groundwater and could diffuse away from the contaminated area [3]. Moreover, oxygen can react with reduced compounds such as Fe^{2+} and Mn^{2+} , which are normally present in hydrocarbon contaminated groundwater [6]. The limitations of the aerobic approaches for the stimulation of the microbial degradation may be overcome by developing new technologies. A strategy can be the use of bioelectrochemical systems (BESs) [7]. A BES is a device in which microorganisms degrade the organic matter in anaerobic conditions by using an electrode (anode) as final electron acceptor and generating an electrical signal [8]. Several advantages can be obtained by using BESs for the bioremediation. BES-based approaches can be cost effective because the anode is a virtually inexhaustible electron acceptor and the electron flux can be maintained constant over time [6]. When graphite electrodes are used, the contaminants can be adsorbed on the electron acceptor generating an area with high metabolic activity [6]. The electrical signal produced can be used in biosensors for the real time monitoring of the microbial activity [9,10].

Bioelectrochemical enhanced bioremediation could be particularly suitable for creating reactive zones in the aquifer where contaminants are efficiently removed. Particularly BESs could contribute to overcome some current limitations of biobarriers (BBs) applications. Indeed, BBs are an innovative strategy for *in-situ* bioremediation of contaminated groundwater. A BB is a passive system built by placing a permeable material in the contaminated aquifer to intercept the contaminated plume [11]. The support material (e.g. volcanic pumice) is colonized by microorganisms able to degrade the contaminants [12]. Oxygen can be supplied to stimulate the aerobic degradation in BBs but, due to the porosity of the support material, microenvironments with a low oxygen concentration may be present [13]. An alternative approach may be the stimulation of the microbial metabolism in anaerobic BBs by placing electrodes directly in the support material.

Electrodes have been shown to effectively stimulate the biodegradation of toluene and benzene in marine sediments and groundwater [6,14,15], however mixtures of hydrocarbons (e.g. gasoline) or single compounds, are often present in contaminated environments. BTEX compounds are among the main constituents of gasoline [16] but, to our knowledge, no information about the degradability of BTEX mixtures in BESs is available so far [7].

The main goal of this study was to investigate the degradability of a BTEX mixture at different voltages by using an anode as

solid electron acceptor. Furthermore, the microbial communities enriched during the treatment were characterized by sequencing of the 16S rRNA gene.

2. Materials and methods

2.1. Bioelectrochemical reactor set-up, operation and experimental conditions

Single chamber BES reactors [17] were constructed by using 120 mL serum bottles. Each bottle was filled with 30 g of commercially available volcanic pumice (Euroterriflora) as support material for microbial growth. Pumice was grinded and sieved prior to use in order to select particle size in the range of 2–5 mm. Volcanic pumice was chosen because it is a suitable support material for BBs [12]. Reactors were flushed with N_2 for 30 min in order to remove oxygen. After flushing, each reactor was sealed with a thick butyl rubber stopper (Rubber BV, Hilversum, Netherlands) and refinery wastewater (85 mL, COD 720 mg O_2/L , total P 1 mg/L, total N 20 mg/L) was injected as microbial inoculum. Reactors were flushed prior to the addition of the microbial inoculum in order to avoid stripping of the organic contaminants in the refinery wastewater. Graphite plates (10 cm^2 , geometric area) connected to stainless steel wire (1 mm) were used both as anode and cathode. No catalysts were used on the cathode surface. The stainless steel wire and the electrical connections were covered with a heat shrinkable tube to prevent corrosion. The experiment was performed by applying three voltages (0.8 V, 1.0 V, 1.2 V) in duplicate using an external self made power supply. The current production was monitored with a data logger (Squirrel SQ2010, Grant, Cambridge, UK), data were recorded every 1 min and the average calculated every 30 min was reported. Furthermore, two open circuit controls (OC) and one abiotic control for each external voltage applied (i.e. 0.8 V, 1.0 V and 1.2 V) were set up. The reactors were run for few days in order to allow microbial colonization, a mixture of BTEX was then spiked and the concentration of hydrocarbons was monitored over time. At the end of the experiment, samples of both the electrodes and the pumice were collected and stored at -20°C for the microbial communities characterizations.

2.2. Chemical analyses

BTEX concentration was monitored by a GC-FID (Agilent 6890N) equipped with a headspace autosampler (Agilent 7697A). BTEX removal rate was estimated by the first order kinetic constants calculated during the second and the third batch cycles in order to minimize the effect of adsorption onto the electrodes. The first order kinetics constants were calculated by linear regression of time vs $\ln(C/C_0)$ where C is the measured concentration of each compound at each sampling time and C_0 is the initial concentration. The R^2 values are summarized in Table S1.

The determination of sulfate concentration was performed by ion chromatography (Thermo Scientific Dionex ICS-1100) with a detection limit (DL) of 0.1 mg/L. Before the analysis, several samples were diluted with deionized water.

Total phosphorus and total nitrogen were measured in the refinery wastewater by spectrophotometry following standard methods [18].

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