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Cleaning-up atrazine-polluted soil by using Microbial Electroremediating Cells

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HIGHLIGHTS

- MERC system allows the *in situ* bioremediation of polluted environments.
- Atrazine-polluted soil can be cleaned-up by stimulating electroactive bacteria trough bioelectroventing.
- The atrazine removal was well correlated with the soil ecotoxicological analysis.

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ABSTRACT

Biodegradation of pollutants in soil is greatly limited by the availability of terminal electron acceptors required for supporting microbial respiration. Such limitation can be overcome if soil-buried electrodes accept the electrons released in the microbial metabolism. We propose the term bioelectroventing for such a environmental treatment. The process would be performed in a device so-called Microbial Electroremediating Cell. Indeed, our studies demonstrate that the presence of electrodes as electron acceptors effectively stimulated by 5-fold the biodegradation rate of the herbicide atrazine (2-chloro-4-ethylamino-6-isopropyl amino-1,3,5-triazine) in comparison with soil natural attenuation. Furthermore, a different set of toxicological test using *Pseudokirchneriella subcapitata* green alga e, *Salmonella typhimorium* bacteria and *Sorghum saccharatum* plant seeds respectively, confirm that atrazine-polluted soil can be effectively cleaned-up in short time by the use of MERCs.

1. Introduction

The organic pollutants can be degraded in nature by microorganisms making biodegradation the main mechanism for their removal in the environment (Head, 1998; Semple et al., 2001). Frequently the lack of suitable electron acceptors limits microbial respiration and a variety of organic pollutants persist in water-logged soil or sediment (Liu and Suflita, 1993). Such a TEA limitation can be overcome using electrically conductive material like the electrodes used in Sediment Microbial Fuel Cells (sMFC). These bioelectrochemical devices consist of a sediment- or a soil-buried electrode (anode) acting as electron sink coupled to the microbial oxidation of organic matter. This anode is connected through an external resistance to a cathode where electrons are finally consumed by an electron acceptor as oxygen so electrical energy can be harvested (Tender et al., 2002; Venkata Mohan et al., 2009; Domínguez-Garay et al., 2013). If sMFC are allocated in polluted environments then microbial biodegradation can be enhanced. In that sense, Zhang et al. (2010) demonstrated for the first time that graphite electrodes could serve as an electron acceptor for the degradation of toluene and benzene in polluted slurries. Since then, several studies have reported the biodegradation enhancement of pollutants of different chemical nature: PAHs (K. Chandrasekhar, 2012; Morris and Jin, 2012; Wang et al., 2012; Yan et al., 2012; Lu et al., 2014; Rodrigo et al., 2014; Zhang et al., 2014; Li et al., 2015, 2016; Sherafatmand and Ng, 2015; Venkidusamy et al., 2016), petroleum hydrocarbons (Huang et al., 2011; Daghio et al., 2016), pesticides (Liang et al., 2014), chlorinated organics (Cao et al., 2015;

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Chun et al., 2013; Yu et al., 2016) and herbicides (Rodrigo et al., 2016).

On top of sMFC, a variant of environmental fuel cells was recently design to enhance *in situ* bioremediation through generation of current instead of harvesting energy as MFCs typically do (Rodrigo et al., 2014). Moreover, not just pollutant removal but true clean-up of a DBT-polluted soil after MERCs treatment was demonstrated by ecotoxicological analysis (Rodrigo et al., 2014). More recently, Rodrigo et al. (2016) also reported how selecting an electrode potential as high as 600 mV (vs Ag/AgCl) led to a positive impact in the pollutant mineralization. Such a bioelectrochemical approach to accept the electrons from microbes metabolism was named for first time with term bioelectroventing due to the similarities with bioventing where oxygen is artificially applied as electron acceptor (Kabelitz et al., 2009; Frutos et al., 2010). In order to cope with electron acceptor limitations, additional biostimulation tasks consist of supplying humic acids (Lovley, 2000) or nitrates (Yu et al., 2014). However, nutrients, oxygen, electron acceptors or donors are exhaustible compounds in soil that requires a constant additional supply and its availability depends on the appropriate environmental conditions (pH, temperature, moisture, etc). So thus, bioelectroventing-based remediation overcomes this limitation due to the electrode that behaves as a never-ending electron acceptor in anaerobic soils, allowing microorganisms to perform oxidative metabolism beyond the natural conditions. Moreover, the competitive role of an electrode may reduce the formation of hydrogen sulphide and even methane that cause secondary pollution concerns (Pandey, 2012). Interestingly, wild environmental conditions can be easily restored after the treatment completion just by removing the electrode.

The absence of suitable terminal electron acceptors in an environment like soil might be responsible of the limited *in situ* biodegradation of Atrazine (2-chloro-4-ethylamino-6-isopropyl amino-1,3,5-triazine), a member of chlorinated *s*-triazine group of herbicides, which is moderately mobile and highly persistent in the environment (Iriel et al., 2014). Although atrazine was banned in the European Union in 2003 (Bethsass and Colangelo, 2006), it is still widely used around the world due to its low cost and high effectiveness for control of weeds in crops such as corn and sorghum. Therefore it often appears in ground and surface water, exposing a risk to human health. However, in spite of its recalcitrance recent studies have demonstrated that atrazine biodegradation is feasible (Solomon et al., 2013). In soils, atrazine degradation is mainly a result of microbial activity (Mudhoo and Garg, 2011) and a large variety of microorganisms such as *Pseudomonas* sp ADP (de Souza et al., 1998), *Agrabacterium radiobacter* J14a (Struthers et al., 1998) or *Nicordioides* (Topp et al., 2000) degrade this herbicide through co-metabolic processes that lead to the formation and accumulation of atrazine metabolites. Atrazine biodegradation pathway seem to follow *N*-dealkylation and dechlorination processes prior to ring cleavage (Fang et al., 2015). Either single species or microbial consortia are responsible for reactions that involves three hydrolases genes atzA, atzB, atzC that encode for dehalogenate and dealkylate atrazine in a stepwise fashion (Smith et al., 2005).

Numerous studies have indicated that atrazine inhibits growth and photosynthesis of freshwater algae and algal responses to atrazine vary widely depending upon concentrations used, duration of exposure, and algal species tested (Tang et al., 1997; Weiner et al., 2004). Furthermore, atrazine and other triazinic compounds have showed genotoxic and mutagenic actions in *Drosophila*, yeasts and plants, but not mutagenic actions in bacteria (de Campos Ventura et al., 2008). Recently, atrazine and its chlorometabolites have been reported to affect the development and behaviour in the early life stages of zebrafish (Liu et al., 2016).

Several authors have already studied changes in the yield of chlorophyll fluorescence in the presence of herbicides. Conrad et al. (1993), reported that some triazines and derivatives of phenylurea led to variations in the yield of *in vivo* fluorescence for PSII chlorophyll-a (Conrad et al., 1993; Iriel et al., 2014).

So, the main aim of this work was to apply a MERC treatment for stimulating the biodegradation of atrazine in soil. Moreover, a set of several toxicological analyses, including ecotoxicity, genotoxicity and phytotoxicity tests have revealed how the MERC-assisted treatment outperforms natural attenuation to accelerate the clean-up process of an atrazine-polluted soil.

2. Material and methods

2.1. Chemicals

Atrazine (CAS No. 1912-24-9, purity >97%) was obtained from TCI. Na_2HPO_4 and NaH_2PO_4 were used to make the phosphate buffer solution, with purity >98% and purchased from Scharlau. Deionized water was used to prepare all media and solutions and Methanol from Sigma Aldrich (purity > 99.9%) was used to soil extractions.

2.2. Soil sampling

The laboratory-scale experiments were developed using a single soil collected from an uncontaminated soil on an alluvial plain in Calasparra (Murcia, SE Spain), deposited by the Segura River and developed on rice fallow land. A complete description of the physical and chemical characteristics of soil is provided in the Supporting Information (SI) section.

2.3. Construction and operation of the Microbial Electroremediating Cells (MERCs)

MERC systems were constructed under a multichamber configuration in plastic containers. Each watertight compartment was 4 × 6.5 × 3.5 cm (wide, length and deep) and it was filled with 60 g of sieved dry paddy soil (≤2 mm) (Fig. S1a and S1b).

Plain graphite plates (*Mersen*, 3 × 3 cm, 0.5 cm thickness) were used as electrode for the anodes that were buried in the middle of the soil layer. Copper wires used for connections were sealed with a conductive epoxy resin (Circuit Works) and isolated with a nonconductive epoxy resin (Araldit Ceys). Graphite felt (Mersen, 4 × 6.5 × 0.5 cm; 0.7 m^2/g of surface area) was used as cathode electrode and were allocated in the flooded deionized water of the MERC. The electrodes were connected through a 3 KΩ resistor and the cell voltage was monitored with a multimeter (7700, Keithley instruments).

The MERC assays were compared with other experimental conditions to estimate the adsorption of the contaminant to the soil and to the electrode. So thus, electrode-free assays (natural attenuation) were compared with non-connected MERC assays (open circuit). Additionally, an electrode-free sterilized soil was compared with an electrode-free soil (natural attenuation). Sterlization by autoclaving was not performed to avoid physical perturbation of the soil. Instead, chemical sterilization by adding 3 ml of 100 mM $HgCl_2$ was performed to discard the existence of abiotic electrochemical reactions (Table 1). Sterilization was confirmed by analysing the bioelectrochemical response after adding acetate to both a MERC an $HgCl_2$-supplemented MERC (Figs. S3 and S4).

Each assay condition was simultaneously replicated three times at room temperature, and was stabilized for two weeks. After this stabilization period and to estimate the presence or absence of biological activity a pulse of acetate 20 mM (1 mL) was spiked to a