Soil Biology & Biochemistry 116 (2018) 131–138

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Anodic shifting of the microbial community profile to enhance oxidative metabolism in soil

Jose Rodrigo Quejigo [a, b], Ainara Domínguez-Garay [a], Ulrike Dörfler [b], Reiner Schroll [b], Abraham Esteve-Núñez [a, c, *]

[a] University of Alcalá, Alcalá de Henares, Madrid, Spain
[b] Helmholtz Zentrum München, Munich, Germany
[c] IMDEA-WATER Parque Tecnológico de Alcalá, Madrid, Spain

ARTICLE INFO

Article history:
Received 23 February 2017
Received in revised form
30 August 2017
Accepted 10 September 2017

Keywords:
Bioelectroventing
Isoproturon
Mineralization
Bioremediation
Microbial fuel cell
Ecotoxicology

ABSTRACT

The biodegradation of pollutants in soil is limited by the availability of terminal electron acceptors required to support microbial respiration. Microbial Electroremediating Cells (MERCs) consist of a variety of bioelectrochemical devices that aim to overcome electron acceptor limitation and maximize the biodegradation of pollutants in the environment. This electrode-based method to stimulate the oxidative metabolism of environmental microbial populations is referred to as *bioelectroventing*. The current research uses MERCs principles, under different configurations, for stimulating native soil bacteria to achieve the complete removal of the herbicide isoproturon (IPU). Our studies conclude that the application of a high anodic potential (+600 mV versus Ag/AgCl) to contaminated soils increases, not only IPU-removal, but also leads to an effective clean-up as demonstrated by soil ecotoxicological analysis after treatment. Furthermore, electrode potential differences induced taxonomical shifts in the microbial community as exposed by the high-throughput sequencing analysis. We also used microbial community diversity as reporter of the electrode's influence. Our results showed that the electrode impacted the communities as far as 0.5 cm away. The data provided here is evidence that polarized electrodes are a cost-effective and environmentally friendly strategy to select microbial communities for the successfully bioremediation of isoproturon-polluted soils.

1. Introduction

Environmental pollution by xenobiotic compounds is a matter of global concern (Folberth et al., 2009). Biodegradation based on microbial metabolic activities is the primary mechanism for pollutant removal in the environment due to the biodiversity and catabolic potential of the microbial communities. However, the bioremediation of a pollutant depends on environmental conditions, the existence of indigenous degrading species, and the nature and chemical structure of the compound being degraded. In environments like soil, the absence of suitable terminal electron acceptors (TEA) to sustain microbial respiration might be responsible for the limited anaerobic biodegradation of pollutants (Larsen and Aamand, 2001; Megharaj et al., 2011).

Microbial Electrochemical Systems or Microbial Electrochemical Technologies (METs) have been shown as an alternative to the classic bioremediation strategy of supplying electron acceptors like oxygen (bioventing) (Kabelitz et al., 2009; García et al., 2010), humic acids (Lovley, 2000) or nitrates (Yu et al., 2013) to name a few. Rodrigo et al. (2014) referred to the microbial electrochemical devices that aim to maximize current production (Amperes) through maximizing metabolic degradation of organic/inorganic soil pollutants as Microbial Electroremediating Cells (MERCs). In MERCs, electroactive microorganisms oxidize the organic pollutant as an electron donor and use the anode as an inexhaustible electron acceptor. This strategy is called *bioelectroventing*, in allusion to the similarities with the traditional bioremediation technique *bioventing* where oxygen is artificially applied as electron acceptor. Likewise, bacteria can also use electrodes as an electron source for reducing pollutants (Rosenbaum et al., 2011).

The electrode's potential governs the microbes' electron releasing capabilities, determining from a thermodynamic point of view, the metabolic pathway able to be used and the theoretical

* Corresponding author. University of Alcalá, Alcalá de Henares, Madrid, Spain.
E-mail address: abraham.esteve@uah.es (A. Esteve-Núñez).

https://doi.org/10.1016/j.soilbio.2017.09.012
0038-0717/

energy gain from the biocatalyst (Schröder, 2007). To date, most studies on electrode-based biostimulation used a soil-buried electrode with a negative potential as a consequence of the biochemical environment around it, which can be insufficient or inappropriate to drive the transformation of many recalcitrant organics (Zhao et al., 2006). A higher anode potential may increase the amount of energy, per electron transferred, available for growth and cell maintenance, resulting in higher microbial density and current generation (Aelterman et al., 2006; Finkelstein et al., 2006; Busalmen et al., 2008). Actually electrodes artificially polarized at potentials as high as +600 mV (versus Ag/AgCl) had a high impact on the microbial degradation activity in herbicides-contaminated soils (Rodrigo Quejigo et al., 2016; Domínguez-Garay et al., 2017). So, the electrodes not only overcame TEA limitation but also allow for the controllability of the bioremediation processes by engineering the environmental redox conditions and selecting microbial activities that remediate contaminants of concern.

However microorganisms involved in MERCs are poorly understood. The entire microbial community comprises a complex network of upstream fermenters that generate electron donors and downstream electron consumers that produce methane and/or electricity as end products. So, MERCs performance not only relies on electroactive bacteria's ability to transfer electrons from substrates to an electrode, but on non-electrochemically active microorganisms and their immense ensemble of syntrophic interactions (Zhi et al., 2014). Despite the inherent complexity of these systems, understanding the link between microbial community and the electrocatalysis of pollutants in MERCs will help us to better understand, not only the electrode's effect on microbial community structure, but also to optimize our envisioned application of these systems (Daghio et al., 2016).

The absence of suitable TEAs in anaerobic and strongly reductive environments like flooded soils might be responsible for the limited biodegradation of isoproturon (IPU) (Larsen and Aamand, 2001). Isoproturon is one of the most extensively used herbicides in agriculture for pre- and post-emergence control of annual grasses and weeds in winter cereals. As a consequence, the presence of IPU in groundwater may exceed the approved critical value for drinking water (0.1 μg L^{-1}) set by the European Community Drinking Water Directive (Folberth et al., 2009), leading to a significant impact on ecosystems and hazards to human health.

In the current work we explore the communities of heterotrophic bacteria participating in bioelectroventing for cleaning-up IPU-polluted soil, and how they are affected by electrode potential and their distance from the electrode.

2. Material and methods

2.1. Chemicals

3-(4-isopropylphenyl)-1,1-dimethylurea (IPU), monodemethyl-isoproturon (MDIPU) [3-(4-isopropylphenyl)-1-methyl-urea], 2-OH-Mono-demethyl-Isoproturon (2-OH-MIPU) 3-(4-(2- hydroxyisopropylphenyl))1-methylurea, didemethyl-isoproturon (DDIPU) [3-(4-isopropylphenyl)-urea] and 4-isopropyl-aniline (4-IPA) were purchased from Dr. Ehrenstorfer (Augsburg, Germany; purity 99.5%). All other chemicals and solvents were of analytical grade and were purchased from Merck (Darmstadt, Germany).

2.2. Soil

The soil material was an Aric Anthrosol from an agricultural field (Hohenwart; latitude: 48.250, longitude: 11.567, elevation 472 m) in Germany without IPU-history and with an organic matter content of 0.99%. A complete physical-chemical analysis of this soil was reported by Grundmann et al. (2011). Soil samples were taken from 0 to 20 cm and were stored in plastic bags at −20 °C according to guidelines of the Organization for Economic Cooperation and Development (OECD, 1995). The soil samples were unfrozen and equilibrated 2 weeks before the start of the experiment following the incubation protocol specified by Rodrigo Quejigo et al. (2016).

2.3. Spiking of the soil samples

45 μL of 19 mM ^{14}C-IPU standard was applied to an aliquot of 3 g dried-and-ground soil and homogeneously mixed. After evaporation of the organic solvent (methanol), the soil aliquot was mixed with 32 g (dry weight equivalent) of equilibrated soil with the goal to distribute the pollutant homogenously, resulting in a concentration of 5 (±0.1) μg g^{-1} soil (dry weight). The spiked soil sample was transferred to an opaque glass flask of the laboratory system described below, compacted to a soil density of 1.3 g cm^{-3} and flooded (water holding capacity + 35 mL extra deionized water). Water evaporation was compensated for 3 times per week by the addition of deionized water.

2.4. Laboratory system

The removal experiment was conducted in a laboratory system built in approximation to the OECD guideline for testing of chemicals 304A (OECD, 1981). It consisted of opaque glass flasks (250 mL volume; neoLab, Heidelberg, Germany), which were closed with a rubber stopper (neoLab, Heidelberg, Germany). A hollow needle (neoLab, Heidelberg, Germany) conducted through the rubber stopper allowed a constant supply of O_2. Actually, it is common practice in Microbial Electrochemical Systems to expose the cathode electrode to atmospheric oxygen (He et al., 2007; Song et al., 2011) given that oxygen reduction reaction is the dominant cathodic process.

2.5. MERCs: Operating conditions

MERCs were assembled in the laboratory system (Fig.S1B and C, in Supplementary Information (SI)). The electrodes used in this experiment were carbon felt (Sofacel, Barcelona, Spain), as it showed no IPU-adsorption and very adequate mechanical properties to conform to the system (Rodrigo Quejigo et al., 2016). The electrodes were located at the bottom of the soil layer (anode) and above the water body (cathode). The geometrical area of the electrodes was 39 cm^2 (surface area: 0.7 m^2 g^{-1}). Microbial Electroremediating Cells were operated under 2 different conditions: 1) systems operated at a poised anode potential of +600 mV versus Ag/AgCl reference electrode (RE-5B, BASi, United Kingdom) (+199 mV vs. SHE) by using a potentiostat (NEV2 nanoelectra) which are designated as **pol-MERCs** 2) systems where the electrodes were connected by a copper wire using a 56 Ω external resistor (R) with the redox potential of the anode set spontaneously by the redox potential differences across sediment/water, which are designated simply **MERCs**.

Electrode-free controls (Fig. S1A in SI) were assembled in the laboratory system without electrodes and under the same water content, temperature and IPU-concentrations as the electrode-assisted treatments.

Abiotic reactions on IPU were evaluated by using sterile free-electrode control. The soil was sterilized with γ-irradiation in a Gammacell 220 cobalt-60 irradiation unit during 96 h at a rate of 8.33 Gy min^{-1} for a total γ-ray dosage of 60 KGy. Biologic activity was tested by dish-plate inoculation. Elutes of soil were prepared using phosphate buffer (pH 8) in 1:10 soil-water ratio. 1:10 and 1:100 dilutions were carried out and incubated at 30 °C in LB agar