Vol. 19, Sup. 1 (2020) 111-121
Revista Mexicana de Ingeniería Química

Biotechnological processes improved with electric fields: the importance of operational parameters selection

Procesos biotecnológicos mejorados con un campo eléctrico: la importancia de la selección de parámetros operacionales

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Received: May 8, 2020; Accepted: July 2, 2020

Abstract

The application of electric fields on a porous media has been a useful tool for biotechnological processes improvement, mainly in environmental engineering; however, the success in its reproducibility depends on the standardized methodology rather than empirical results. This work describes three experimental cases: *Aspergillus brasiliensis* attached to perlite for emulsifier protein production, *Metarhizium anisopliae* as a biologic control of insect pests and the characterization of a hydrocarbon contaminated soil. The standardized methodology is independent of the biotechnological purposes and consists of: (i) establishment of current density in which porous media behave as an electric resistance (0-2.5, 0-0.6 and 0-0.85 mA cm^{-2}, for perlite, rice-corn mixture and soil, respectively). (ii) establishment of a current density in which cell potential is constant, to make sure that no gradient of nutrients concentration is formed and (iii) pH gradient evaluation as a consequence of water oxidation/reduction electrochemical reactions and the charge transport capability across the porous media. As a result of the aforementioned standardization three sections of packed porous media on electrochemical cell would be obtained: anodic (acidified), middle (neutral) and cathodic (alkalinized), which have different physicochemical properties and promote also different metabolic responses when they are used as supports for solid-state culture.

Keywords: electric field, solid surface modifications, metabolism modifications, pH gradient, electric resistance.

Resumen

La imposición de un campo eléctrico en un medio poroso es una técnica utilizada para mejorar procesos biotecnológicos, principalmente en ingeniería ambiental. La reproducibilidad en los resultados depende de la estandarización metodológica, más que los resultados empíricos. El presente trabajo describe tres casos experimentales: *Aspergillus brasiliensis* inmovilizado en agrolita para producir proteínas emulsificantes, *Metarhizium anisopliae* como control de plagas de insectos y la caracterización de un suelo contaminado con hidrocarburos. La estandarización, independientemente del objetivo biotecnológico, consiste en: (i) establecer un intervalo de densidad de corriente en la que el medio se comporte como una resistencia eléctrica (0-2.5, 0-6 y 0-0.85 mA cm^{-2}, para agrolita, mezcla arroz-maíz y suelo, respectivamente), (ii) establecer una densidad de corriente en la que el potencial de celda sea constante, para garantizar que no existan gradientes de concentración de nutrientes y (iii) evaluar el gradiente de pH formado por las reacciones de oxidación/reducción de agua y la capacidad de transferencia de carga a través del medio poroso. Como resultado de la estandarización se obtienen tres secciones de medio poroso empacado en la celda: anódica, media y catódica, con características fisicoquímicas diferentes que a su vez provocan modificaciones de respuestas metabólicas, cuando se utilizan como soportes en cultivos sólidos.

Palabras clave: campo eléctrico, modificaciones superficiales en sólidos, modificaciones metabólicas, gradiente de pH, resistencia eléctrica .

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https://doi.org/10.24275/rmiq/Bio1689
ISSN:1665-2738, *issn-e*: 2395-8472

1 Introduction

The use of electric fields on biological and non-biological targets is widely known in biotechnology and environmental engineering. The focus of this utilization has been as a technique for soil decontamination relying on one basic principle: pollutant displacement triggered by water electrolysis (Acar & Alshawabkeh, 1993). Pollutants' displacement and removal extents strongly depend on contaminant properties and soil characteristics (Virkutyte *et al*., 2002). Electrochemical remediation has been coupled with many other technologies to improve its results (Martínez-Prado *et al*., 2014). For example, surfactants and/or co-solvents have been added to enhance the desorption of organic pollutants by increasing their solubilization into the aqueous phase (Martínez-Prado *et al*., 2017); the use of oxidation/reduction agents which favor chemical modifications of the contaminants (Huang *et al*., 2012); and the use of biologic agents (such as microorganisms or plants) which not only promote the desorption of contaminants but also can remove pollutants through their metabolic activity (Gill *et al*., 2014). In this sense, the center of attention of most of the electrochemical remediation laboratory-scale studies has been effects of electric fields on organisms' metabolisms modifying of the operational parameters in electrochemical cells (*i.e.* intensity of the electric current, time of exposure, geometry, and material of electrodes).

For example, research by Jackman *et al*. (1999) has mainly focused on metabolism, and the operational parameters they used (20 mA, 3 V, 200 A m^2) were set according to those used in soil remediation. Wang *et al*. (2019), evaluated the degradation of phenanthrene and the growth rate of *Pseudomonas* sp. using cylindrical graphite electrodes and varying the intensity of the applied electric field (0.1, 0.2 and 0.3 V). Araújo *et al*. (2004), characterized the effects of electric potential (0.75 V) during the growth of *Saccharomyces cerevisiae* in yeast extract peptone dextrose culture medium. Guo *et al*. (2014) evaluated the decontamination of a clay soil contaminated with total petroleum hydrocarbons (TPH) in an electrochemical cell (1 V cm^{-1}) provided with 25 cylindrical graphite electrodes, they observed an enhanced decrease in TPH concentration around the electrodes.

A lack of methodology when choosing operational parameters and systems' heterogeneity seems to be the common ground for all these studies. The scarce information about the experimental parameters selection ignores the fact that the reported modification of physicochemical and biological properties is mainly related to the characteristic of the systems where electric field are being applied (soils or solid-state cultures). The diverse nature of these systems represents a challenge for defining the experimental parameters, and it is imperative that operational conditions allow the distinction between the effect of electric field from other effects taking place simultaneously.

In this line of thought, the selected operational parameters that yield enhanced metabolic responses as well as decreases on pollutants concentrations should consider the responses of porous media to electric fields. Therefore, the objective of this study is to propose an experimental strategy that relates all these factors with aims on generalizing the usage of electric fields on complex porous media systems with biotechnological applications. This was accomplished by revising three cases of study: one with a mineral porous media (perlite), one with an organic porous media (rice-corn stubble), and one with a complex porous media (soil).

2 Materials and methods

2.1 *Preparation of porous media*

Before electrochemical experiments, the three different porous media described in this work were individually conditioned. More detailed information for each porous medium and analytical methods used for fungal biomass growth, sporulation and carbon source consumption can be consulted in the works published by Velasco-Alvarez *et al*. (2011) and Sánchez-Vázquez *et al*. (2017, 2018).

2.1.1 *Perlite*

Perlite, a glassy volcanic rock composed of a mixed oxide consisting mainly of SiO_2 and Al_2O_3 (Alkan *et al*. 2005), was washed with hot distilled water and dried at room temperature. The support was sieved (particle size 1.19-1.68 mm) and impregnated with hexadecane dissolved in 1.9 mL hexane for a final concentration of 180 mg of hexadecane per g of dry support (gds). Hexane was evaporated in a fuming chamber for 24 h at room temperature. Moisture was adjusted to 75% with a liquid culture medium (2.3 mL gds^{-1}). The medium was composed of 21.2, $NaNO_3$; 3.0, KH_2PO_4; 0.9, $MgSO_4$; 3.0, KCl g of salt per L^{-1}, and 6 mL L^{-1} of a trace element solution containing (g L^{-1}), 0.100, $FeSO_4$ $7H_2O$; 0.015, $CuSO_45H_2O$; 0.161, $ZnSO_4$ $7H_2O$; 0.008, $MnSO_47H_2O$. The pH of the medium was adjusted to 5 with 0.1 mol L^{-1} H_3PO_4 and autoclaved at 120 ºC for 15 min.

2.1.2 *Rice-corn stubble mixture*

The corn stubble was grounded, dried, and sieved (0.66-0.77 mm). Parboiled rice (Verde Valle®) was used. A mixture of parboiled rice:corn stubble in a 9:1 ratio was used as the organic support for solid-state culture. The mixture was hydrated with distilled water (3.3 mL gds^{-1}) 24 h prior sterilization. The pH of the mixture was adjusted to 5 with 0.1 mol L^{-1} HCl, and finally, the mixture was autoclaved at 120 ºC for 15 min.

2.1.3 *Soil*

Air-dried soil was sieved (0.4-2 mm), then autoclaved two times at 120 ºC for 15 min. Soil samples were spiked with a hydrocarbon mixture (HCM) composed of hexadecane, phenanthrene, and pyrene (100:1:1 w/w) dissolved in a 1:1 mixture of hexane and acetone. Samples were spiked at a concentration of 25,000 mg of the HCM per kg of soil for each experiment. Hexane and acetone were evaporated in a fuming chamber for 12 h at room temperature. Sterile distilled water was used to hydrate soil at field capacity (46.24 ± 2.3 %).

2.2 *Electrochemical cell devices*

The electrochemical cell device used for this study was made of acrylic glass tubing (18 cm long and a diameter of 6.5 cm). It was provided with three separate sections: one central compartment where the porous medium was packed, and two separate compartments placed at both ends of the cell, these were filled with the electrolytic solutions (20 mL, 0.1 mol L^{-1} KH_2PO_4). The three compartments were separated through electrodes made of Ti and coated with RuO_2. The electrodes (Ti plates coated with RuO_2) were placed at both ends of the electrochemical cell. These types of electrodes are known to favor water oxidation/reduction reactions, without additional electrochemical reactions, and exhibit high corrosion resistance and stability (Trasatti, 2000; Freitas *et al*., 2007). Each electrode was shaped like a semicircle plate with 14.13 cm^2 cross-sectional area (Figure 1). The shape of these electrodes is meant for creating two environments inside the reactor: i) a bed for placing the porous media for solid-state fermentation experiments, and ii) a headspace, to allow a correct gas exchange for biological cultures. The central compartment was divided into three equidistant longitudinal sections between anode and cathode (4 cm per section; see Fig. 1). The following response variables were measured in triplicate for each section: pH, production of biomass, HXD degradation, spores/conidia production, biosurfactant production, and the mortality percentage.

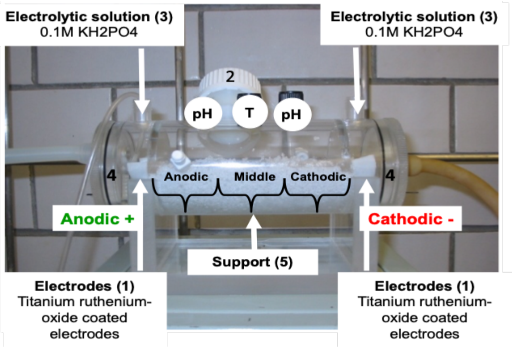

Figure 1. Electrochemical cell device. (1) Titanium ruthenium-oxide coated electrodes; (2) hermetic valve; (3) electrolytic solution inlet; (4) reservoir for electrolytic solution; (5) porous medium packing section. The electrochemical cell was divided into three equidistant longitudinal sections between anode and cathode: (i) anodic section; (ii) middle section; and (iii) cathodic section. The electrodes were placed at both ends of the electrochemical cell, keeping the electrolyte solution separate from the porous medium. (Velasco-Alvarez, *et al*., 2011).

The experimental device used for soil samples was designed as a slight modification from the previously described. Since the experiments with soil samples were abiotic, the headspace was unnecessary, therefore electrodes were shaped as complete circles with a cross-section area of 28.25 cm^2 and one only chamber was created inside the reactor, which was completely packed with soils samples. Both anodic and cathodic compartments were filled with electrolyte (0.1 M Na_2SO_4), which was circulated through a peristaltic pump at 80 mL h^{-1}.

2.3 Electrochemical cell characterization

The characterization of the electrochemical cell was performed for each of the three different porous media used.

For perlite, the electrochemical cell was packed with 15 g of preconditioned perlite (see Section 2.1.1). Subsequently, it was inoculated with a suspension of, 2.0 $\times 10^7$ *Aspergillus brasiliensis* ATCC 9642 spores per gram of dry support (Sánchez-Vázquez *et al.* 2017). For rice-corn stubble, the electrochemical cell was packed with 28 g of the preconditioned mixture of substrates (see section 2.1.2). Subsequently, it was inoculated with a suspension of *Metarhizium anisopliae* var. *lepidiotum* (Postgraduates College Collection, Texcoco, Mexico) 1×10^6 conidia per gram of dry support and 270 μL of 0.5% chloramphenicol were added. For soil samples, the electrochemical cell was packed with 300 g of contaminated sterile soil (see section 2.1.3), which was hydrated with sterile distilled water at field capacity.

Subsequently, characterization was performed for each solid inside electrochemical cells by applying different current densities. Responses for each porous media were identified for each current density by the evolution of the cell potential and the evolution of temperature. Electric current application was controlled by a potentiostat/galvanostat, changes on cell potential were recorded by a high impedance multimeter and changes on temperature were measured using a thermometer.

2.4 Statistical analysis

All measurements were carried out in triplicate. The electrical resistance of the porous media is determined by linear regression and it is presented as estimated value with standard error.

3 Results and discussion

3.1 Porous media as a resistance for electrical conductivity

According to previous reports (Velasco-Alvarez *et al.*, 2011) the functionalities of solid-state culture could be improved by applying an electric field. In this work culture media was improved only when the porous media acted as an electrical resistance. Under this assumption, the electric field would only behave as an abiotic stress factor which modifies the biochemical responses *e.g.* diminishing biomass production and enhancing nutrients uptake. In this way, these biochemical responses are physical phenomena solely associated with the magnitude of electric field applied.

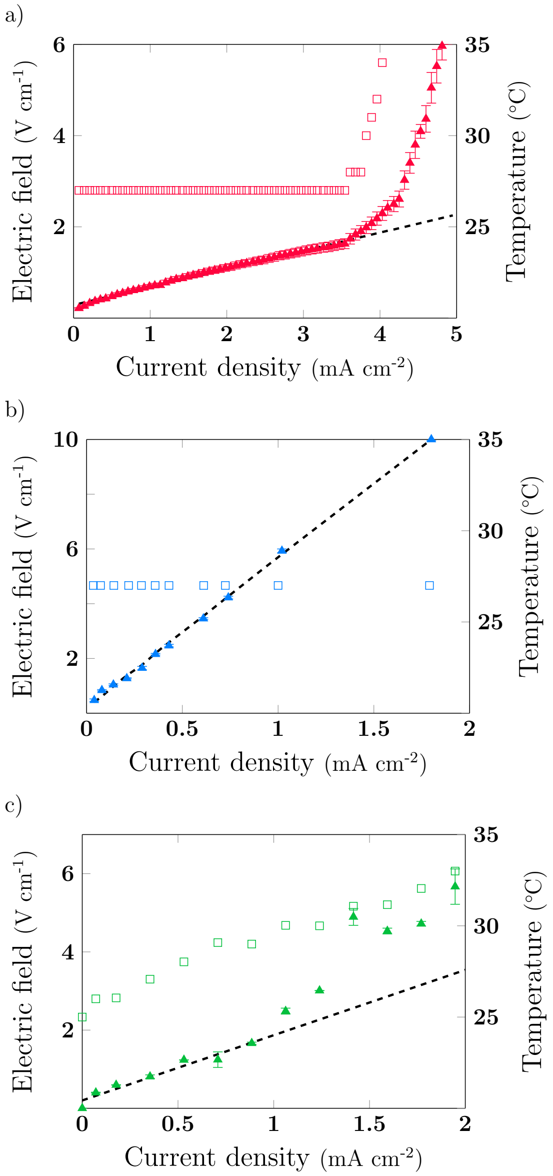

Figure 2. Variation of the electric field (filled triangles ▲) and temperature (empty squares □) as a function of the applied current density in three different porous media: a) *Aspergillus brasiliensis* ATCC 9642 on perlite, impregnated with n-hexadecane (180 mg gds^{-1}) and hydrated with culture medium (2.3 mL for gds^{-1}, 75% moisture); b) *Metarhizium anisopliae* on rice:corn stubble, hydrated with distilled water (3.3 mL gds^{-1}, 73% moisture); (c) Soil, hydrated with sterile distilled water at field capacity (46.24 ± 2.3%).

To this end, the selection of operational parameters for electrochemical reactors, where solid-state cultures are conditioned, is crucial. In this sense, the definition of the magnitude of the applied electric field, acting as a mild stress factor, is decisive in restricting the electrochemical phenomena occurring in the porous media. The behavior of different porous media acting as electric resistances is analyzed next (Figure 2).

Perlite was the porous media that showed the lowest resistance (4.65 $\times 10^3 \pm$ 0.065 Ω cm^2) (Figure 2a), this behavior may be caused by the high water-holding capacity of this porous material and because the hydrating solution consisted on a mineral medium designed for microbial consumption of hydrocarbons. Differently from perlite, the rice-corn mixture showed the most resistive behavior, with a resistance of 66.04 $\times 10^3 \pm$ 0.47 Ω cm^2 (Figure 2b). This high resistance was possibly related to the fact that only water was used as a hydrating solution. Additionally, rice particles are bigger than perlite and have a higher tendency to absorb moisture, consequently, ions were less available generating a major electric resistance. Soil also shows a lower resistance to the passage of the electric current, with a resistance of 29.52 $\times 10^3 \pm$ 0.32 Ω cm^2 (Figure 2c). As the rice-corn mixture, the soil sample was only hydrated with water, nevertheless, its resistance was lower. This less resistive behavior may be related to the natural ion content of the soil, which could be desorbed from soil particles, solubilize in the hydrating water, and be transported through the soil.

Electric resistance of any porous media is determined by the physicochemical characteristics of solid particles, their empty spaces, as well as the processes taking place at solid/liquid interface also known as the electrical double layer, which is formed when the charged surface comes in contact with the ionic substances present in the solution surrounding the pores *e.g.* mineral medium in a solid-state culture or solubilized compounds in natural porous media as a soil matrix or rice-corn mixtures (Alizadeh *et al*., 2019).

In this way, for the same applied current, the electrochemical cell voltage developed by the different porous media could be totally diverse (Figure 2). Consequently, this type of characterization should be mandatory when making comparisons on the effect of electric field over chemicals and microorganisms interacting with a porous media.

Another effect of electric fields applied to porous media is the generation of electroosmotic flow; this phenomenon can generate nutrient gradients due to rapid movement of nutrients, and then, heterogeneity and deficiency on microbial growth or physicochemical changes on porous media properties. In order to discard this phenomenon, cell potential variations were evaluated on applied current time, see Figure 3.

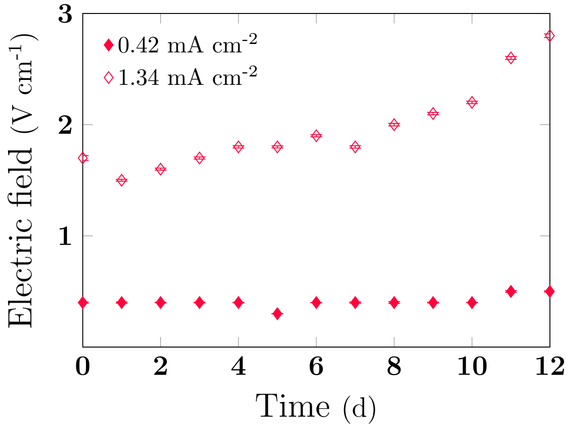

Figure 3. Cell electric field (V cm^{-1}) as function of time during separate experiments applying two different current densities: 0.42 and 1.34 mA cm^{-2}. The porous media used was perlite impregnated with n-hexadecane (180 mg gds^{-1}), culture medium (2.3 mL gds^{-1}) and inoculated with a suspension of 2.0 x 10^7 spores gds^{-1} (*Aspergillus brasiliensis* ATCC 9642).

Electric field intensity was independent from time (Figure 3) when the applied current density was within the operating values (inside the Ohmic zone in Figure 2a, 0.42 mA cm^{-2}). Meanwhile, the electric field intensity increased sharply with time when the applied current density was outside the Ohmic zone (Figure 3, 1.34 mA cm^{-2}). This behavior could be associated with the modification of processes controlling the charge transfer through the porous medium. The polarization curve (Figure 2a) allowed selecting the Ohmic behavior zone. Two current densities were selected from this experiment (0.42 and 1.34 mA cm^{-2}) and validated with *Aspergillus brasiliensis* metabolic responses, in both cases lethal effects, neither CO_2 production nor spores germination, on *A. brasiliensis* were observed. Therefore, the current density (0.42 mA cm^{-2}) was selected from the characterization of the electrochemical cell (Ohmic zone) since the cell potential is kept constant over time (Figure 3), the metabolic responses of the fungus exposed at these conditions are reported in (Table 1).

As this behavior is maintained, meaning the cell potential is kept constant while the electric current density is applied, it can be inferred

Table 1. Metabolic responses of *Aspergillus brasiliensis* ATCC 9642 by a low-intensity electric field application.

Variable	**Anodic section**	**Middle section**	**Cathodic section**	**Control (without electric current)**
pH	3.1 ± 0.2	6.7 ± 0.6	10.3 ± 1.1	5.9 ± 0.4
Biomass production (mg (gds)$^{-1}$)	25 ± 1	65 ± 7	29 ± 2	196 ± 7
Hexadecane degradation (%)	98 ± 0.8	96 ± 1.3	97 ± 1.2	81 ± 1.2
Spores production (gds^{-1})	$2.8 \pm 0.4\times10^{7}$	$6.3 \pm 0.5\times10^{4}$	Not detected	$4.6 \pm 0.3\times10^{9}$

that ionic compounds are not being transported by electroosmosis, but only by a continuous electromigration process. Then, it is possible to establish operating conditions under which the porous medium act as an electric resistance, with a homogeneous nutrient distribution. Also, the electric field can be used assuring its effect only as a stress factor for microbial metabolism stimulation. On the other hand, the electrochemical semi-reactions: water reduction and oxidation could generate a chemical gradient which could affect the microbial metabolism or physiology.

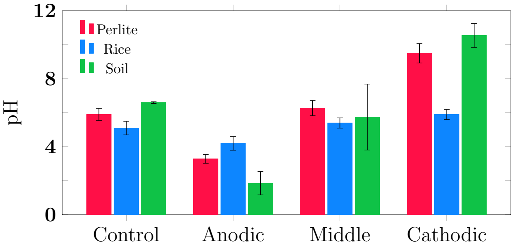

Figure 4. pH modification in three zones of the electrochemical cells packed with different substrates: *Aspergillus brasiliensis* ATCC 9642 on perlite, impregnated with n-hexadecane (180 mg gds^{-1}) and hydrated with culture medium (2.3 mL for gds^{-1}, 75% moisture); *Metarhizium anisopliae* on rice, hydrated with distilled water (3.3 mL gds^{-1}, 73% moisture); Soil, hydrated with sterile distilled water at field capacity (46.24 ± 2.3%).

3.2 *pH gradient generation*

It has been demonstrated that an electric field could modify microbial activity and viability near electrodes (Lear *et al.*, 2004), possibly due to the hydrated porous media at the vicinity of electrodes underwent electrochemical reactions that allowed the continuity of the electric circuit: near the anode, oxidation of water favored the formation of H_3O^+, while reduction of water near the cathode caused a rise of pH due to the formation of OH^-. Electrochemical reactions can generate a pH gradient, which allows the identification of three zones with different chemical potential (Figure 4). It is worth mentioning that the electrolytic solutions in the anodic and cathodic wells were not in contact with the porous medium and that the pH gradient was only generated by electrochemical reactions (oxidation/reduction of water) associated to the charge transfer between electrodes and electrolyte into the porous media packed inside the electrochemical cell.

Three defined sections can be easily identified along electrochemical cell: (i) anodic section, where the solid medium was acidified, (ii) cathodic section, where the medium was alkalinized, and (iii) middle section, where no significant differences were observed for pH. This gradient promoted chemical or biochemical phenomena that affected each porous medium differently, modifying its associations with organic matter and/or microorganisms. In this work three experimental cases are studied.

3.2.1 *Physiological response of Aspergillus brasiliensis growing on perlite as porous support*

In previous works, *Aspergillus brasiliensis* has been grown on perlite as porous inert support during the application of an electric current density and using hexadecane as a carbon and energy source (Velasco-Álvarez *et al.*, 2011). In this work, fungal biomass growth, sporulation, and carbon source consumption were evaluated in the three defined electrochemical

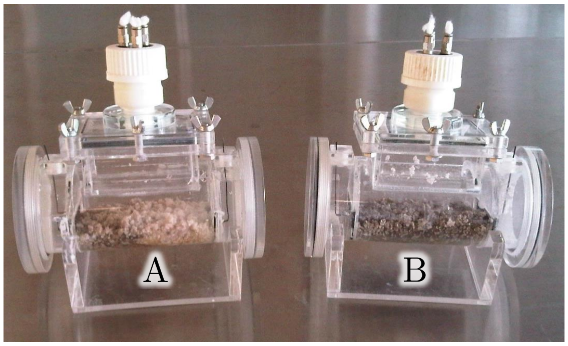

Figure 5. *Aspergillus brasiliensis* ATCC 9642 growth and sporulation after 12 d, with electric field application (A) and without (B).

cell sections (Figure 5) to elucidate the metabolic fungal response due to the electric field application.

According to the previous section, the solid-state culture was conducted under a constant current density, *i.e.* porous media (perlite) behaved as an electric resistance. However, the transference of charges between the porous medium and the electronic conductors (electrodes) was achieved through electrochemical water oxidation, generating OH^- at the cathodic section and H_3O^+ at the anodic section. The formation of these species created a pH gradient, which coupled with an electric field, synergistically promoted different metabolic responses by *A. brasiliensis* (Table 1). The mentioned metabolic responses are directly associated with the electric field application and not with the pH alterations, as it was demonstrated in previous work (Velasco-Alvarez *et al*. 2011).

Sánchez-Vázquez *et al*. (2018) proposed *A. brasiliensis*, grown on perlite and pre-treated in presence on electric field, as a supported biological catalyst (BC), for both emulsified hexadecane degradation and emulsifier protein (EP) production, in liquid culture. In this way, the BC produced in the three sections (anodic, middle, and anodic) of the electrochemical cell were separately transferred to an airlift bioreactor to evaluate each one metabolic capability. The electric field application on fungal biomass grown on perlite as porous medium, shown to promote surface modifications that improved biomass attachment to the support, as well as physiological responses when this biomass was transferred from solid to liquid medium.

EP production differed as a function of the used section (Figure 6). The anodic section was able to produce the EP with the highest emulsifying capabilities (Figure 6Ba), achieving a specific emulsifying activity of 37.6 ± 4.0 U mg^{-1}, which was 1.88-fold higher than that observed when the three sections were used mixed in the liquid medium. These metabolic modifications could be observed in other similar systems like a filamentous fungus growing on natural supports such as rice-corn mixture.

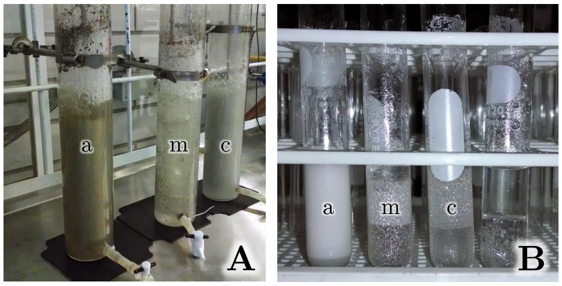

Figure 6. Biosurfactant production in liquid medium (A) by using the anodic (a), middle (m) and cathodic section (c); of the perlite/*Aspergillus brasiliensis* ATCC 9642 packed in the electrochemical cell and emulsion formed mixing an aliquot of cell-free extract and hexadecane-β-methylnaphthalene (B) (1:1 v/v).

3.2.2 Entomopathogen conidia production and infective efficiency

Metarhizium anisopliae conidia is an infectivity unit used in biological control to decrease populations of insect pests, like *Tenebrio molitor*. The effects of electric fields in these infecting capabilities have been also studied by the work group. In these studies, no significant differences were observed between the production of conidia in the presence and absence of the electric field after 8 days. However, total the possible metabolic response lies in the structural molecules biosynthesis during sporulation, since mortality of *T. molitor* larvae provoked by conidia produced in presence of the electric field was 40% higher compared to that provoked by the conidia control (Table 2).

pH did not show drastic changes during the application of the electric field (Table 2). It is known that during the application of the electric field water electrolysis reactions promote an acidic front at the anode and an alkaline front at the cathode, and when both fronts meet, they neutralize each other (Acar & Alshawabkeh, 1993). However, the pH gradient depends on the relative mobility of hydronium and hydroxyl ions through the support (Pamukcu & Wittle, 1993). Therefore, the greater the resistance, the less movement of the ions through the support, and pH gradients are less drastic. Rice is highly resistive to the

Table 2. Response variables of the conidia harvested after 8 days of culture in the presence or absence of the electric field.

	Application of the electric field					
	Anodic section		*Middle section*		*Cathodic section*	
	Without	With	Without	With	Without	With
pH	5.2 ± 0.1	4.2 ± 0.4	5.3 ± 0.2	5.4 ± 0.3	5.3 ± 0.1	5.9 ± 0.3
Conidia (gds^{-1})	4.9×10^8 $\pm 4.0\times10^7$	4.4×10^8 $\pm 2.6\times10^7$	4.9×10^8 $\pm 2.2\times10^7$	4.9×10^8 $\pm 3.4\times10^7$	4.8×10^8 $\pm 2.5\times10^7$	5.6×10^8 $\pm 6.6\times10^7$
Mortality (%)	53 ± 5	78 ± 5	53 ± 5	92 ± 8	53 ± 5	94 ± 5

Mortality expressed as the % of dead larvae after the 14th day of the bioessay(Results awaiting publication)

passage of electric current (Figure 2b), which could be associated with the low conductivity and the low availability of free water. Rice has a high capacity to absorb and retain water (2.8 mL per g of rice), and the amount of free water to carry out the ionic conduction is low. As observed with *M. anisopliae*, the pH changes were not drastic during the application of the electric field (Figure 4) because the ion conduction in the system was very low, therefore effects on conidia may be only attributed to stress induced by the electric field.

Both *A. brasiliensis* on perlite and *M. anisopliae* on rice-corn mixture have been metabolically modified by the electric field. Whilst *M. anisopliae* infective capabilities were improved, *A. brasiliensis* became a better producer of emulsifier protein. These two improvements can be interpreted as changes in a biochemical aspect; however, physicochemical modifications were also observed when surface properties of porous media promoted the attachment of biomass to perlite (Sánchez-Vázquez *et al.* 2017). These changes could be also observed by our group in electrochemical experiments using natural soil.

3.2.3 Changes on soil properties due to pH gradient

Soil characteristics dramatically changed after the electrochemical treatment. Whilst the pH of soil at the middle section remained close to the initial pH, ranging from 6.6 ± 0.05 down to 5.75 ± 1.94, the pH of soil at the anodic and cathodic sections evolved down to 1.86 ± 0.69 and up to 10.55 ± 0.70, respectively. pH gradient formation seemed to influence soil particles stability, with a slight disruption of soil aggregates (Figure 7) which lead to an increment of Dissolved Organic Matter (DOM).

Matsumoto *et al.* (2018) reported that rapid acidification of soil causes a shift on soils zeta potential by the liberation of Al^{+3} from clay minerals, which consequently leads to a decrease on aggregation; while alkalization of soil causes repulsion between negatively charged soil particles resulting on soil erosion.

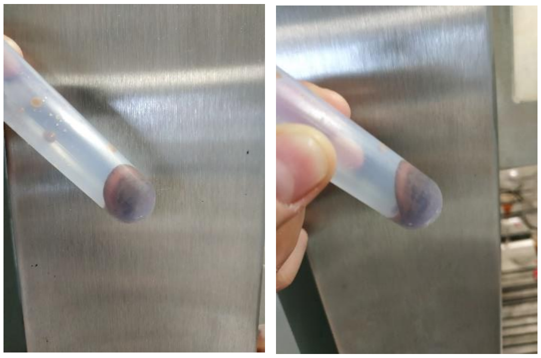

Figure 7. Disruption of soil aggregates after electrochemical treatment.

Figure 8. Soil organic matter solubilization in samples before and after electrochemical treatment through sequential solvent extractions. (a. before, b. after-anode, c. after-middle, d. after-cathode.

DOM was barely observable in samples before electric field application (Figure 8a). On the contrary, observable DOM was increased in samples after that. Electrochemical phenomena occurring in soil seemed to have a priming effect on Solid Organic Matter

(SOM), promoting its disruption. Nevertheless, this breakdown depended on the pH conditions of each section.

Under acidic conditions, (anodic section) observable DOM seemed to increase (Figure 8b). It is known that acidic environments in soil favor protonation of carboxylic groups in Humic Acids (HA), modifying the colloidal properties of these organic molecules (Terashima *et al*., 2004). On the contrary, acidic conditions provoke the development of hydrolysis reactions for Fulvic Acids (FA), inducing its breakdown and shifting its structure towards smaller molecular weight compounds that can dissolve along DOM (Calace *et al*., 2001).

Even though the pH at the middle section of the electrochemical cell was not significantly modified (Figure 8c), observable DOM was increased with respect to samples prior EC treatment (Figure 8a). Electric fields occurring in soils are known to modify the distribution of DOM as well as the colloidal properties of humic substances (HA and FA) (Übner *et al*., 2004; Cérémonie *et al*., 2008), which may have led to a redistribution on organic matter present in these fractions. Under similar operational conditions (*i.e.* below 1 V cm^{-1}), Pazos *et al*. (2012) found that soil characteristic (*e.g.* available nutrients, conductivity, buffering capacity) are no significantly modified and effectiveness decontamination is accompanied by preservation of soil functionality.

Reduction of water molecules increases the concentration of OH^- at the cathodic section, creating an alkaline environment. At high pH values, deprotonation of hydroxyl groups of SOM makes their charge more negative and repulsive forces are increased, consequently influencing the release of DOM (Rashad *et al*., 2010; Curtin *et al*., 2016).

So far, the success of remediation technologies has been measured by the achievement of high removal rates, supposing a decrease on contaminants concentration as a lessening on environmental risk. However, and depending on operational conditions, these high efficiencies implicate modifications on soil properties that in some cases cause loses of primal soil functions. A wrongful selection of operational conditions during electrochemical treatment for the decontamination of soils leads to the spatial re-distribution of resources, which may affect soil functionality after electro-remediation (O'Brien, 2017). Therefore, the two main challenges of electrochemical technologies are the careful control over the electro-kinetic phenomena and the enhancement of soil buffering capacity for diminishing pH jumps near electrodes or the implementation of polarity reversal strategies. By controlling these factors, soils ecological functions may be preserved, while acceptable rates of removed contaminants are achieved.

Conclusions

The application of electric field has been used for improvement of biotechnological processes obtaining historically positive results. Most of researches reported in the literature have empirically selected the operational conditions; however, some physicochemical aspects must also be considered to apply an electric field and observe reproducible results. Since the electric resistance showed by porous media depends on particle size, porosity, empty spaces, attached ionic species and ionic strength of hydrating solutions, this work proposes a previous characterization in order to stablish an electric current density range in which the porous media shows an Ohmic behavior. After the operating conditions are established, a generated pH gradient must be evaluated, this gradient allows identifying at least three sections into the porous media: anodic section that is alkalinized by generation of OH^-, cathodic section that is acidified by generation of H_3O^+, and middle in which both species are encountered maintaining a neutral environment. These three sections have different physicochemical properties which promote different metabolic responses when the porous media are used as support for solid-state cultures, such as biomass production and substrate consumption yields modification, the improvement of some metabolites production, or the modification on SOM properties that can promote changes on the interactions formed between contaminants and soils.

Acknowledgements

The three experimental works were financed by CONACyT (scholarships number 203321, 290414 and 611112; CONACyT Ciencia Básica projects number 257394 and 287972-Z) and PEMEX Refinación.

Dedication

The three case studies presented in this work correspond to the experimental findings from three doctoral theses held under the direction of Mariano

Gutiérrez-Rojas during the last 15 years of his career. These findings accrue the dreams of Mariano, but they also diversified his lines of research in Laboratorio de Residuos Sólidos y Bioingeniería Ambiental (Solid Wastes and Environmental Bioengineering Laboratory) of UAM-I. Unfortunately, Dr. Gutiérrez-Rojas passed away on February 11th, 2020. Thereby, the results here presented are the outcome of intense and passionate discussions where Mariano shared his infinite love and dedication to science. His lessons resonated in the authors' souls and served as an inspiration to compose the current paper, which is meant to be read as a poem, wishing it echoes throughout the moon.

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