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Mobilisation of bacteria in a fine-grained residual soil by electrophoresis

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ABSTRACT

An investigation of electrokinetic bacterial mobilisation in a residual soil from gneiss is presented here. The experimental program aimed at assessing the efficacy of electrophoresis against the electro-osmotic flow to transport endospores of *Bacillus subtilis* LBBMA 155 and nitrogen-starved cells of *Pseudomonas* sp. LBBMA 81. Electrokinesis was performed on a low hydraulic reconstituted clayey soil column submitted to a 5 mA electrical current for 24 h. Cells were coccoid-shaped and characterised as possessing low surface hydrophobicity and less than 1 μm in diameter. Distribution coefficient for *B. subtilis* in the soil was between 16.8 and 19.9 times higher than that for *Pseudomonas* sp. Distribution coefficient for *B. subtilis* between eluate and anionic exchange column was 11.8 times higher than that for *Pseudomonas* sp. After the electrokinesis, it was shown that cells and endospores were distributed hyperbolically through the soil probe and moved against the electro-osmotic flow; however, endospores were transported throughout all soil core and starved cells only till half of its length. The higher transport efficiency of *B. subtilis* endospores was attributed to their higher negative charge on cell surface. These results demonstrate that electrokinesis can be used for bacteria transport in soils with low hydraulic conductivity, even against the electro-osmotic flow.

1. Introduction

Residual soils are generally poor in nutrients, a characteristic that restricts the use of natural attenuation as a bioremediation tool in sites heavily contaminated by organic pollutants. In such environments, the biodegradation process can be accelerated by adding essential inorganic nutrients, such as phosphate and nitrate. However, this process must be strictly controlled in order to attain the most favorable C:P and C:N balance. In addition, natural attenuation also depends on the microorganisms' ability to degrade contaminants [1,2]. When autochthon populations are not capable to degrade the contaminant(s) of concern, bioaugmentation is an alternative. In soils with low hydraulic conductivity, amendments with inorganic compounds and exogenous microorganisms can be a difficult task that may render bioremediation impractical. In this case, the development of strategies that associate technical and economical feasibility with transport of nutrients and selected microorganisms through porous matrices are significant.

Electrokinesis is a promising *in situ* technique that emerged over the past few decades. It involves the use of a direct current electric field across electrodes placed in the soil. The technique derives its name from the three major associated phenomena, which are electro-osmosis, electrophoresis and electromigration [3].

The main mechanisms which electrokinesis exploits are the electrolysis of water introduced at an inert anode to produce hydrogen ions and the electro-osmotic flux of the acid front together with ion migration through the soil mass to the cathode. This acid flushing can desorb/solubilise contaminants and transport them through the soil mass to a point where they can be pumped out and treated.

Other studies have demonstrated that electrokinesis can be used to transport bacteria through a soil porous matrix [4–8]. In these attempts, the main phenomenon used was the electro-osmosis, which indicates that the porous matrices used in those studies were characterised by high hydraulic conductivity.

Not only is the cell mobilisation strategy important for a successful bioaugmentation, but also for the physicochemical characteristics of the cell surface. For instance, restriction to the transport of bacteria through soil has been attributed mainly to cell adhesion to soil particles [9]. Various characteristics are associated with bacterial adhesion, such as cell shape, dimension,

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Table 1
Petroleum hydrocarbon degrading bacteria

Isolate	Identification	Origin[a]	Substrate[b]
LBBMA 81A	*Pseudomonas* sp.	1	MMMP
LBBMA 155	*Bacillus subtilis*	2	MMP

[a] Isolate origin: (1) landfarming—Gabriel Passos Refinery (REGAP), Minas Gerais; (2) coastal marsh—Duque de Caxias Refinery (REDUC), Rio de Janeiro.

[b] Abbreviations refer to (MMMP) isolated in mineral medium added of molasses and 1% petroleum (v/v) and (MMP) isolated in mineral medium added of 1% petroleum (v/v).

and arrangement [10], hydrophobicity [11] and surface electric charge [12]. Moreover, the transport of bacteria through porous systems can be facilitated by exposing the cells to nutrient starvation, aiming to reduce adhesion of the bacterial cells to surfaces [13].

This study reports the potential of bioaugmentation by electrophoretic mobilisation of bacteria in a low permeable soil. Furthermore, the importance of the cell-surface characteristics on bacterial adhesion and cell distribution in porous media is discussed.

2. Material and methods

2.1. Bacterial strains

Bacterial strains used in this study belong to the culture collection of the Laboratório de Biotecnologia e Biodiversidade para o Meio Ambiente (LBBMA) at the Department of Microbiology, Federal University of Viçosa (UFV), Minas Gerais, Brazil (Table 1). These isolates were selected for their capacity to use petroleum hydrocarbons as carbon and energy sources [13].

Bacterial identification was performed by fatty acid methyl esters (FAME) profiles, using the Sherlock Microbial Identification System® (MIDI, Newark, DE, EUA) (TSBA50 reference library).

2.2. Preparation of starved cell

Stock cultures of bacterial strains (Table 1) were activated in solid NMP mineral medium [14], after they were inoculated in 75 mL of the same culture media and incubated at 30 °C and 150 rpm until the end of the logarithmic growth phase. The cells were collected using centrifugation at 6000 × *g* and 4 °C for 10 min. The pellet was washed three times in 0.85% sterile saline. Finally, the cells were centrifuged and suspended in the NMP media lacking ammonium sulfate (C:N ratio of 1320:1). Cell concentration was adjusted to 10^8–10^9 CFU mL^{-1}. Starved cells of *Pseudomonas* sp. LBBMA 81A were obtained in the N-limited NMP after incubation for 72 h; *Bacillus subtilis* LBBMA 155 cells were maintained in this medium for 2 weeks, in order to obtain endospores.

Cell and endospore suspensions used for determination of surface characteristics and for electrokinetic transport assays were centrifuged at 6000 × *g* and 4 °C for 10 min and washed three times in 0.85% sterile saline. Finally, cells were suspended in PBS [15] to a cell concentration required for each experiment.

2.3. Cell-surface characteristics

2.3.1. Cell hydrophobicity

Hydrophobicity of N-starved vegetative cells and spores was determined by measuring cell adhesion to a hydrocarbon phase (ABH test), using xylene as apolar phase [15]. Xylene (0.4 mL) was added to a 4.8 mL aliquot of bacterial suspension with DO_{600} at 0.5. Samples were vortexed vigorously for 15 min. The aqueous phase was removed for DO_{600} measurement and cellular hydrophobicity was calculated by the following formula:

$$\text{Hfb} = \frac{DO_0 - DO_f}{DO_0} \times 100 \quad (1)$$

where Hfb is the cell-surface hydrophobicity, DO_0 is the optical density of the aqueous phase before vortexing with the apolar phase and DO_f is the optical density of the aqueous phase after vortexing with the apolar phase.

2.3.2. Cell-surface charges

Cell-surface charges were determined by a modification of the electrostatic-interaction chromatography method described by Pedersen [16] and Gusils et al. [12]. Poli-Prep Biorad (0.8 cm × 4.0 cm) chromatographic columns were filled with 2 mL of PBS-hydrated anionic (Sephadex CM-50) or cationic (Sephadex DEAE-50) exchange resins. For each isolate, a cell suspension in PBS with DO_{600} adjusted to 1.0 (DO_i) was used. The bacterial suspension (0.5 mL) was added to each column, following elution with 3 mL of PBS at 0.2 mL min^{-1}. Eluate DO_{600} (DO_f) was measured and the bacterial distribution coefficient between eluate and column was calculated using:

$$\text{KdC} = \frac{C_c}{C_e} \quad (2)$$

where KdC is the bacterial distribution coefficient between eluate and column, and

$$C_c = \frac{DO_i - DO_f}{V_c} \quad (3)$$

$$C_e = \frac{DO_f}{V_e} \quad (4)$$

where C_c is the cell concentration in the ionic exchange resin, C_e is the cell concentration in the eluate, V_c is the volume of the resin in the column (2 mL) and V_e is the volume of the eluate (3.5 mL).

In this study, it was assumed that the bacterial distribution coefficient between eluate and ionic exchange resin expresses a relative cell-surface charge [17]. Cell surface positive (KdCC) and negative (KdCA) charges are obtained in the cationic and anionic exchange columns, respectively.

2.3.3. Cell adhesion to soil

Adhesion of bacterial cells to soil was evaluated by a modification of the differential centrifugation technique [18]. A 6 mL sample of cell suspension containing 10^9 CFU mL^{-1} PBS was mixed with 6 g of dried sterile soil in a 50 mL polypropylene centrifuge tube. The mixture was vortexed vigorously for 1 min and left to stand for 5 min, followed by an addition of 15 mL of PBS. After mixing at 2000 rpm in an orbital shaker for 1 min, the solution was left to stand for 5 min and centrifuged at 200 × *g* for 3 min. The supernatant was used for determination of viable cell concentration by the drop plate method [19]. A second washing was performed by adding 15 mL PBS aliquot, followed by the same procedure described above, except for the centrifugation time, which was increased from 3 to 5 min. It was assumed that the difference between the number of cells remaining in the soil pellet after the first washing and of cells remaining in the soil pellet after the second washing represents the population of cells with weak adhesion to the soil particles. The cells adhered to the soil after the second wash represent the population strongly adhered to the soil [18].

Distribution coefficient of bacteria in the soil was calculated according to the following equation:

$$\mathrm{KdA} = \frac{C_s}{C_w} \quad (5)$$

where,

$$C_s = \frac{N_t - N_s}{W} \quad (6)$$

$$C_w \frac{N_s}{V} \quad (7)$$

and, KdA is the distribution coefficient ($mL\,g^{-1}$), C_s is the cell concentration on the solid phase ($CFU\,g^{-1}$), C_w is the cell concentration in the supernatant ($CFU\,mL^{-1}$), N_t is the total cell number adhered to the soil sample (CFU), N_s is the total cell number in the supernatant (CFU), W is the soil mass (g) and V is the volume of fluid (mL).

Two cell distribution coefficients were considered in this study: one relative to weakly adhering cells (KdA1), obtained from the first soil washing step, and another relative to strongly adhering cells, obtained after the second washing step (KdA2).

Distribution coefficient is a relative value representing the ratio between the number of bacteria adhering to the solid phase and the bacteria remaining in suspension. This coefficient may discriminate between microbial strains with relatively close adhesion capabilities, since it is expressed in a numerical range varying from zero to infinite [20], as opposed to adhesion percentages, which are expressed within a range between 0 and 100.

2.3.4. Cell diameter

Cell diameter was determined from cell micrographs obtained by scanning electronic microscopy, according to the method described by Luo et al. [21]. Cells were collected in cellulose Millipore membranes (0.22 μm), washed gently with a 100 mmol L^{-1} (pH 7.0) phosphate buffer and fixed with 2.5% glutaraldehyde and a 1% osmic acid solution. The specimen was dehydrated by using sequential ethanol concentrations ranging from 30 to 100% in 20 or 15% increments with 20 min of exposure per concentration, and then the ethanol was replaced by acetate isoamyl ester. After dehydration, samples were dried to the critical point (Bal-Tec, CPD 030) and finally metallised (Balzers, FDU 010) for scanning electronic microscopy (LEO VP1430).

2.4. Soil characterisation

The soil used in this study is a residual soil from gneiss from Duque de Caxias, RJ. Petrographic microscope study of the coarse-grained fraction detected the presence of quartz, feldspars, biotite and garnet. X-ray diffraction using the oriented aggregate method identified the presence of kaolinite, gibsite and traces of illite in the fine-grained fraction. Table 2 presents its physicochemical soil characteristics.

2.5. Electrokinetic cell

The cell (Fig. 1), made of Perspex® was designed to test reconstituted soils. Parts were assembled by means of stainless steel screws and sealed by 'o' rings.

The specimen holder of the reconstituted material cell, shown in Fig. 1(d), consisted of a tube with inner diameter of 38 mm and length of 50 mm. Electrodes were made from stainless steel 316 and to make them work properly, direct contact with the specimen was avoided. They were situated 88.5 mm from the specimen to achieve a homogeneous current density over the end surfaces of the sample. Electrode chambers were connected between the electrodes and the specimen holder and consisted of Perspex® tubes with inner diameter of 40 mm and length of 96.5 mm. At the top of these chambers, PVC couplings connected two graduated 10 cm^3 Pyrex® pipettes, with a 0.1 mL resolution. These pipettes were used to measure electro-osmotic flow.

Table 2
Physicochemical soil characteristics used in cell adhesion assays

Physicochemical soil characteristics	Value
Organic matter ($dag\,kg^{-1}$)	5
Grain density ($kg\,m^{-3}$)	2.73
Grain relative density ($kg\,m^{-3}$)	2.74
pH	4.9
Coarse gravel (%)	0
Medium gravel (%)	2
Fine gravel (%)	2
Coarse sand (%)	5
Medium sand (%)	5
Fine sand (%)	13
Silt (%)	35
Clay (%)	38
Liquid limit (%)	61.2
Plastic limit (%)	39.6
Plasticity index (%)	21.6

2.6. Soil specimen preparation

Reconstituted specimens were prepared using the apparatus exhibited in Fig. 2. It consisted of a perforated plate made of Perspex® that was attached to the specimen holder. At the top of the specimen holder a cylinder made of PVC, with identical inner diameter, was attached. The required stress was accomplished by the placement of weights on the top of a PVC piston that was inserted into the PVC cylinder.

The residual soil, at a moisture content of 1.25 of its liquid limit ω_L (achieved mixing the soil with PBS [15]), was squeezed into the specimen holder and the PVC cylinder. The desired vertical stress was applied by a dead weight system. At the end of consolidation, the specimen was cut to the desired length and it weighed in at an average of 100 g.

The soil probe was placed in the specimen's holder, which was then connected to the electrodes chambers. Filter papers were used at both ends of the specimen to avoid material loss and contamination of the electrode fluids by soil particles. The electrode chambers were then filled with sterile PBS [15]).

2.7. Cell mobilisation by electrokinesis

Bacterial cells or endospores (10^7) were filtered through a 0.22 μm Millipore [15] membrane. After connecting the soil probe reservoir to the electrolytic chambers, the membrane was placed in contact with the soil surface at the cathode end. The soil was submitted to a 5 mA current for 24 h. The current density and time parameters were chosen after the study of Luo et al. [21], in which it was demonstrated that this current during 24 h does not change the surface property of bacteria. Next, the soil probe was removed, cut in five 10-mm sections in a laminar flow chamber and each section was submitted to a bacterial cell count. Each section was homogenised by vigorous vortexing of 4 g of soil in 39 mL of PBS [15] containing 0.1% sodium pyrophosphate for 5 min. Serial dilutions of bacterial suspensions were inoculated in Petri dishes containing NMP mineral medium, and the plates were incubated at 28 °C. Colony counts were performed 48 h after inoculation. For transport efficiency calculations, the cell recovery efficiency of the adopted

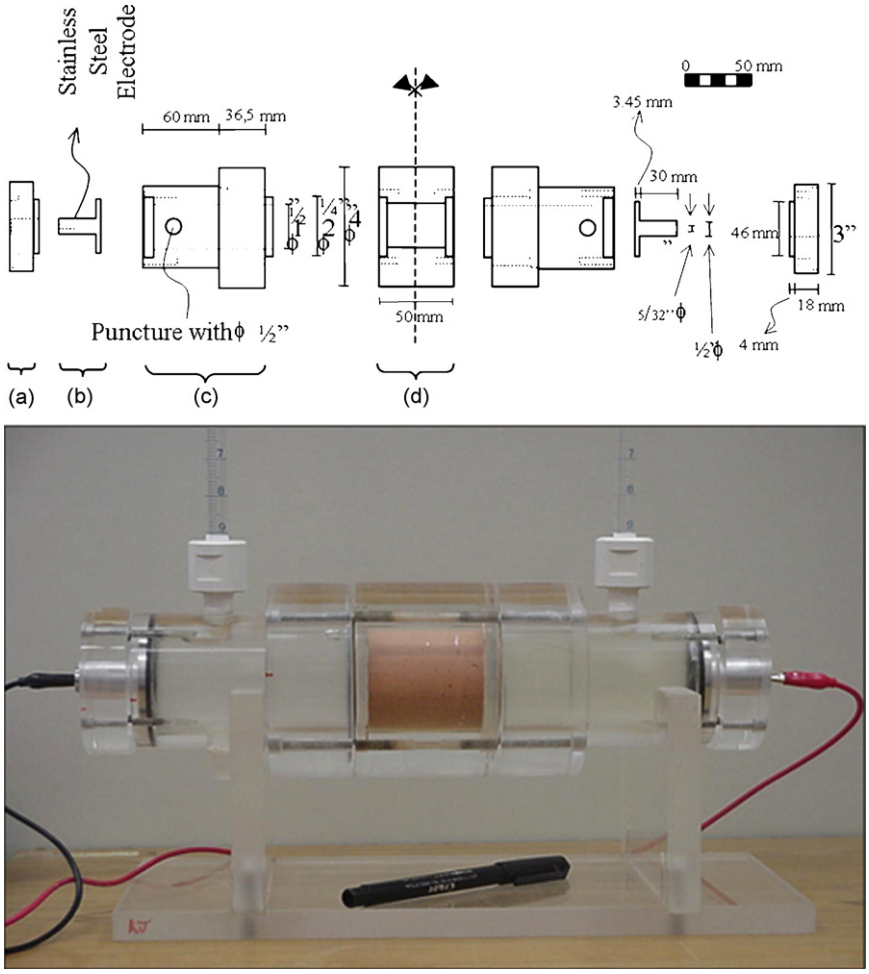

Fig. 1. Electrokinetic cell details: (a) electrode holder; (b) electrode; (c) reservoir (electrolytes); (d) soil sample reservoir. Adpated from Rivas [30]. Bars indicate the standard deviation of the samples.

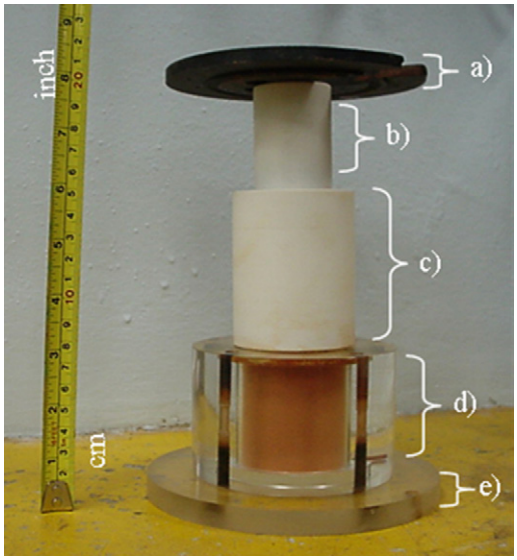

Fig. 2. Apparatus used to reconstitute soil specimens. (a) Weights; (b) piston; (c) PVC cylinder; (d) soil specimen reservoir; (e) perforated plate. Bars indicate the standard deviation of the samples.

plate-counting method (Section 2.9) was used. Additionally, pH (NBR 7953), moisture content (NBR 6457) and soil density of the soil slices were also analysed.

2.8. Quality controls

2.8.1. Survival of the N-starved cell in PBS

This experiment was performed to check the viability of the N-starved cells of *Pseudomonas* sp. in PBS for 24 h, the same time period that the electrokinetic assay lasted. A sample of 10^8 cells was suspended in 10 mL of PBS and counted using the above-mentioned plate-counting method. The cell suspension was sampled each hour in the first 6 h and then every 3 h for the remaining time.

2.8.2. Cell recovery efficiency from the soil

For packed soil control, the soil column was inoculated with 10^7 CFU and submitted to packing, according to the procedures described in Section 2.6. At the end of 24 h, the soil column was fractioned and the recovery of inoculated microorganism was evaluated by the procedures described in Section 2.7. For unpacked soil, 10^7 CFU were mixed with 100 g of soil with 35% humidity. Recovery efficiency of the inoculated cells was checked immediately after soil inoculation and 24 h later.

2.8.3. Contamination control

The electrokinetic cell was made of Perspex®, which cannot be heat-sterilised. This control was performed using the same aseptic

procedures adopted in the electrokinetic assays, except for the addition of bacteria, to verify whether soil sterilisation, soil manipulation or the electrokinetic assay procedures were appropriated to avoid soil contamination.

2.8.4. Passive cell transport in the soil core

This control was performed in order to check cell migration without electrical current application. The assay was conducted under the same conditions described in Sections 2.6 and 2.7, except for the absence of electric current.

2.9. Other determinations

During the electrokinetic studies, the volume of percolated liquid was determined. Additionally, at the end of the 24 h electrokinetic assays, the following characteristics were determined: total energy spent (W), soil humidity (NBR 6457), and pH (NBR 7353) in the soil bed and electrolyte solutions.

2.10. Statistical analysis

A split-plot design was used, with three replicates. Data were submitted to analysis of variance and the means of isolates were compared by F test ($p < 0.05$). The means of cell recovery from control packed soil, unpacked soil, and from packed soil after electrokinesis assays were compared by Tukey's test ($p < 0.05$).

3. Results and discussion

3.1. Cell-surface characterization

Nitrogen-starved cells of *Pseudomonas* sp. LBBMA 81A and endospores of *B. subtilis* LBBMA 155 showed no differences between their cell-surface hydrophobicity (Table 3). Both were characterised as highly hydrophilic. Both N-starved cells of *Pseudomonas* sp. LBBMA 81A and endospores of *B. subtilis* LBBMA 155 were characterised as coccoid-shaped, 0.64–0.67 μm in diameter. N-starved vegetative cells of *Pseudomonas* sp. LBBMA 81A differed from *B. subtilis* LBBMA 155 endospores by having a lower anionic cell surface, slightly higher cationic cell surface and a lower adhesion to soil particles than *B. subtilis* LBBMA 155 endospores (Table 3).

It has been shown that bacterial adhesion may be affected by cell hydrophobicity [11] and by cell-surface charge [12]. Because the cells did not differ in characteristics such as hydrophobicity and cationic charges, the higher adhesion of *B. subtilis* LBBMA 155 endospores to soil particles was attributed to the higher anionic surface charge in these cells (Table 3), compared to N-starved cells of *Pseudomonas* sp. LBBMA 81A.

Modification of cell-surface characteristics in *Pseudomonas* spp. in response to starvation has already been described [22–24].

Table 3
Characteristics of starved vegetative cells of *Pseudomonas* sp. LBBMA 81A and of *Bacillus subtilis* LBBMA 155 endospores

Properties	*Pseudomonas* sp. LBBMA 81A	*Bacillus subtilis* LBBMA 155
Hfb	1.62 (0.50)A	3.29 (1.04)A
KdCC	1.049 (0.010)A	0.125 (0.009)B
KdCA	5.879 (0.251)B	69.49 (10.98)A
KdA1	32.32 (1.54)B	541.4 (8.09)A
KdA2	47.49 (1.52)B	945.6 (34.4)A
CD	0.64 (0.04)A	0.67 (0.02)A

Hfb: Hydrophobicity; KdCC: cationic cell charge; KdCA: anionic cell charge; KdA1: weak adhesion; KdA2: strong adhesion; CD: cell diameter. Values in parenthesis represent the standard errors. Averages followed by the same letter do not differ by the F test ($p < 0.05$).

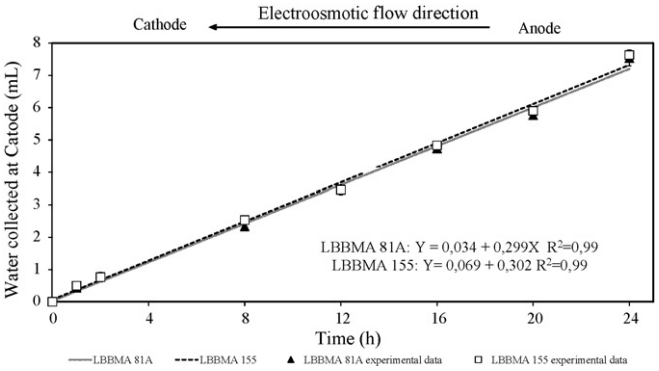

Fig. 3. Percolated liquid volume in electrokinetic transport assays of *Pseudomonas* sp. LBBMA 81A and *Bacillus subtilis* LBBMA 155 isolates. Bars indicate the standard deviation of the samples.

Shrinkage of *Pseudomonas* spp. is well documented in literature [23,24]. This phenomenon increases surface area to volume ratio, making the transport of nutrients into the cell easier and reducing energy consumption for nutrient uptake [22]. Additionally, nitrogen starvation decreases hydrophobicity and cell-surface charge of the genus *Pseudomonas* due to alteration of the quantity and quality of proteins and fatty acids on the cell surface [22]. Nutrient starvation not only causes modification of cell-surface characteristics, but also increases the resistance of the cells to environmental stresses [25]. However, surface characteristics of *B. subtilis* endospores are still poorly reported [26,27]. *B. subtilis* endospores are characterised by a protein-rich coat. This structure confers great resistance against hard environmental conditions. Additionally, it possesses enzymatic activity capable of signalling when environmental conditions for endospore germination are favorable [26,27].

3.2. Electrokinetic physicochemical parameters

There was no difference ($p < 0.05$) in energy consumption during electrokinetic tests with or without bacterial cells. It may be suggested that, under the present conditions, bacterial cells did not alter the electric conductivity of the soil. The average energy spent on each trial was 0.602 W (data not shown).

The volume of percolated liquid increased linearly during the electrokinetic tests as shown in Fig. 3, and it was not influenced by the bacterial strain. An anode–cathode direction of electro-osmotic flow was observed, as in previous studies [28,29]. The observed flow direction probably occurred due to the net negative charge of the clay minerals that constitute a great part of the soil matrix (i.e., kaolinite, Table 2).

The use of electrical fields through soils, without pH control, may elevate the pH gradient throughout the soil matrix, due to the formation of an acid front in the cathode–anode direction [26]. Data obtained in this study demonstrate that PBS at low concentration (0.1 mol L^{-1}) is effective to keep pH values close to 6.5 through most of the soil probe (Fig. 4). High pH values close to the anode electrolyte have already been demonstrated [30]. The cathode electrolyte remained close to the neutrality (6.8 ± 0.08). pH control during electrokinetic application, such as for bioaugmentation or biostimulation, must be considered, since abrupt pH changes may affect cell physiology of autochthonous and allochthonous microbial populations. Additionally, extreme pH values may reduce soil microbial populations considerably [5,6,31].

Moisture content is also a relevant soil physicochemical factor to be considered since metabolic activity and bacterial dispersion through porous medium are dependent on water availability [7,32,33]. There was no difference in moisture content between

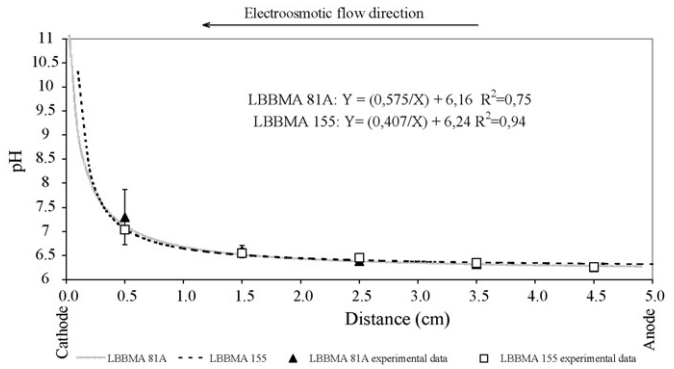

Fig. 4. pH values throughout the sample at the end of starved *Pseudomonas* sp. LBBMA 81A cells and *Bacillus subtilis* LBBMA 155 endospores electrokinetic assays. The distance is expressed from the sample surface facing the cathode electrolyte. Bars indicate the standard deviation of the samples.

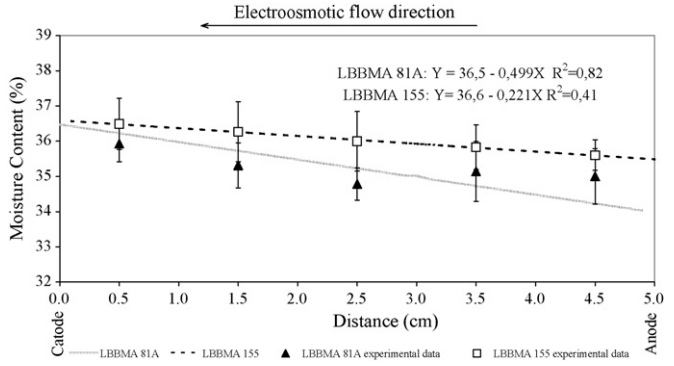

Fig. 5. Sample humidity of *Pseudomonas* sp. LBBMA 81A and *Bacillus subtilis* LBBMA 155 isolates at the end of the electrokinetic transport assays. The distance is expressed from the sample surface facing the cathode electrolyte. Bars indicate the standard deviation of the samples.

electrokinetic tests conducted with N-starved cells of *Pseudomonas* sp. LBBMA 81A or with *B. subtilis* LBBMA 155 endospores (Fig. 5). The analysis of water content in the soil probe used for transport of *B. subtilis* LBBMA 155 showed an elevated error (R^2 = 0.41). This error may be understood by observing the variation of density values across the soil specimens used in the transport tests (Fig. 6). The 60 kPa tension used to prepare the soil specimens was applied on the anode side, which probably caused higher densities close to the cathode side of the soil specimens. Density variation between

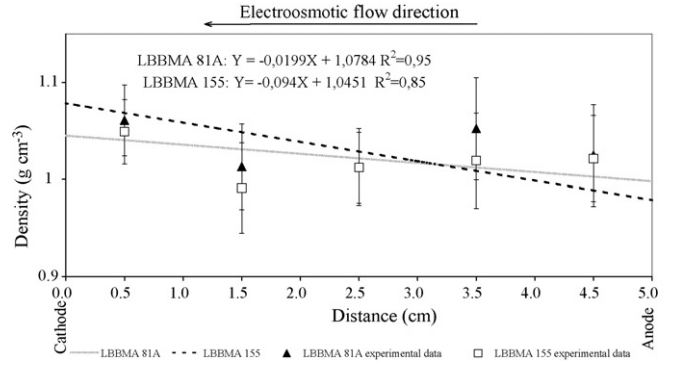

Fig. 6. Apparent density throughout the sample of *Pseudomonas* sp. LBBMA 81A and *Bacillus subtilis* LBBMA 155 isolates at the end of the electrokinetic transport assays. The distance is expressed from the sample surface facing the cathode electrolyte. Bars indicate the standard deviation of the samples.

Table 4
Recovery (% of total added to the soil) of starved *Pseudomonas* sp. LBBMA 81A and *Bacillus subtilis* LBBMA 155 endospores in deformed soil

Genus/species	Isolate	CRC 0 h	CRC 24 h
Pseudomonas sp.	LBBMA 81A	54.5 (1.52)Aa	49.5 (1.36)Ba
Bacillus subtilis	LBBMA 155	44.6 (0.49)Ab	42.7 (0.21)Bb

CRC 0 h: Deformed soil recovery assay, immediately after inoculation. CRC 24 h: Deformed soil recovery assay, 24 h after isolate inoculation. Values in parenthesis represent the standard error. Averages with the same capital letter, within the lines, and small caption, within the columns, do not differ by the *F* test ($p < 0.05$).

anode and cathode was more expressive in the soil probe used in the *B. subtilis* LBBMA 155 transport test (Fig. 6). Higher density implies that pore volume at the cathode side must be lower than at the anode side, and this variation could explain the elevated error obtained for water content along the soil probe (Fig. 5).

3.3. Quality controls

A significant decrease in the viability of the *Pseudomonas* sp. N-starved cell during the 24 h incubation in PBS (data not shown) was not observed.

Cell recovery from soil decreased after 24 h of contact with soil particles (Table 4). This is attributed to an increase in cell adhesion to soil particles, or to a decrease in cell viability [9].

The recovery of N-starved cells of *Pseudomonas* sp. LBBMA 81A during the electrokinetic assay was near 50%; *B. subtilis* LBBMA 155, cell recovery was near 43% (Table 5). These data are similar to the results obtained in the soil adhesion assays (Table 3) and to those of cell recovery from deformed soil samples (Table 4), when a higher adhesion of *B. subtilis* LBBMA 155 endospores to soil particles was demonstrated.

Cell recovery from packed soil samples did not differ from that obtained during deformed soil assays, independent of electric current application (Table 5). Therefore, it can be inferred that neither packing nor electric current affected cell recovery from soil by the method proposed by Jones et al. [15].

The analysis performed to validate the aseptic procedures used during the electrokinetic assays did not detect any contamination.

The assays conducted to verify the passive cell transport under the same conditions of the electrokinetic assays demonstrated that the cells were not transported in the absence of electric current (data not shown). These results indicates that the mobilisation of cells observed during the electrokinetic assays (Section 3.4) were due to the presence of the electric field.

3.4. Cell mobilisation

Electric current caused *B. subtilis* LBBMA 155 endospores and N-starved cells of *Pseudomonas* sp. LBBMA 81A to move through the soil probes (Fig. 7). *B. subtilis* LBBMA 155 endospores, despite their higher adhesion to soil particles (Table 3), were transported with

Table 5
Pseudomonas sp. LBBMA 81A and *Bacillus subtilis* LBBMA 155 isolate cell recovery (% of total added to the soil) in deformed soil assay, packed soil without current application and in electrokinesis transport assay

Genus/species	Isolate	CRC 24 h	RC ESC	RCEE
Pseudomonas sp.	LBBMA 81A	49.5 (1.36)Aa	50.9 (5.27)Aa	50.9 (5.27)Aa
Bacillus subtilis	LBBMA 155	42.7 (0.21)Ab	41.1 (1.75)Ab	44.1 (0.77)Ab

CRC 24 h: Cell recovery from deformed soil 24 h after inoculation. RC ESC: Cell recovery from packed soil without current application. RCEE: Cell recovery from soil in electrokinesis transport assay. Averages with similar capital letter within lines do not differ by Tukey's test ($p < 0.05$); averages with small caption within columns do not differ by the *F* test ($p < 0.05$).

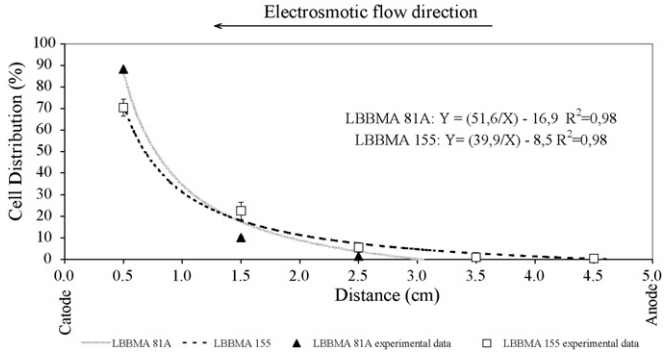

Fig. 7. Cell distribution (% of recoverd cells) and viable cell concentration in different sample sections, of *Pseudomonas* sp. LBBMA 81A and *Bacillus subtilis* LBBMA 155 isolates at the end of the electrokinetic transport assays. The distance is expressed from the sample surface facing the cathode electrolyte. Bars indicate the standard deviation of the samples.

higher efficiency, and were distributed throughout the entire soil probe (Fig. 7). It was shown that endospores of *B. subtilis* LBBMA 155 and N-starved cells of *Pseudomonas* sp. LBBMA 81A have similar dimensions, cell-surface hydrophobicity and positive charges (Table 3), but differ greatly in cell-surface negative charges. The finding that endospores of *B. subtilis* LBBMA 155 are transported more efficiently than N-starved cells of *Pseudomonas* sp. LBBMA 81A indicates that, during application of electric current, cell transport is dictated mainly by the intensity of negative charges on cell surface. Another important finding concerning the mobilisation of the cells is that it occurred against the electro-osmotic flow, which means that the cells were transported by electrophoresis.

During the last decade, different studies demonstrated the feasibility of cell mobilisation through soil matrices [4,6–8]. Recently, electro-osmosis was shown to be a promising phenomenon to deliver cells by electrokinesis in model aquifers [7,8]. This study shows that in clayey soils with low hydraulic conductivity electrophoresis of endospores or starved cell may be an alternative to inoculate bacteria in soils.

3.5. Conclusions

The results of our experiments showed that electrokinesis could be used to transport bacterial cells and endospores through low permeability clayey soils and that in our experimental conditions electrophoresis was the main phenomenon responsible for cell mobilisation.

It was also demonstrated that bacteria with higher negative net on its surface could be better transported throughout the soil matrix when electrophoreses was the major transport phenomenon.

We conclude that electrokinesis could be a promising alternative for bioaugmentation to treat contaminated sites or for microbial transport in microbial enhanced oil recovery.

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