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ORIGINAL PAPER

Electrokinetic remediation of gasoil contaminated soil enhanced by rhamnolipid

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Abstract Electrokinetic remediation (EKR) has been investigated as one of the best technologies in soil remediation but its applications for organic contaminants have been limited due to low solubility of organics in water and their non-ionic nature. The use of biosurfactants may increase the remediation efficiency by increasing the solubility of organics. The purpose of the introduction of complexing substances is to enhance the EKR process forming complexes and/or increasing the electro-osmotic flow. In this study, the removal of gasoil from a soil using electrokinetic method was investigated in the presence of Rhamnolipid at various concentrations. EKR experiments were undertaken on a gasoil contaminated soil (20,000 ppm). Graphite carbon electrodes were used to provide an electrical direct current (ddp 30–60 V). Results showed that increasing the dose of Rhamnolipid, the efficiency of gasoil removal increased up to 86.7%. Moreover, the lower concentration of the gasoil observed in the liquid phase at the higher concentration of the biosurfactant clearly indicated that the Rhamnolipid addition can enhance gasoil biodegradation.

Keywords Electrokinetic · Biosurfactants · Contaminated soils · Gasoil remediation

1 Introduction

Throughout the world, soil contamination has become a widespread and pervasive problem. In order to further protect public health and the environment, pollution must be prevented and sites with existing contamination need remediation. In recent years, increasing interest has been developed for the use of electro-bioremediation, a hybrid technology of bioremediation and electrokinetics for the treatment of soil contaminated with hydrophobic organic compounds.

1.1 Electrokinetic remediation technique

Several studies have demonstrated the effect of an electric field to the soil to improve the removal of organic pollutants [1–4] and have summarized the state of knowledge on physical, physico-chemical [5], engineering [6] and remediation aspects [7]. Electrokinetic remediation (EKR) is substantially based on a low level direct current of the order of some mA cm^{-2}, which crosses the area comprised between electrodes to remove contaminants. The low-level direct current results in physicochemical and hydrological changes in the soil mass, and leads to species transport by coupled mechanisms, such as electroosmosis, electrophoresis, electromigration and water electrolysis. Electroosmosis and electrophoresis are defined as the mass flux of pore fluid and charged particles, under an electric field, respectively. For non-ionic substances electroosmosis is the principal transport mechanism. Electromigration is the movement of ionic species in an electric field to the electrode of opposite charge. Electrolysis reactions at the electrode are needed to be considered together with the mass flux of species. Electrolysis of water produces hydrogen ions in the anodic compartment, which causes an acidic front that advances across the soil towards the

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cathode desorbing contaminants from the surface of the soil particles. But at the same time, the water reduction at the cathode generates a basic front that moves towards the anode which can cause the precipitation of the metal ions in the soil regions close to the cathode and therefore impair their removal. Also both fronts meet at some point in the soil and the neutral pH with low ion concentration can cause a sharp decrease of the soil conductivity. Moreover, these changes in the pH may potentially influencing biodegradation of organic in the soil. Although these limitations, EKR offers the following advantages as compared to the other remediation techniques: (1) simplicity in equipment requirements, (2) safety, (3) wide range of contaminated media, (4) wide range of contaminants, (5) flexibility and (6) cost effectiveness.

1.2 Bioremediation of hydrophobic organic contaminated soils

Bioremediation of soil contaminated by organic chemical pollutants benefits considerably from the use of soil microorganisms to metabolize the organic chemical compounds. Previous studies have demonstrated that the pollutants biodegradation is affected by its bioavailability defined as “the fraction of a chemical in a soil that can be taken up or transformed immediately by living organisms”. Two factors have been considered necessary to determine the chemical bioavailability: (i) the rate of mass transfer of the compound from the soil to the catabolically active cells and (ii) the rate of the compound uptake and metabolism by the cells, which in turn acts as driving force for the mass transfer to the cells. As a consequence of progressive binding with soils, residual organic contaminants are very slowly released so becoming unavailable in order to be biodegraded [2]. In contrast with these limitations, the advantages of bioremediation are its relatively low cost, the mild conditions of the process, and the subsequent low demand for energy or landfill space compared with conventional technologies.

1.3 Use of biosurfactants to improve electro-bioremediation techniques

Removal of organic pollutants can be facilitated through the disruption of the bond established between the contaminant and soil particles [8]. Solvents are the chemical agents that have shown good success in other techniques [9–12] like soil washing. However, the solvents used themselves become pollutants and will require application of treatment and/or recovery technology. Surfactants are chemical compounds containing a hydrophobic and hydrophilic moiety [13]. They are compounds with a hydrophilic head and a hydrophobic tail: the hydrophilic head can be negatively charged (cationic), positively charged (anionic), neutral (nonionic), or zwitterionic (plus and minus charge). The compositional features render them favourable for alignment at interfaces. This fact facilitates removal of inorganic and organic chemical pollutants, through micellar solubilisation and emulsion formation in combination with lowered surface tension [14]. The mechanism by which these changes in interfacial energies are obtained relies on the concentration of the surfactants at the interfaces between immiscible bodies, e.g., liquid–solid, liquid–liquid and vapour–liquid. A correlated relationship exists between the concentration of surfactants and the surface tension. As the concentration increases, the surface tension is reduced until a minimum value. The concentration of surfactants at that point is defined as the critical micelle concentration (CMC) [8, 14]. For this reason surfactants can prove to be very useful in releasing absorbed organic pollutants and in enhancing in situ biodegradation. Although surfactants have been effective in removing contaminants, large quantities of these chemicals are required; the main factor that should be considered when selective extracting agents are used include effectiveness, cost, public and regulatory perception but also biodegradability and degradation products, toxicity to humans, plants, animals and ability to recycle [15]. Biosufactants are synthesized as metabolic products of yeast and/or bacteria [16]. They can be categorized into groups known as glygolipids, phospholipids, fatty acids, neutral acids and are predominantly anionic or neutral. Biosurfactants are amphiphilic [17], one of the group that has been studied extensively is the Rhamnolipids produced by Pseudomonas aeruginosa, is a naturally occurring extracellular glycolipid that is found in the soil and on plants. Rhamnolipids are powerful bio-surfactants that provide a great antibacterial and antifungal activity, and low toxicity levels, which make them an attractive alternative to the known petroleum derived surfactant used in the pharmaceutical industry, the petroleum industry, agriculture, personal care products, animal cleaning products, and other applications. The rapid removal of them from the environment to avoid secondary pollution will make its application more safe and wide [18–20].

The present work studies the feasibility of the use of a biological surfactant Rhamnolipid, in electrokinetic experiments as assisting agent for gasoil.

2 Materials and methods

2.1 Experimental cell

The experiments were performed in a methacrylate prototype, showed in Fig 1, consisting of a central cylindrical

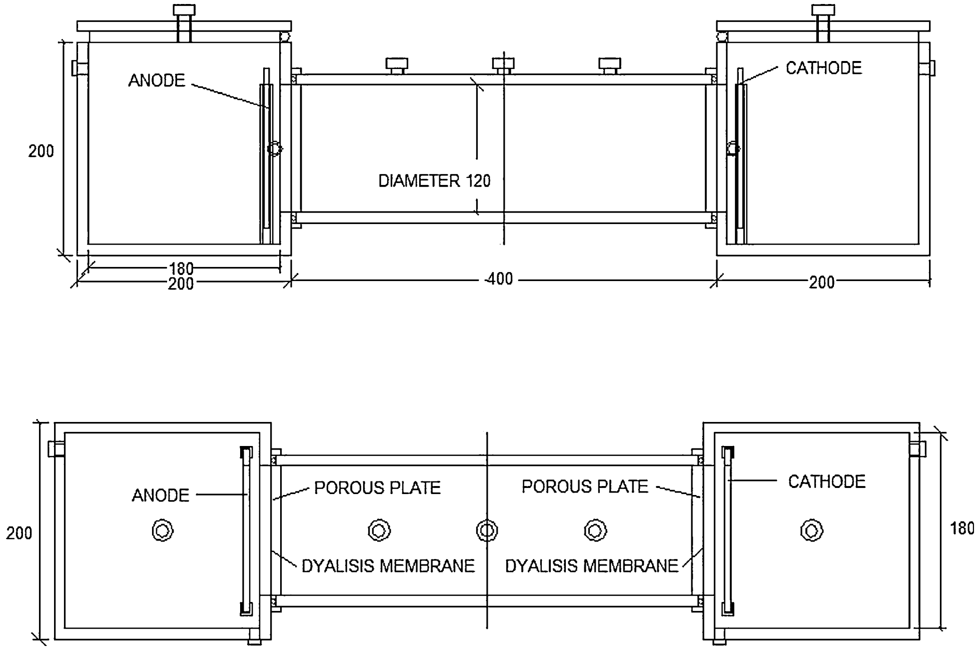

Fig. 1 Methacrylate prototype—dimensions are in mm

cartridge (inner diameter 120 mm, length 400 mm), two square electrode compartments (with about 6 L of working volume) filled up with electrolyte solution (0.1 N of citric acid) and a power supply. Two square graphite electrodes, placed in each compartment, were used as the working electrodes. A sand bed, a porous plate and a dialysis membrane at either end separated the central cartridge from the ionic solution which contained the anode and the cathode in order to prevent the passage of soil into the neighbouring chambers. Three graphite rods to measure the voltage were placed along the soil sample.

2.2 Soil

The soil used was taken from Alcalá de Henares (Central Spain) and its properties, measured in previous studies, are summarized in Table 1.

Prior to use, the soil was sieved (<2 mm) and then (4.3–4.5 kg) added in layers to each of four cartridges and lightly compacted. Then it was made up to a moisture content of 20% using a mix of distilled water, gasoil and Rhamnolipid (4% w/v of Rhamnolipid in culture broth from Jeneil Biosurfactant Co., LLC [21] provided by Idrabel Italia) at different concentrations.

2.3 Experimental set up

Table 2 shows all the experimental conditions for each test. The treatment time was of 15 days for the tests A and of 10 days for the tests B. The test 1, 3 and 4 were conducted in the same conditions. Only for the experimental set A, the test A2 was performed at 60 V.

Table 1 Soil properties

Parameters	Soil
Porosity (%)	19.25
Real density (g cm^{-1})	1.81
USDA classification	Sandy clay loam
pH	8.06
Soil respiration rate (mg CO_2 5 g $soil^{-1}$ h^{-1})	1.2
Soil β-galactosidase activity (μmol *p*-nitrophenol g $soil^{-1}$ h^{-1})	0.0122
$CaCO_3$ (%)	5.6
N (%)	0.08
O.M. (%)	1.18
Olsen-P (mg kg^{-1})	16
Ca (mg kg^{-1})	4826
Mg (mg kg^{-1})	634.5
Na (mg kg^{-1})	72.55
K (mg kg^{-1})	382.5

The gasoil used in the experiments is defined as Industrial gasoil, Gas oil no. 2, according to European Directive about Dangerous substances 67/548/EEC (Anexo I, no. 649-224-00-6).

2.4 Analytical measurements

During the treatment the electrolyte pH and conductivity and the voltage between anode, cathode and lengthways the cartridge were measured daily. After 15 days, a part of the samples were dried at 60 °C in order to measure changes in

Table 2 Experimental conditions

Test	Gasoil (ppm)	Rhamnolipid (g/kg)	Time (days)	Voltage (V)
A1	20,000	1	15	30
A2	20,000	1	15	60
A3	20,000	2	15	30
A4	20,000	0 (Control)	15	30
B1	20,000	1	10	30
B3	20,000	2	10	30
B4	20,000	0 (Control)	10	30

soil parameters: pH, conductivity, humidity and nutrient content [22]. The nutrients content was measured in the final electrolyte solutions, too. Gasoil content was analyzed with Dexsil's PetroFLAG equipment using wet samples [23]. For the test B, samples were prior centrifuged at 4,000 rpm and then the soil and the supernatant (liquid phase) was analyzed separately by the same procedure in order to consider which fraction of gasoil was desorbed from the soil to the liquid contained in the cartridge. The organic carbon content was measured by wet oxidation, by Walkley and Black's method [24]. The rate of organic matter was calculated multiplying the percentage of carbon by the Van Bemmelen's factor [25]. The respiration rate and the *β*-galactosidase enzymatic activity were also measured in the soil at the end of the process. These biological properties can be used as indicators of the soil degradation since they are more sensible than the chemical–physical properties to the changes produced by the anthropic activities and the environment. The respiration rate was determined using BacTrac analyzer, bases itself on an impedance method [26, 27], while, in order to measure the *β*-galactosidase activity, the Apte and Batley's method was used [28].

3 Results and discussion

3.1 pH and conductivity trend

The trends of pH at cathode compartment for the tests A and B are shown in Figs. 2 and 3. As expected, the pH at

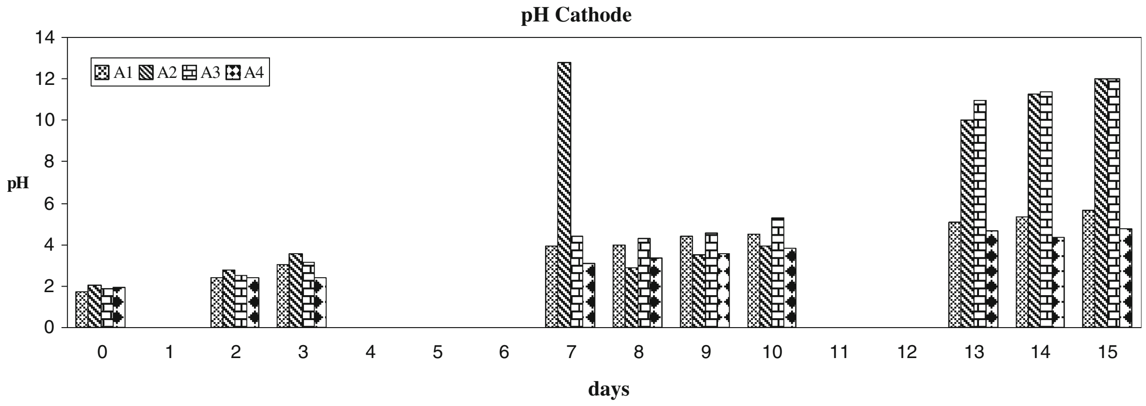

Fig. 2 pH of the electrolyte solution at cathode compartment: test A

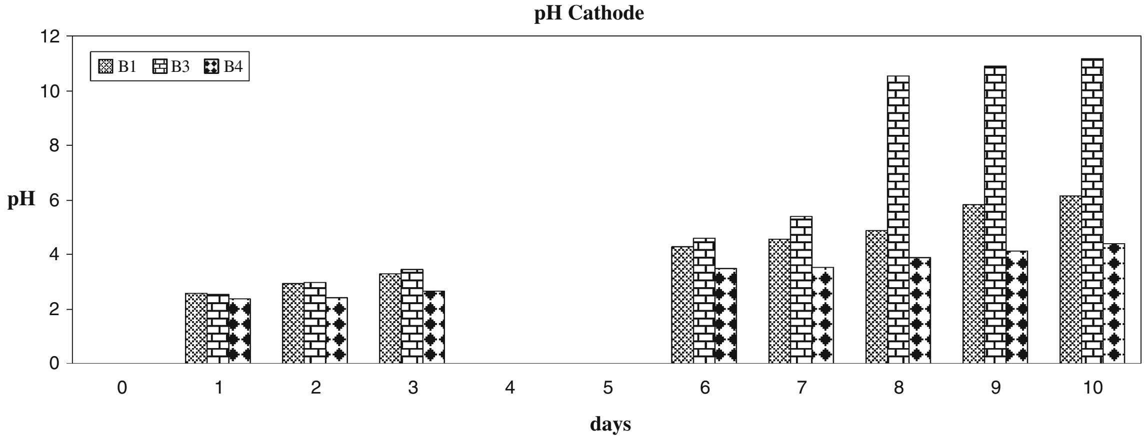

Fig. 3 pH of the electrolyte solution at cathode compartment: test B

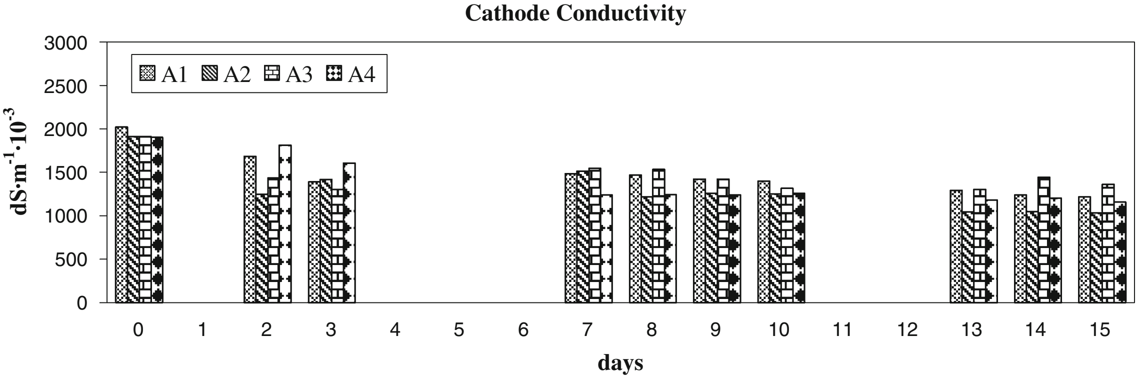

Fig. 4 Conductivity of the electrolyte solution at cathode compartment: test A

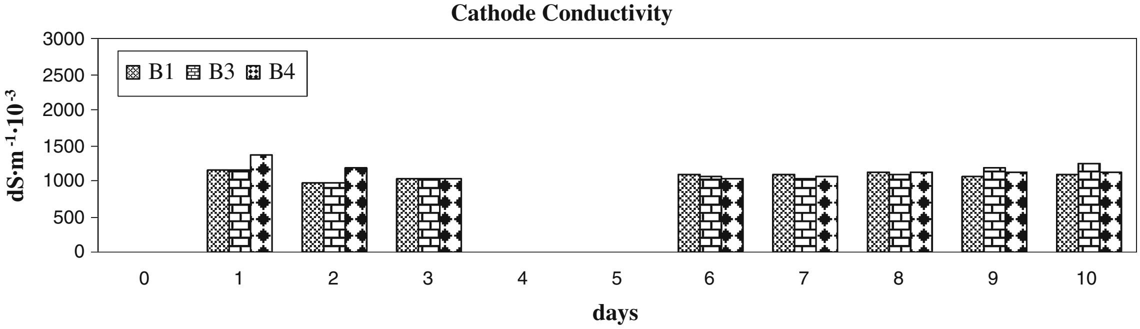

Fig. 5 Conductivity of the electrolyte solution at cathode compartment: test B

cathode compartment risen during the time up to 12 because of the electrolysis of the water at the cathode, with the production of OH^-. As showed in Fig. 2, at cathode compartment for the test performed at 60 V, just before one week the pH was risen up to 12 so it was necessary to restore the normal value, replacing the acid solution, in order to go on with the treatment.

At the same time a great variation of conductivity was not observed, as showed in the Figs. 4 and 5 (from 2.020 dS m^{-1} up to 1.034 dS m^{-1} for the tests A and from 1.357 dS m^{-1} up to 0.962 dS m^{-1} for the tests B). This fact can be attributed to separate effects: the precipitation, in the form of salts, of selected cations of the soil, and the smaller relative conductivity of the ions OH^- compared to ions H^+.

At anode compartment the trends of pH and conductivity were quite different: the pH did not show an appreciable variation, as showed in the Figs. 6 and 7, whilst a great change in conductivity was observed, as showed in the Figs. 8 and 9.

The initial pH of the electrolyte solution was about 2, so it was very difficult to appreciate the changes caused by the production of H^+, due to the electrolysis of water at the anode.

At the same time, as result of the high relative conductivity of H^+ ions, a great increase of conductivity was observed at anode compartment (from 1.912 dS m^{-1} up to 4.890 dS m^{-1} for the tests A and from 1.439 dS m^{-1} up to 2.760 dS m^{-1} for the tests B). Also in the case of conductivity, for the test A2, after one week of treatment it was necessary to replace the acid solution owing to the too high value reached. As showed in the Fig. 4, after a week, a high conductivity was reached also for the test A3 but in this case the value remained quite stable until the end of the experiment.

3.2 Chemical and biological properties

One of the principal aims of new remediation techniques is to base themselves on non invasive technologies in order to take in account the future use of the soil after the remediation: “Remediation shall consist of actions on the soil aimed at the removal, control, containment or reduction of contaminants so that the contaminated site, taking account of its current use and approved future use, no longer poses any significant risk to human health or the environment” [29]. For this reason is important to use a treatment that preserves the principal characteristics of the soil, such as enzymatic activity, respiration rate and content of nutrients as much as possible. Regarding this, as showed by a comparison between Tables 1 and 3, the principal

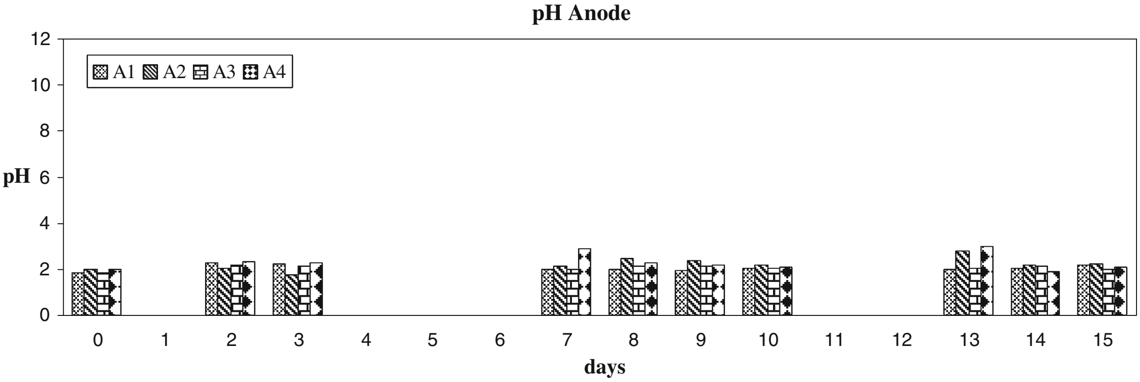

Fig. 6 pH of the electrolyte solution at anode compartment: test A

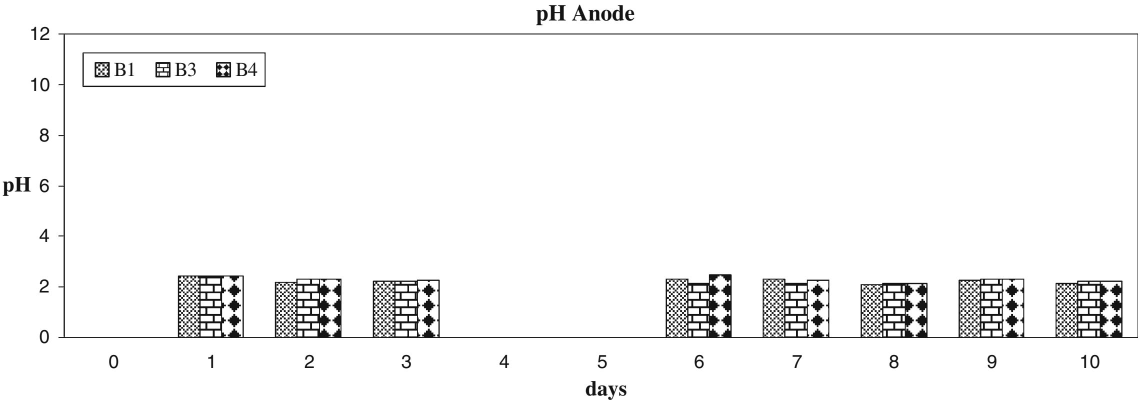

Fig. 7 pH of the electrolyte solution at anode compartment: test B

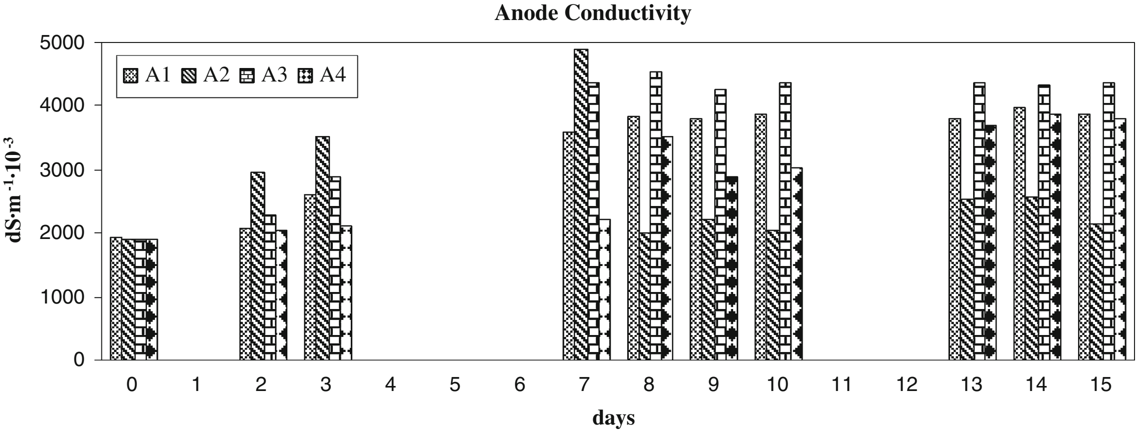

Fig. 8 Conductivity of the electrolyte solution at anode compartment: test A

characteristics of the soil, nitrogen, carbonates, respiration rate and *β*-Galactosidase activity, were quite stable. This unchanged condition clearly indicates that the treatment had none negative impact on the soil from the point of view of the microbial activity and the content of nutrients in the assayed conditions. An increase of the organic matter content was observed, probably due to the effect of the gasoil and of the mixture of Rhamnolipid and its culture broth.

The content of cations in the soil after the treatment can be evaluated from two different points of view: the presence in the final catholyte solution of cations extracted

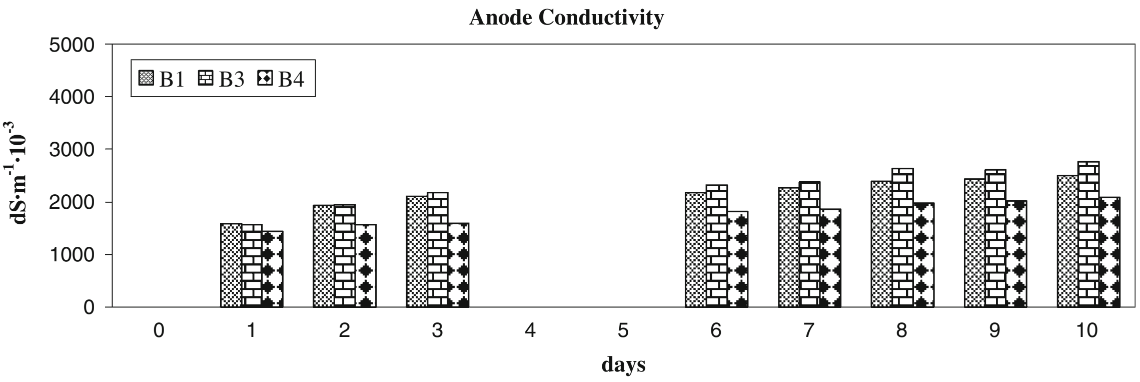

Fig. 9 Conductivity of the electrolyte solution at anode compartment: test B

Table 3 Soil characteristics at the end of the treatment

	A1	A2	A3	A4	B1	B3	B4
O.M. (%)	3.15	3.22	3.12	2.11	3.32	3.12	3.11
Carbonates (%)	5.3	5.4	5.3	5.6	5.1	4.7	4.8
Respiration rate (mg CO_2 5 g soil^{-1} h^{-1})	1.3	1.45	1.2	1.2	1.3	1.65	1.7
N (%)	0.08	0.08	0.08	0.08	0.08	0.08	0.08
β-Galactosidase activity (µmol *p*-nitrophenol g soil^{-1} h^{-1})	0.0345	0.0158	0.0324	0.0327	0.0317	0.0324	0.0294

Table 4 Cations concentration in the final electrolyte solutions at cathode compartment

	Tests						
	A1	A2	A3	A4	B1	B3	B4
K (mg L^{-1})	34	52.7	35.4	18.3	24.04	36.58	18.24
Na (mg L^{-1})	138.2	152.4	200.5	41.1	136	224	48.5
Ca (mg L^{-1})	323.6	1185.1	553	528.7	456	611	491
Mg (mg L^{-1})	132.1	263.4	160.9	153.6	147	134	143

Table 5 Cations concentration in the final electrolyte solutions at anode compartment

	Tests						
	A1	A2	A3	A4	B1	B3	B4
K (mg L^{-1})	0.39	0.17	0.04	0.11	n.d.	0.14	0.09
Na (mg L^{-1})	1.04	0.39	0.7	0.27	0.1	0.34	0.26
Ca (mg L^{-1})	3.13	0.4	1.6	0.21	0.26	0.3	0.18
Mg (mg L^{-1})	0.6	0.17	0.42	0.04	0.005	0.06	0.07

from the soil and the content of cations in the soil matrix after the treatment. The concentration of cations in the electrolyte solutions at cathode and anode compartments at the end of the treatment are shown in Tables 4 and 5. These quantities represent the available fraction of the cations that were mobilized during the process and passed in the liquid phase.

In the Table 6 the concentrations of the cations in the available fraction of the soil at the end of the process are shown: this final value is constituted partly by the available fraction that was not mobilized from the soil and partly by the unavailable fraction that was extracted and passed in the available fraction, due to the effect of the direct current on soil aggregates [30, 31].

In conclusion, despite the high concentrations found in the electrolyte solutions at cathode compartment, the net loss is not so high because the cations mobilized from the available fraction were replaced by the cations extracted from the unavailable or residual fractions. Phosphorus content increases in the soil treated with the biosurfactant due to the organic matter supply in these treatments. No effect in the values of this parameter was observed when the process is applied without rhamnolipid.

3.3 Gasoil remediation

As regards to gasoil remediation, Table 7 shows the results obtained in the tests A and B, respectively. The removal efficiency was calculated with the Eq. 1.

Table 6 Cations concentration in the initial and final soil

	Tests							
	Initial soil	A1	A2	A3	A4	B1	B3	B4
Na (mg L^{-1})	72.6	21.9	19.9	22.5	22.4	21.4	24.2	15.1
Mg (mg L^{-1})	634.5	467.5	307.6	435.4	472.3	405	353	395
K (mg L^{-1})	382.5	298	321	287	250	257	248	228
Ca (mg L^{-1})	4826	4450	4732	3061	2755	4339	4082	4574.5
P (mg L^{-1})	16	27	23	27	16	27	29	18

Table 7 Removal Efficiency and supernatant concentration

Test	A efficiency (%)	B efficiency (%)	B supernatant (mg L^{-1})
1	54.8	75.0	356
2	51.4	–	–
3	84.6	86.7	298
4	39.1	65.9	560

Equation 1: Removal Efficiency

$$\text{Efficiency}\,(\%) = \frac{20000\,(\text{ppm}) - \text{final concentration in the sample}\,(\text{ppm})}{20000\,(\text{ppm})} \times 100$$

For the set A of test, as expected, increasing the dose of Rhamnolipid, the efficiency of gasoil removal increased: from 39.1% in the test performed without the biosurfactant (Rh) up to 84.6% in the test with the higher dose. The gasoil removal was due both to the electroosmosis provoked by the current and the enhanced of gasoil solubilisation induced by Rhamnolipid. Regarding the test A2, the one performed at 60 V, taking in account that its removal rate of gasoil was lower than the prototypes performed at 30 V and the loss of cations at the end of treatment was comparable with the other experiments, we can conclude that it is useless to increase the voltage without benefits and with higher consumption of energy and electrolyte solution. The same trend of the tests A for removal efficiency was observed in the tests B, though higher values (up to 86.7%) were generally calculated; in this series, as mentioned in Sect. 2.4, samples were prior centrifuged and then the soil and the supernatant (liquid phase) were analyzed separately in order to consider which fraction of gasoil was desorbed from the soil to the liquid contained in the cartridge.

Although Rhamnolipid increased gasoil solubility, results, reported in Table 7, show that the concentration of the gasoil in the supernatant, so in the liquid phase, was lower at the higher concentration of the biosurfactant. Previous studies [32–34] showed that the way how and the extent to which degradation of hydrocarbon is enhanced by Rhamnolipid depends on the availability of the substrate. It was demonstrated [35–37], that Rhamnolipid enhanced two different processes that are relevant for remediation of soil: the mass transfer of entrapped or residual substrate from matrices to the aqueous phase and the biodegradation of the substrate as a separate liquid phase. Some authors [38] demonstrated that Rhamnolipid may have enhanced uptake of hydrocarbons either by enhancing uptake of dissolved, emulsified or solubilised substrate by the cells or enhancing uptake of substrate from hydrocarbon droplets after promoting attachment of the cells to these droplets. Alternatively, Rhamnolipid may extract LPS (lipopolysaccharide) [33, 35, 39], an important hydrophilic component of the cells surface, and thereby increase attachment of cells to hydrocarbon droplet.

4 Conclusions

In this study the electrokinetic removal of gasoil was evaluated through the application of Rhamnolipid as biosurfactant. Gasoil removal (up to 86.7%) was due both to the electroosmosis provoked by the current and the enhancement of gasoil solubilisation induced by Rhamnolipid. Furthermore, Rhamnolipid can also enhance gasoil biodegradation through two mechanisms: the first is the increasing of hydrocarbon solubility thus increasing the bioavailability to degrading cells and the second is the interaction with the cells, causing their surface to become more hydrophobic and associate more easily with hydrophobic substrates. Regarding the final condition of the soil, from the point of view of its future use, the principal microbiological characteristics of the soil, enzymatic activity and respiration rate, appear unchanged as well as the content of nitrogen, carbonates and organic matter; furthermore the net content of salts, disguised as cations, did not decrease after the treatment: the loss of cations of the available fraction, extracted from the soil and passed in the liquid phase, was offset by the extraction of the cations from the unavailable fraction into the available fraction. In this sense, electro-bioremediation can be suggested as a friendly environmental technology for soil remediation.

5 Further development

The next stage of this study will be focused on the check of the hypothesis developed in this stage such as the analysis of the intermediate products produced by the degradation of gasoil and the monitoring of the microbial activity, for example measuring the consumption of oxygen content in the prototypes. In the case of Rhamnolipid a potential degradation rate of this component will be measured at the end of the treatment in order to verify if the biosurfactant is partly degraded in the time of the treatment. Further development are focused in the production of the surfactants by autochthonous microorganisms, since the cost of the commercial product could be limit the application of this technology in field experiment.

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