

Biosurfactant Facilitated Emulsification and Electro-Osmotic Recovery of Oil from Petrochemical Contaminated Soil

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Petroleum industries are burdened with the problem of handling petroleum products, petroleum waste products and refinery byproducts such as large quantities of oil waste. Improper management of these products and their wastes present an environment hazard when they end up in the atmosphere, water and land due to their hazardous constituents. An evaluation to determine the possibility of enhancing the electrokinetic process by application of a biosurfactant producing strain for remediation of petroleum contaminated soil through oil recovery and hydrocarbon degradation was studied at a bench scale. A DC powered electrokinetic reactor consisting of electrode/electrolyte compartments and a medium chamber was used under voltage variations of 10 V and 30 V with an electrode spacing of 185 mm. Biosurfactant with its producing microbes and biosurfactant free cells were introduced in the soil chamber after which the reactor was left to run for 10 days under the electric field. The technology was able to achieve the highest oil recovery of 75.15 % from the soil in 96 hours at 30 V. The microorganisms were able to survive under the electric field there by leading to further reduction of the carbon content in the reactor.

1. Introduction

The petroleum industry is a diverse and vital part of the global economy. The petroleum industry, also known as the oil industry includes the global processes of exploration, extraction, refining, transporting and marketing. The products and wastes generated from the production or use of such products are usually composed of petrochemical pollutants such as petroleum hydrocarbons (PHC's). These are generally classified into four fractions, including aliphatics, aromatics, nitrogen sulphur oxygen (NSO) containing compounds, and asphaltenes. The greatest concern regarding contamination by hydrocarbons lies in the mutagenic, carcinogenic and toxic characteristics of such contaminants (Caravaca and Roldán, 2003). Environmental pollution by oil containing substances may have prolonged effects after the contamination event with some ecosystems such as mangrove swamps and salt marshes experiencing the effects for decades after the event (Kingston, 2002).

In the recent years the electrokinetic process has been advancing into a promising technology that has the potential to remove organic pollutants from contaminated soil much as it requires more innovative improvements to be effectively applied extensively on a field scale (Popov et al., 2008). The electrokinetic method employs the use of a low-intensity direct current across an electrode pair on each side of a porous medium, causing electro-osmosis of the aqueous phase, migration of ions and electrophoresis of charged particles in the colloidal system to the respective electrode, which depends on the charge of ions and particles (Yang et al., 2005). The application of the electric field leads to the movement of colloidal particles and solid phase towards the anode area as a result of electrophoresis while the separated liquid phase (water and oil) moves towards the cathode area as a result of electroosmosis. In such for electrokinetic process improvement, Electorowicz and Hatim (2000) argue that the electrokinetic treatment performance can be affected by several factors such as resistance, pH, electrical potential, and spacing between electrodes and suggest that this process may be improved through the use of surfactants or reagents to increase the contaminant removal rates at the electrodes. Mulligan (2009) highlights that a surfactant is usually an amphiphilic compound whose molecule consists of a hydrophobic tail and a hydrophilic tail. The hydrophilic tail

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makes surfactant molecule dissolve in the water phase and increases solubility of PHCs, while the hydrophobic tail makes it tend to gather at the interfaces to decrease the surface or interfacial tension and thus enhance the mobility of PHCs. Surfactants are mainly applied to increase the solubility and mobility of the contaminants in the electrokinetic system (Reddy and Saichek, 2004). Due to low solubility and hydrophobicity properties of organic contaminants, it is usually complex to remove them from a solid matrix unless a surfactant is applied to act as a flushing agent (Wang et al., 2007). Through micellisation, surface tension reduction, solubilisation and increased adsorption, surfactants increase the rate of contaminant removal by altering the surface properties of the matrix leading to an enhanced electroosmotic flow (EOF) (Gomes et al., 2012). The use of synthetic surfactants is however associated with a range of problems such as environmental toxicity and resistance to biodegradation (Mulligan et al., 2001). As compared to chemical surfactants, biosurfactants have received increasing attention since they exhibit greater environmental compatibility, more diversity, better surface activity, lower toxicity, higher demulsification ability, higher selectivity, and higher biodegradability (Bezza and Chirwa, 2017). The aim of this study is to evaluate the possibility of using biosurfactants and biosurfactant producing microbes as an enhancement for the electrokinetic process in oil recovery and bioremediation.

2. Methodology

2.1 Petroleum contaminated soils

The soil used in these experiments composed of 71 % sand, 20 % silt and 9% clay obtained from Pretoria, South Africa. The soil had initial total organic carbon content of 4.03 % and particles sizes of 74.13% > 425 μm , 21.45 % between 425-300 μm and 4.42 % < 300 μm . This soil was sieved using a 2 mm sieve to remove large coarse materials such as leaves and stones. The soil was then spiked with waste oil obtained from a tribology laboratory at the University of Pretoria to achieve 150 mL/kg of soil contamination after homogenous mixing using an overhead stirrer and kept for 14 days before experiments.

2.2 Microbial culture, media and growth conditions

Plate count agar, nutrient agar and nutrient broth were prepared by dissolving the amounts indicated on the bottle in distilled water followed by autoclaving at 121 °C in order to sterilize for 15 min. The agar was poured on to the agar plates between 40-50 °C. The pure microbial culture of *Pseudomonas aeruginosa* used in this study was sourced from a sample of petrochemical contaminated soil in South Africa and identified using the 16S ribosomal RNA (rRNA) sequencing as reported by Lutsinge and Chirwa (2018). The mineral salt medium (MSM) sterilized by autoclaving at 121 °C for 15 min was used for the growth and production of biosurfactants. The medium was prepared as was reported by Trummler et al. (2003) by dissolving in 1 L of distilled water: 6.0 g $(\text{NH}_4)_2\text{SO}_4$; 0.4 g $\text{MgSO}_4 \times 7\text{H}_2\text{O}$; 0.4 g $\text{CaCl}_2 \times 2\text{H}_2\text{O}$; 7.59 g $\text{Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$; 4.43 g KH_2PO_4 ; and 2 mL of trace element solution.

2.3 Experiment set up

A pure strain of *pseudomonas aeruginosa* was inoculated in 150 mL of nutrient broth for 24 hours. The cells were obtained from the broth by centrifugation at 9000 rpm at 4 °C for 10 minutes. 30 g of the cells were used in experiments where biosurfactant free cells were required. On the other hand, in experiments where cells with biosurfactant were required, 3 % (v/v) of the microbial suspension was transferred to 250mL of mineral Salt medium supplemented with oil and incubated on rotary shakers for 144 hrs. The isolate was then tested for biosurfactant production before being transferred to the electrokinetic reactor to make sure that cells had started producing biosurfactants. Under both conditions, the inoculum was mixed with the contaminated soil using an overhead reactor for 30 minutes to obtain a homogenous mixture.

The inoculum was screened using the drop collapse method and the oil spreading test to confirm biosurfactant production. In the drop collapse method, 2 L of mineral oil was added to each well of a 96-well micro titer plate. The plate was equilibrated for 1 h at room temperature, and then 5 μl of the culture was added to the surface of oil (Bodour and Miller-Maier, 1998). The shape of the drop on the surface of oil was inspected after 1 min. The result was negative if the drop remained beaded while the result was positive if the drop collapsed. Cultures were tested in triplicate. Oil spreading test was done as described by Morikawa et al. (2000) in which 50 mL of distilled water was added to a large petri dish (25 cm diameter) followed by the addition of 20 μl of oil to the surface of the water. 10 μl of culture were then added to the surface of oil. The diameter of the clear zone on the oil surface was measured and related to the concentration of biosurfactant. Mineral salt medium and distilled water without cells were used as controls for both screening tests.

2.4 Electrokinetic set up

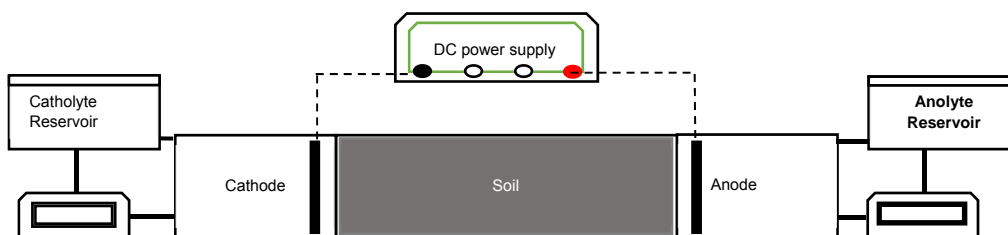


Figure 1: Schematic view of the electrokinetic reactor

4 experiments were carried out under the conditions shown in the table 1. 2000 g of soil spiked with oil was treated for all the experimental conditions described in triplicates. The electrokinetic reactor was meticulously constructed from acrylic glass to make 3 compartments; a soil compartment (160.5 mm × 150 mm × 150 mm) and two electrode compartments (150 mm × 90 mm × 150 mm) so that one of them constituted the anode and the other one the cathode with outlets to electrolyte overflow reservoirs. Graphite electrodes (100 mm long × 20 mm diameter) were located into the electrode compartments at specified distances apart and connected to the DC power supply (0-30 V, 0-3 RS-IPS 303A). Distilled water was used as the electrolyte with the electrode-medium compartment interfaces fixed with Whatman microfiber glass filters (GF/A) to allow electroosmotic flow across the cell. The medium compartment was divided into seven sections normalized to the nearest cathode to allow measurements of pH, bacterial counts and total carbon. Electroosmotic flow, pH, current measurements and bacterial counts were made every after 24 hours. To determine the number of viable cells, 10 mL of an aliquot were picked from each of the seven sections in the soil compartment at 10 mm, 30 mm, 50 mm, 70 mm, 100 mm, 130 mm and 160 mm normalized distances from the cathode including samples from the anode and cathode compartments every after 48 hours to determine colony forming units (CFU) at each section as described by (APHA, 2005).

Table 1: Experimental conditions

Experiment	Microbial conditions	Voltage (V)	Distance between electrodes	Time to run experiment (hours)	Wire Material used in connections
A	250 mL of biosurfactant + cells	30	185 mm	240	Copper
B	250 mL of biosurfactant + cells	10	185 mm	240	Copper
C	30 g of bacterial cells	30	185 mm	240	Iron
D	30 g of bacterial cells	10	185 mm	240	Iron

2.5 Total Carbon analysis

Solid samples were picked from each of the seven sections in the soil compartment at 10 mm, 30 mm, 50 mm, 70 mm, 100 mm, 130 mm and 160 mm normalized distances from the cathode after 240 hours. The samples were air dried for 5 days and grinded to the smallest particles using a mortar and pestle. The fine samples were ready for analysis in the Shimadzu Total Organic Carbon Analyzer after they were sieved to remain with particles small enough to go through a 600 µm mesh. The solid sample boats were decontaminated of carbon residue by brush washing under flowing tap water followed by rinsing with distilled water. The boats were then soaked in 2 M hydrochloric acid for 10 minutes and heated in a furnace at 900 °C for 10 minutes and left to cool before running a sample.

3. Results and Discussions

3.1 Oil Recovery

Oil was observed to coalesce vertically in the soil compartment as it oozed out of the solid matrix. The highest oil recovery comparing experiments run under different voltages was observed in experiment A and B both of which were under application of biosurfactants in the first 96 hours as compared to those in which biosurfactant free cells were inoculated as seen in table 2 and 1. Most of the oil recovered remained in the soil compartment after 96 hours with miniature amounts moving as part of the electroosmotic flow to both the anode and cathode compartments. The volume of the electrolyte increased in the cathode compartment as it reduced in the anode compartment indicating that the electroosmotic flow was towards the cathode with dominance of water and very low oil volume. With the surface charge of soil being predominantly negative, the electroosmotic flow is expected to flow towards the cathode (Yang et al., 2005). The net negative charge on

the soil surface is as a result of both the variable and permanent charge emanating from the ionisable hydrogen ions and isomorphous substitution respectively. The variable charge is therefore dependant on solution pH since it varies depending on the sorption and desorption of H^+ and OH^- ions on the soil surfaces from the pore fluid (Park et al., 2009). With oxidation and reduction reactions happening in the electrode compartments, there is a formation of the acid front at the anode and an alkaline front at the cathode on immediate application of an electric field but as ions start migrating, the pH dynamically changes across the system as the H^+ ions move towards the cathode; This explains why the electroosmotic flow towards the cathode reduced as the acid front moved further away from the anode area towards the cathode with a possibility of reversed electroosmosis (from cathode towards the anode) due to the change in the soil surface charge influenced by reduction in pore fluid pH. The experiments were however stopped before the acid front covered more than 15 mm from the anode compartment to observe a significant increase in the volume of the anolyte as a result of reversed EOF.

Table 2: Volume of oil recovered in the soil compartment and that transferred to the electrode compartments due to electroosmotic flow in 96 hours

Experiment	Anode (mm ³)	Soil Compartment (mm ³)	Cathode (mm ³)	Total Oil Recovery (%)
A	54000	144450	27000	75.15
B	13500	96300	13500	41.10
C	27000	96300	27000	50.10
D	27000	72225	13500	37.58

From equation (1) it can be concluded that the higher the viscosity of the liquid the lower the electroosmotic flow; With viscosity of water being generally low than that of oil, it can explain why the electroosmotic flow was more dominated by water as compared to oil leading to horizontal stagnation of most of the recovered oil in the soil compartment instead of moving into the electrode wells. This is also in agreement with Yang et al. (2005) who argues that the process of electroosmosis can be affected by viscosity and molecular size of the water or oil. The larger the size of the molecules the lower the electroosmotic rate since the liquid phases may not easily go through the filter to the electrode chambers. This can affect the rate of oil recovery opposed to dewatering as oil has larger molecules which means it's out competed by water which has smaller ones. The difference in the oil recovered in the first 96 hours can only be explained by the activity of the biosurfactant which demulsified the contaminated soil leading to more oil recovered as compared to when cells were inoculated into the reactor. The oil recovered in experiment C and D was basically because of electro-demulsification since the two experiments failed the biosurfactant test in the first 144 hours. The anolyte in the system became more turbid with time due to the movement of colloids towards the anode well; a process known as electrophoresis. These coagulated and sedimented in the compartment forming a very observable yet so distinct difference between the anolyte and the catholyte since the catholyte was quite clear.

3.2 Voltage and Current

The highest applied voltage of 30 V produced the highest current of 2.44 mA as compared to the lower electric potential of 10 V which produced 0.79 mA. Figure 2 shows that the highest current values were registered at the beginning of the experiment and started diminishing with time. The high current values observed during the initial stages of the process were due to the high electromigration of ions in the system which continues until equilibrium is reached due to reactions between the ions and the compounds in the system (Pham et al., 2009). In most electrokinetic reactors, electric current increases quickly during the first few hours and then gradually thereafter. This is due to resistance in the interface between electrodes and the electrolyte which increases because of concentration polarization and water dissociation and because ions with positive or negative charges move to the two ends of the electric cell as a consequence of electro dialysis, which results in the drop of ionic strength in soils and the current (Wang et al., 2007). Comparing experiments run with similar voltages, experiment C and D produced higher currents than experiment A and B respectively but they did not produce higher oil recovery than the later signifying that biosurfactant had produced a significant baseline recovery that couldn't be overrun by a small change in current. In the same vain the electroosmotic flow was highest during the beginning of the experiment and reduced with reduction in current. Considering Helmholtz–Smoluchowski theory represented by equation (1) to include electric field (E_x), electroosmotic flow (EOF), dielectric constant (D), vacuum permittivity (ϵ_0), and fluid viscosity (μ), It is in agreement with the results since electroosmotic flow is directly proportional to the electric field applied.

$$EOF = \frac{-D\epsilon_0 Z}{\mu} E_x \quad (1)$$

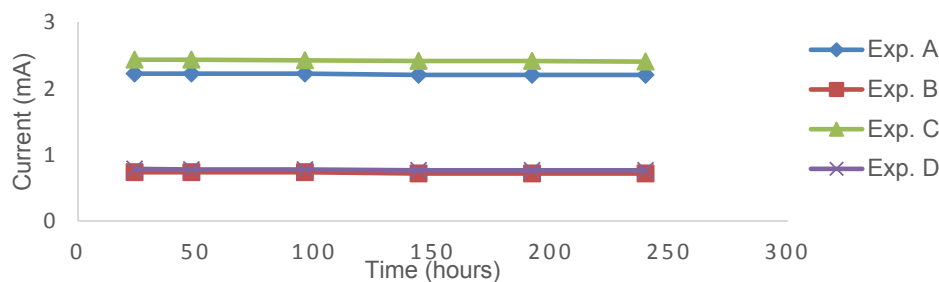


Figure 2: Time course of current during the electrokinetic process

3.3 In situ microbial growth

Figure 4 shows a substantial number of bacteria in different sections of the soil matrix. This is an indication that the bacteria were able to survive under application of the electric field considering electroosmosis, pH, electrical potential and temperature variations can lead to the death of the microorganisms due to the electro-halo-thermal environment that may not favour their survival by damaging their cell membranes (Lear et al., 2007). Bacteria growth was not inhibited by the electric field since the bacteria showed normal growth variations with time. The viable cells in every particular section of the soil matrix was rather greatly influenced by the pH. The bacteria were able to move to the electrode wells and the viable cell counts increased with time as opposed to the beginning of the experiment. The bacteria in the system is most affected by electroosmosis because it's their main transport system much as they are also transported by electrophoresis (Mena et al., 2016). Figure 2 shows that there are substantial variations in the cell counts along the normalised distance from the cathode. It is known that bacteria can grow under a wide range of pH values but the optimum pH conditions for *pseudomonas aeruginosa* are pH 7 (Das and Mukherjee, 2007). With highly variable pH gradients in the bio-electrokinetic reactors ranging from as high as 11.78 to as low as 2.3 the highest colony forming units were identified in sections of the soil matrix whose pH was between 9 and 6. These were areas between 50mm and 100mm normalised distances from the cathode. The strong growth patterns indicate that the bacteria obviously contributed to the degradation of the hydrocarbons by utilising the organic compounds as substrate leading to a 71.4% reduction in total carbon from 0.238 mg of carbon/mg of soil to 0.068 mg of carbon/mg of soil.

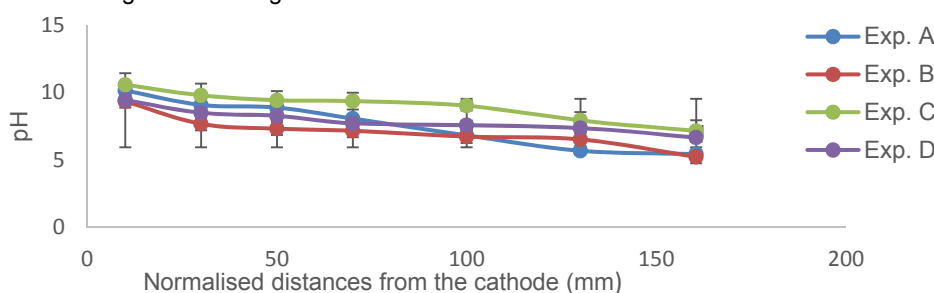


Figure 3: Average pH distribution up to the end of bio-electrokinetic treatment.

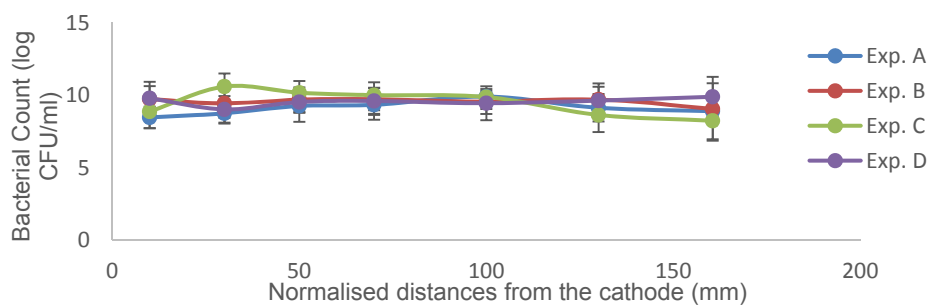


Figure 4: Average bacterial counts up to the end of the experiment.

4. Conclusion

Biosurfactants have the capacity to accelerate oil recovery in the electrokinetic process but the system is mostly affected by the voltage gradient since the highest voltage had the highest oil volume recovered. EOF of oil is possible but is highly affected by the filter pores and oil viscosity. The survival and growth of bacteria under the electric field applied gives promising results for in situ biosurfactant production. A study is however being made to substantiate the effect of different biosurfactant concentrations to the process.

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