Title:

Electrokinetic biosparging of toluene in groundwater

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Abstract:

Electrolysis of water occurs when electrokinetic techniques are used to remediate contaminated soils and groundwater. Under an electric field, generation of hydrogen and oxygen gases, and hydroxyl and hydrogen ions, occurs at the electrodes. By orienting electrodes vertically, oxygen has been generated at the base of aqueous solutions and saturated soil specimens, which then rises in the form of fine bubbles through the overlying media. Three sets of experiments were performed to explore the ability of this oxygen flow to encourage removal of dissolved phase toluene by both sparging and biosparging. Low electric currents of 10 to 50 mA were found to be sufficient to generate appreciable quantities of oxygen. These in turn were found to stimulate more rapid growth of bacteria (*Pseudomonas putida* mt-2) in uncontaminated aqueous media with and without the presence of gravel. In addition, bubble generation was found to cause abiotic removal of the volatile toluene in coarse-grained soils (sand and gravel) but not in fine-grained sand. Finally, removal of toluene from aqueous solution was achieved through the combined action of sparging and enhanced biodegradation (biosparging).

Keywords: 002.3 Bioelectrokinetics, 019 Land Contamination, 019.5Remediation Techniques

Introduction

Electrokinetic techniques are capable of remediating soils and groundwater contaminated with a range of pollutants (Virkutyte *et al*., 2002). Metallic and other charged contaminants can be moved or removed *via* electromigration whilst electroosmotic water flows can flush contamination of many types from fine-grained soils in particular. More recently, the potential of combining electrokinetics with bioremediation has been explored (Wick *et al*., 2007). Bioremediation comprises a range of popular remediation tools that lead to source removal, but can be hindered through factors such as lack of contaminant availability, low mass transfer rates or lack of availability of nutrients and other growth factors.

The use of electrokinetic phenomena to enhance bioremediation has included increasing contaminant availability through mass transfer of contaminants (Harbottle *et al*., 2009; Luo *et al*., 2006) or microorganisms (Deflaun & Condee, 1997; Harms & Wick, 2006), and delivering limiting nutrients (Xu *et al*., 2010). A low intensity electric field can directly and indirectly stimulate microbial activity in aqueous systems (Thrash & Coates, 2008; Jackman *et al*., 1999; Friman *et al*., 2012) although impacts on microbial communities in soil can be reduced (Lear *et al*., 2004 & 2007) and at higher intensity generation of antimicrobial chemical species, as well as the field itself, may negate any positive effects (Martínez-Huitle & Brillas, 2008).

Lack of availability of oxygen often limits microbial growth and contaminant biodegradation in sub-surface environments and its supply will often enhance biological activity. Application of an electric field to an aqueous system can cause electrolysis of water leading to electrode-specific reactions as follows:

Cathodic reaction: $$2H_2O + 2e^- \rightarrow H_{2(g)} + 2OH^-$$ Equation 1

Anodic reaction: $$2H_2O \rightarrow O_{2(g)} + 4H^+ + 4e^-$$ Equation 2

These reactions have a significant impact on electrokinetic remediation processes in soils. For example, generation of a pH gradient can directly affect mobility and removal of certain contaminants. However, the generation of oxygen gas through reduction of water at the anode has the potential to supply oxygen directly to subsurface processes.

Fadlalla & Alshawabkeh (2006) presented evidence for significant increases of dissolved oxygen in clay soils through application of a horizontal electric field. Dissolved oxygen generated at the anode moved into the soil through electroosmotic water flow, and elevated levels were maintained over many weeks, particularly near the anode. The use of electrolysis to generate oxygen in bioreactors has been found to be equivalent to standard aeration techniques, with generation of fine bubbles allowing rapid mass transfer between gaseous and liquid phases (Sadoff *et al.*, 1956; Thrash & Coates, 2008). The presence of a gaseous phase can also lead to abiotic mass transfer and remediation of volatile organic compounds (VOCs). Sparging or biosparging of soils or groundwater through direct injection of air or oxygen below the phreatic surface are established methods of removing VOCs through either volatilisation or stimulating microbial activity (Johnson *et al.*, 1993).

The work reported in this paper has investigated the potential for electrolytic generation of oxygen in aqueous solution and saturated, coarse-grained soils to stimulate microorganisms and abiotically sparge VOCs from solution, using a vertically oriented electrokinetic cell. The vertical orientation allows generation of a bubble column, the extent of which is controlled by electrode dimensions, which would be particularly applicable for treating plumes of mobile pollution in groundwater. The ability of electrokinetics to move ions in the groundwater can also deliver other, ionic, nutrients such as nitrates, or assist in treating mixed contamination, by removing metals through electromigration and so stimulating microbial activity in the oxygen enhanced zone by reducing overall toxicity. A conceptual model illustrating this is presented in Figure 1. Previous work by Wang *et al*. (2007) demonstrated the use of a vertical system for abiotic transport of both metals and organic contaminants.

Methodology

Apparatus

The majority of experiments were performed in Perspex cylinders (diameter 100 mm, height 287 mm). Compressed graphite anodes (area 50 x 45 mm) were sealed to the base of the cylinder whilst stainless steel mesh cathodes (area approximately 50 x 60 mm) were placed below the water level after addition of fluid to give a separation between electrodes of approximately 200 mm. Later experiments were performed in amber glass bottles with a similar arrangement. All containers were loosely closed to minimise loss of toluene through volatilisation. Power was supplied by a benchtop power supply (BST PSD30/3B, maximum 30 V, 3 A) with constant voltage or current facility.

Bacterium

The bacterium, *Pseudomonas putida* mt2 (culture collection accession number NCIMB10432 / ATCC23973), was obtained from the National Collection of Industrial & Marine Bacteria (Aberdeen, UK). It was cultured by inoculating 50 ml of Oxoid CM001 nutrient broth with 1 ml of a stock cell culture and incubating overnight at 30ºC. Prior to use, the fresh culture was centrifuged at 3000 rpm for 20 minutes to concentrate cells, and the supernatant discarded. Cells were resuspended in 5 ml of nutrient solution.

Sampling and Analysis

For toluene extraction, 20 ml samples were obtained using a glass syringe from the midpoint between electrodes. 10 ml aliquots were placed in glass extraction vials and 2 ml dichloromethane (DCM) added, before shaking at 240 rpm overnight. The DCM was then extracted and analysed by GC-MS (Clarus 500, Perkin Elmer) with identification confirmed by use of laboratory-prepared toluene standards. Cell counts were performed by taking 0.5 ml of each sample and centrifuging at 14,000 rpm for 1 minute. After discarding the supernatant, 0.1 ml acridine orange (AO) solution (285 μM), a fluorescent chemical probe which binds to DNA and RNA, was added to the sedimented cells, which were then resuspended on a vortex mixer. After 5 minutes incubation in the dark, the cells were washed three times by centrifuging, removing the supernatant and adding 0.1 ml deionised water before resuspending. A 20 μl aliquot of the resulting suspension was placed on a glass microscope slide, covered with a cover slip and observed on a Nikon LV100D epifluorescence microscope with B-2A filter cube (470 nm wavelength incident light, emission spectrum of AO 520-560 nm). Three random locations on the slide were observed and fluorescent cells counted manually. Counts were back-calculated to obtain the cell density of the original sample, and averaged.

Experimental structure

Three sets of experiments are reported here, as shown in Table 1. Each set of experiments was performed separately with some variation in conditions; however, the use of controls has been employed to permit comparisons to be made. In addition, a preliminary experiment allowed determination of pH and temperature changes with position following application of an electric field (10 mA constant current) to tap water.

Set A: the effect of a vertical electric field on growth of *P. putida* in 1 L nutrient solution without toluene (in g/L deionised water: glucose - 20; $(NH_4)_2SO_4$ - 2; K_2HPO_4 - 6; KH_2PO_4 - 3; NaCl – 3; $MgCl_2$ – 0.093; $CaCl_2$ – 0.011; trace metals solution [$CaSO_4.2H_2O$ - 0.2; $FeSO_4.7H_2O$ - 0.2; $ZnSO_4.7H_2O$ - 0.02; $MnSO_4.H_2O$ - 0.02; $CuSO_4.5H_2O$ - 0.02; $CoSO_4.7H_2O$ - 0.01; $Na_2B_4O_7$ - 0.005; $(NH_4)_6Mo_7O_{24}.4H_2O$ - 0.005] – 1 ml per L [based on Heydorn *et al.* (2000)]) was explored. Two microcosms were used, one with and one without an applied electric field. Three experiments were performed, one in aqueous solution and a 10 mA current (A1), one in aqueous solution with a 20 mA current (A2), and one in saturated particulate medium (gravel - 2-9 mm), again with 20 mA current (A3). Experiments continued for 48 hours.

Set B: these experiments investigated the abiotic removal of toluene contamination (400 mg/L in 1 L tap water), again over 48 hours. Impact of electric field intensity was assessed in four experiments. A 10 V field in aqueous solution only (B1) was compared to 10 and 20 V fields in the presence of solid particulate media (gravel – 2-9 mm [B2]; coarse sand – 1-2 mm [B3]; fine sand – 0.06-0.25 mm [B4]).

Set C: the possibility of combined sparging and biosparging was assessed in 1 L aqueous artificial groundwater (g/L in tap water: $CaCl_2.2H_2O$ – 0.526; $MgSO_4.7H_2O$ – 0.184; KH_2PO_4 – 0.0085; K_2HPO_4 – 0.02175; Na_2HPO_4 – 0.0177; KNO_3 – 0.133 [Lutterodt *et al.*, 2009]) containing *P. putida* (prepared as above) and toluene (200 and 400 mg/L). Four cylinders were used in each experiment, comprising controls and electrokinetic specimens (both with/without bacteria), and experiments continued for 33 hours. A constant current of 50 mA was applied. Two experiments were performed in Perspex cylinders (experiment C1 with 200 mg/L and C2 with 400 mg/L toluene) and two in amber glass bottles (experiment C3 otherwise identical to C2, and C4, which was deaired prior to addition of contaminants and bacteria). The use of amber glass allowed establishment of whether photochemical and sorption losses were significant, whilst deairing would help to establish the true impact of oxygenation by electrolytic means, without being obscured by dissolved oxygen in the system. In experiment C1, an additional cylinder was included with oxygen bubbles only (the cylinder was partially submerged in a much larger container of water; bubbles were supplied by a horizontally aligned pair of electrodes beneath the cylinder, with oxygen from the anode captured and funnelled to the base of the cylinder). This was designed to avoid pH, temperature or kinetic effects associated with the electric field itself, which would arise outside the cylinder and be diluted by the large volume of water in the surrounding container.

Results

Both cell count and contaminant recovery data are presented as a percentage of the count or contaminant recovery obtained at the start of the experiments. In all experiments where the anode was visible, small bubbles (typically approximately 1mm in diameter) were observed forming on the anode surface before rising up through the water column. In some cases the electric field caused slight discolouration of the water. A preliminary experiment to determine pH changes in aqueous solution as a result of the electric field was performed. The greatest effects were seen close to the electrodes, with maximum and minimum (at cathode and anode respectively) of 8.97 and 6.30 observed, compared to an initial pH of around 7.2, although the majority of data were between 6.50 and 8.50. The maximum effect on temperature was observed to be an increase of 0.2ºC.

Effect of vertical electric field on bacterial growth (experiment set A, Figure 2).

Data show increased bacterial cell counts over time in all experiments when an electric field is applied compared to controls. Whilst a lower current of 10 mA caused a small increase in cells per millilitre after 30 hours (20-30% relative to controls; experiment A1), a higher current of 20 mA led to a larger increase occurring more rapidly (up to

400% relative to controls; experiment A2), although there was significant variability in the data from electrokinetic specimens and this effect was not maintained, with no further growth after 16 hours. The presence of gravel appeared to hinder increases in cell numbers; experiments A2 and A3 had the same applied current, but the latter had a similar response to that seen in experiment A1, with a lower current.

Effect of vertical electric field on abiotic removal of toluene (experiment set B, Figure 3).

The presence of an electric field was seen to lead to increased removal of toluene from aqueous solution (experiment B1), and also in the presence of coarse-grained solid materials (B2 and B3), relative to control experiments. Increasing the electric field strength enhanced this effect. The presence of particulate solid media has an impact on toluene removal, with increasingly fine material leading to a reduction in the effect of the field. In gravel (B2), there was a significant enhancement apparent due to the field, with almost complete removal of toluene after 24 hours with 20 V. In coarse sand also (B3), there was a larger reduction in toluene with the field than without, although this effect was less substantial. In fine sand (B4), however, there was no discernable difference between control and electrokinetic experiments. In most cases it was clear that there was substantial loss of toluene through natural volatilisation or other losses within the system.

Combined electrokinetically enhanced sparging and biodegradation of toluene (experiment set C, Figure 4).

In the majority of experiments, a rapid initial decrease in toluene recovery was noted from all treatments, most likely due to abiotic mechanisms such as volatilisation and sorption, as noted above. There is one instance where this did not occur to the same extent, in experiment C4 (with no bacteria or electric field).

In all four experiments, toluene losses in the presence of either bacteria or electric field were faster than in the respective controls. In most cases, the presence of either bacteria or electric field (or both) led to complete removal within the experiment (i.e. by a maximum of 33 hours); with no bacteria or field, this was usually not the case. Removal was considerably faster with a lower concentration of toluene (experiment C1; 200 mg/L).

Experiments C2 and C3 were nominally identical apart from the latter was carried out in amber glass bottles rather than Perspex cylinders. Results indicate relatively little difference between them, although in C2 both bacterial specimens reached zero concentration of toluene by 24 hours, ahead of non-bacterial specimens, whereas in C3 both electrokinetic specimens were lower than non-electrokinetic controls. When the specimens were deaired (C4), background losses were apparently reduced. In amber glass specimens, the electric field caused the most significant toluene losses, with little noticeable effect from bacteria in these specimens. Data from C1 demonstrated little difference between the recovery of toluene when an electric field was applied and when a supply of oxygen bubbles only was supplied.

Discussion

The presence of an electric field stimulated an increase in microbial cell counts in nutrient broth. In purely aqueous conditions, this was linked to the magnitude of the electric field. The exact cause of enhanced growth is unclear, but significant quantities of gas generation were observed and the current applied in these experiments was low compared to other studies (Martínez-Huitle & Brillas, 2008; maximum approximately 2 mA/cm^2) and so unlikely to have significant negative effects. Sadoff *et al.*, (1956) applied up to 430 mA and achieved an increase in cells of more than a factor of five (by dry weight). It is possible that both direct stimulation of cells by the field and the presence of oxygen may have contributed to this effect. In addition, heating effects due to the field are sometimes seen which would stimulate growth, but very little effect was observed here. Changes in pH in the region where samples were obtained were not expected to be substantial based on the outcomes of the preliminary experiment. Error bars presented on Figure 2 show variation in cell number between images observed, and so are a measure of accuracy in analysis rather than in sampling. However, the larger errors noted in experiment A2 are indicative of clumping of cells in the observed samples. The presence of particulate solid media (A3) appeared to hinder cell proliferation (compared to A2), although there was still a small increase over the control specimen. This reduced effect may be linked to a reduction in the area through which current passed and through which oxygen bubbles flowed, and may be strongly affected by heterogeneity in the system – enhancement of activity and growth may be limited to certain areas due to the preferential current and oxygen flow.

Toluene recovery from the majority of specimens in experiment sets B and C, including controls, decreased substantially with time. There is also variability between experiments, indicated by comparison of control specimens. However, the net effects of the field and bacteria in individual experiments can be determined by differences when compared with their respective control samples. Losses from controls are attributed primarily to volatilisation; comparing control data from experiments C2 and C3, in Perspex and glass containers respectively, does not provide evidence for significant sorption to Perspex. Some sorption to microorganisms and electrodes may also occur, but is accounted for in controls. Volatilisation rates will be determined partly by laboratory temperature, and fluctuations may account for a portion of the variability in control data observed.

Sparging experiments (Set B) demonstrated the effect of oxygen generation on abiotic removal of toluene, with a positive link between voltage level and removal efficiency. The presence of particulate media was found to have a direct impact, with a reduced effect when solids were present (comparing 10 V specimens from experiments B1 and B2). This again may be due to the solids limiting the routes through which oxygen bubbles may travel by encouraging preferential flow. Decreasing grain size decreased the removal of toluene relative to controls, most likely due to the decreasing pore size and consequent difficulty that oxygen bubbles would encounter in travelling unimpeded through the pore space. This is likely to restrict bubble flow to a limited number of preferential flow paths within the medium, such that the majority of the pore fluid would not be exposed to gaseous flow. In traditional air sparging, preferential flow also occurs due to heterogeneity in the ground but flow usually takes the form of air channels rather than bubble flow (Johnson *et al.*, 1993). The ability to generate bubbles of a relatively small size may mean that finer grained materials are treatable – this would require further investigation.

Although experiments in set C indicate that combining biotic and abiotic phenomena has a beneficial effect on toluene removal the relative extent to which they occur is unclear. The presence of an electric field appears to be the better predictor of enhanced removal, particularly in experiments C3 and C4 where amber glass bottles were used. The potential for an electric resistive heating effect exists as the current was higher than in the preliminary experiment. However, the maximum applied power was low, at 1.5 W (maximum 30 V, 50 mA) to a 1 litre specimen, with power input less than this for the majority of the experiments. In addition, it was shown in experiment C1 that the effect of electrokinetics was very similar to that of the oxygen bubble supply alone, suggesting that additional effects of the electric field (heating, kinetics) did not have a significant effect. In practice, for longer periods, heating may become more significant, but this will only have a beneficial effect through increasing volatilisation of any VOCs, as well as stimulating microbial activity.

The relatively high levels of toluene used in the experiment set C are likely to have had a negative impact on survival and degradative activity of the *P. putida*, and the use of an artificial groundwater rather than nutrient broth is likely to have reduced activity. Choi *et al*. (2008) found that toluene concentrations of 250 mg/L entirely prevented growth of a related organism although below this growth did occur. The situation in these experiments was different as the bacteria were inoculated rather than grown *in situ*, and so biodegradation may still be possible with these larger numbers. In addition, loss of toluene through volatilisation (either naturally or through sparging) quickly reduced the concentration present, which would quickly bring it to a level where degradation could occur. This may contribute to differences between specimens with and without bacteria tending to be more pronounced later in the experiment.

These experiments demonstrate the ability of the electric field to stimulate microbial growth and to remove toluene abiotically. The combination of biotic and abiotic effects has enhanced toluene removal also, although evidence suggests that electrokinetic effects may play a large role in this and the extent to which bacteria are able to remove the contamination in conjunction with the electric field is uncertain. Nevertheless, the positive effects seen on both abiotic removal and stimulation of bacteria suggest that with further exploration of the test conditions a combined treatment method may be successful.

Practical Relevance and Applications

This paper presents a multi-purpose and robust method for treatment of multiple contaminant types in flowing groundwater. Current in-situ remediation methods may require significant operation and maintenance activities over the long periods of time needed for treatment of contaminant plumes, and may only address certain contaminant types. The technology described has the potential to tackle mixed contamination in a number of ways concurrently, through sparging, biosparging and electrokinetically enhanced bioremediation (through heating, delivery of nutrients and removal of ionic contaminants). The technology is potentially robust, requiring only an electricity supply to static, vertically oriented electrodes; this is a relatively unconventional arrangement but such installations have been made in the past (e.g. Roulier *et al*., 2000).

References

Choi NC, Choi JW, Kim SB and Kim DJ (2008) Modelling of growth kinetics for *Pseudomonas putida* during toluene degradation. *Applied Microbiology and Biotechnology* **81**: 135-141.

Deflaun MF and Condee CW (1997) Electrokinetic transport of bacteria. *Journal of Hazardous Materials* **55**: 263-277.

Fadlalla H and Alshawabkeh AN (2006) Efficacy of electrolytic generation and transport of oxygen for soil remediation. In *Proceedings of the 5th International Congress on Environmental Geotechnics, Cardiff* (Thomas HR (ed)). Thomas Telford, London, UK, pp. 126-132.

Friman H, Schechter A, Nitzan Y and Cahan R (2012) Effect of external voltage on *Pseudomonas putida* F1 in a bio electrochemical cell using toluene as sole carbon and energy source. *Microbiology* **158**: 414-423.

Harbottle MJ, Lear G, Sills GC and Thompson IP (2009) Enhanced biodegradation of pentachlorophenol in unsaturated soil using reversed field electrokinetics. *Journal of Environmental Management* **90**: 1893-1900.

Harms H and Wick LY (2006) Dispersing pollutant-degrading bacteria in contaminated soil without touching it. *Engineering in Life Sciences* **6(3)**: 252-260.

Heydorn A, Toftgaard Nielsen A, Hentzer M *et al.* (2000). Quantification of biofilm structures by the novel computer program COMSTAT. *Microbiology* **146**: 2395–2407.

Jackman SA, Maini G, Sharman AK and Knowles CJ (1999) The effects of direct electric current on the viability and metabolism of acidophilic bacteria. *Enzyme and Microbial Technology* **24**: 316-324.

Johnson RL, Johnson PC, McWhorter DB, Hinchee RE and Goodman I (1993) An overview of in situ air sparging. *Ground Water Monitoring and Remediation* **Fall**: 127-135.

Lear G, Harbottle MJ, van der Gast CJ *et al*. (2004) The effect of electrokinetics on soil microbial communities. *Soil Biology and Biochemistry* **36**: 1751-1760.

Lear G, Harbottle MJ, Sills G *et al*. (2007) Impact of electrokinetic remediation on microbial communities within PCP contaminated soil. *Environmental Pollution* **146**: 139-146.

Luo Q, Wang H, Zhang X, Fan X and Qian Y (2006) In situ bioelectrokinetic remediation of phenol-contaminated soil by use of an electrode matrix and a rotational operation mode. *Chemosphere* **64**: 415-422.

Lutterodt G, Basnet M, Foppen JWA, Uhlenbrook S (2009) The effect of surface characteristics on the transport of multiple *Escherichia coli* isolates in large scale columns of quartz sand. *Water Research* **43**: 595-604.

Martínez-Huitle CE and Brillas E (2008) Electrochemical Alternatives for Drinking Water Disinfection. *Angewandte Chemie – International Edition* **47**: 1998-2005.

Roulier M, Kemper M, Al-Abed S *et al*. (2000) Feasibility of electrokinetic soil remediation in horizontal Lasagna cells. *Journal of Hazardous Materials* **B77**: 161-176.

Sadoff HL, Halvorson HO and Finn RK (1956) Electrolysis as a means of aerating submerged cultures of microorganisms. *Applied Microbiology* **4(4)**: 164-170.

Thrash JC and Coates JD (2008) Direct and indirect electrical stimulation of microbial metabolism. *Environmental Science and Technology* **42(11)**: 3921-3931.

Virkutyte J, Sillanpää M and Latostenmaa P (2002) Electrokinetic soil remediation – critical overview. *Science of the Total Environment* **289**: 97-121.

Wang JY, Huang XJ, Kao JCM and Stabnikova O (2007) Simultaneous removal of organic contaminants and heavy metals from kaolin using an upward electrokinetic soil remediation process. *Journal of Hazardous Materials* **144**: 292-299.

Wick LY, Shi L and Harms H (2007). Electro-bioremediation of hydrophobic organic soil-contaminants: A review of fundamental interactions. *Electrochimica Acta* **52**: 3441-3448.

Xu W, Wang C, Liu H, Zhang Z and Sun H (2010) A laboratory feasibility study on a new electrokinetic nutrient injection pattern and bioremediation of phenanthrene in a clayey soil. *Journal of Hazardous Materials* **194**: 798-804.

Figure captions

Figure 1. Conceptual model of vertical electrokinetic system for enhanced biodegradation, sparging and electromigration.

Figure 2. Bacterial counts with (EK) and without (control) electric field (experiments A1, A2 and A3). [NB error bars represent standard deviation in counts from multiple images (n=3) only].

Figure 3. Fate of dissolved phase toluene subject to electric field in aqueous solution alone (experiment B1), and with gravel (B2), coarse sand (B3) and fine sand (B4).

Figure 4. Concentration of toluene (200 mg/L [experiment C1] and 400 mg/L [C2-C4]) versus time in combined biodegradation and sparging in aqueous solution due to electric field. Experiments C1 and C2 took place in Perspex cylinders, whilst experiments C3 and C4 took place in amber glass bottles (the latter involving deaired water).

Table caption

Table 1. Experimental structure (cc – constant current; cv – constant voltage).

Figure 1

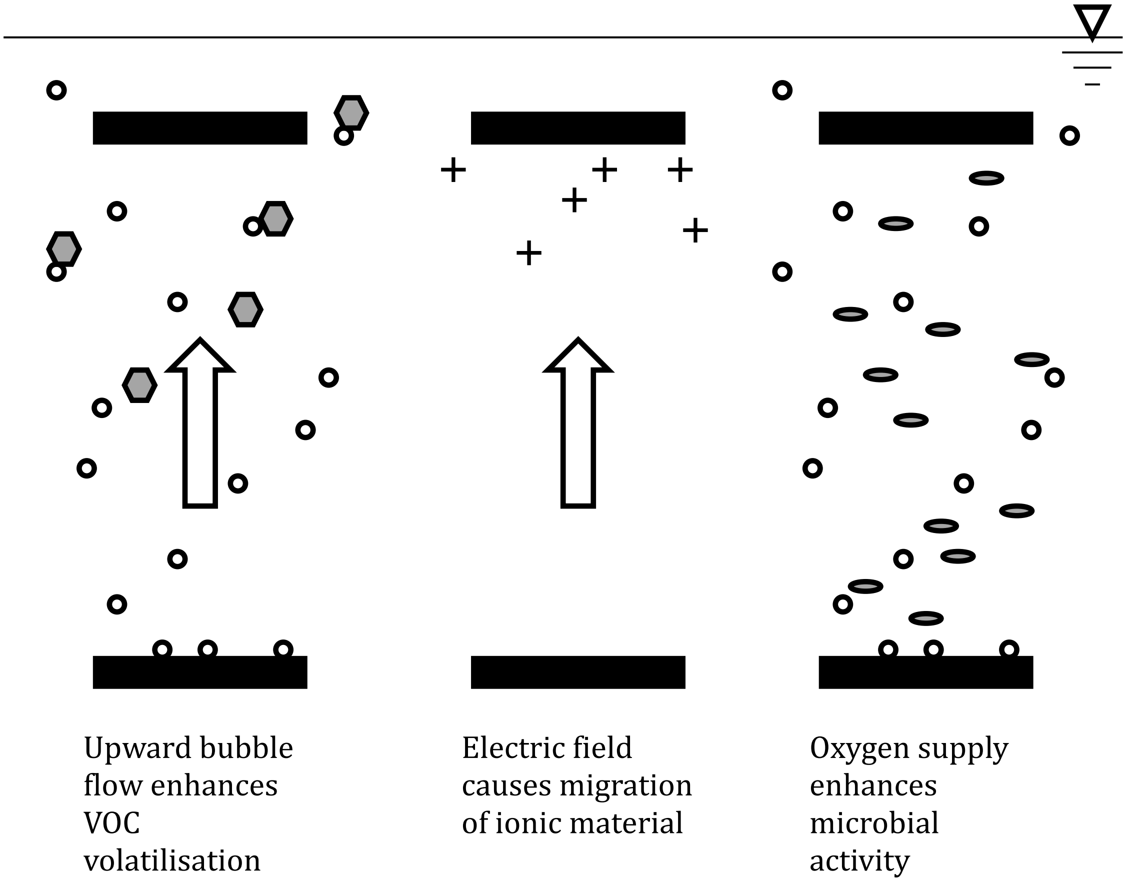
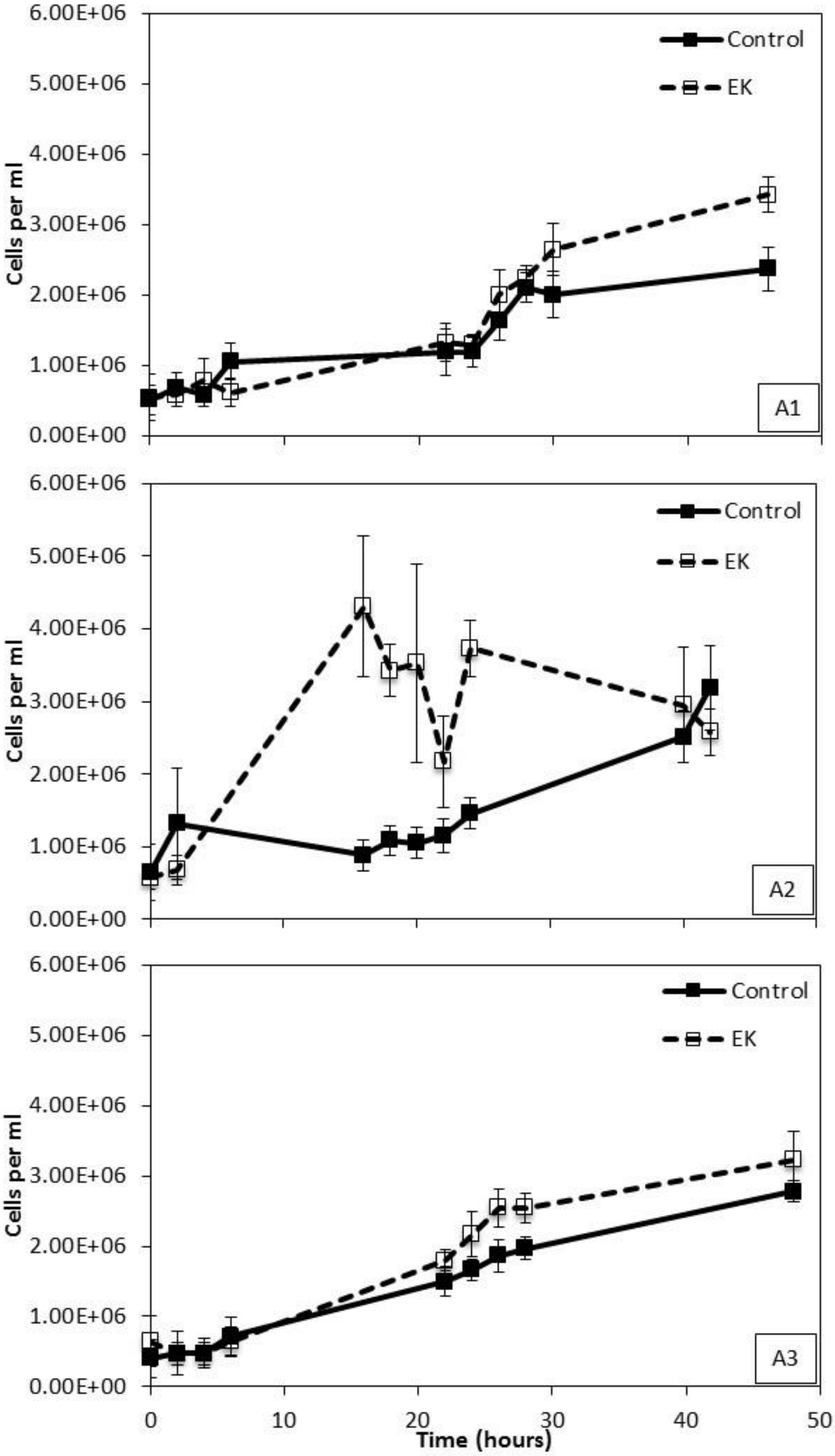
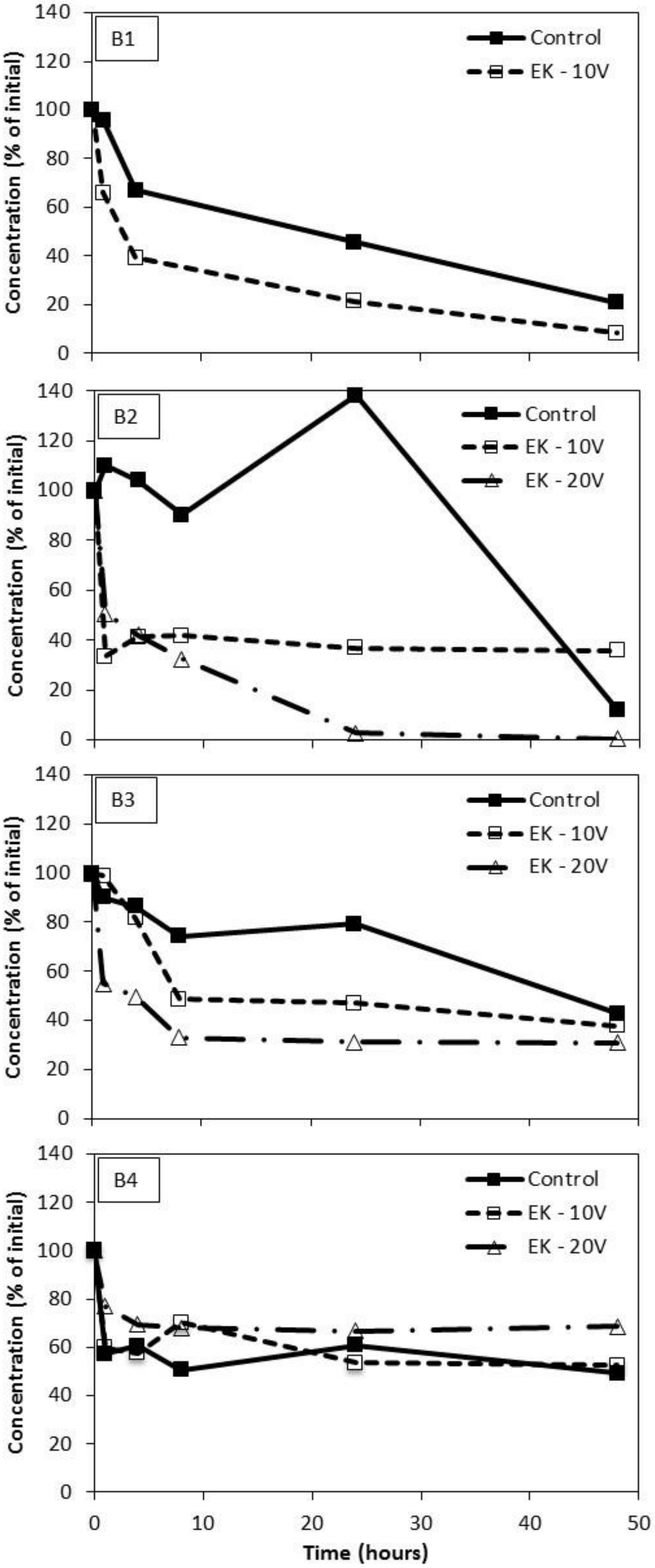

Upward bubble flow enhances VOC volatilisation
Electric field causes migration of ionic material
Oxygen supply enhances microbial activity

Figure 2



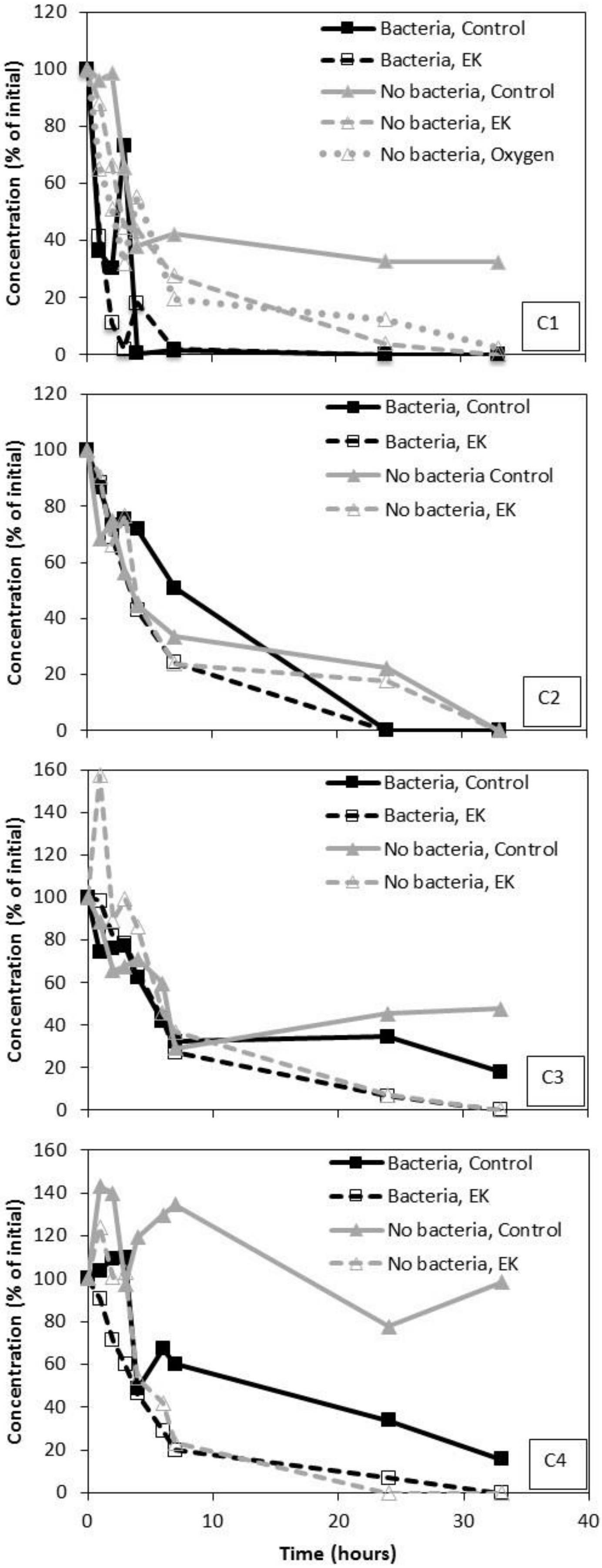
Control
EK
Cells per ml
6.00E+06
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
0.00E+00
A1
A2
A3
0
10
20
30
40
50
Time (hours)

Figure 3



B1
Control
EK - 10V
B2
Control
EK - 10V
EK - 20V
B3
Control
EK - 10V
EK - 20V
B4
Control
EK - 10V
EK - 20V
Concentration (% of initial)
0
20
40
60
80
100
120
140
0
10
20
30
40
50
Time (hours)

Figure 4


Bacteria, Control
Bacteria, EK
No bacteria, Control
No bacteria, EK
No bacteria, Oxygen
C1
No bacteria Control
C2
C3
C4
Concentration (% of initial)
Time (hours)