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1 Title:
2 Electrokinetic biosparging of toluene in groundwater
3
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29 Abstract:
30 Electrolysis of water occurs when electrokinetic techniques are used to remediate
31 contaminated soils and groundwater. Under an electric field, generation of hydrogen
32 and oxygen gases, and hydroxyl and hydrogen ions, occurs at the electrodes. By
33 orienting electrodes vertically, oxygen has been generated at the base of aqueous
34 solutions and saturated soil specimens, which then rises in the form of fine bubbles
35 through the overlying media. Three sets of experiments were performed to explore the
36 ability of this oxygen flow to encourage removal of dissolved phase toluene by both
37 sparging and biosparging. Low electric currents of 10 to 50 mA were found to be
38 sufficient to generate appreciable quantities of oxygen. These in turn were found to
39 stimulate more rapid growth of bacteria (*Pseudomonas putida* mt-2) in uncontaminated
40 aqueous media with and without the presence of gravel. In addition, bubble generation
41 was found to cause abiotic removal of the volatile toluene in coarse-grained soils (sand
42 and gravel) but not in fine-grained sand. Finally, removal of toluene from aqueous
43 solution was achieved through the combined action of sparging and enhanced
44 biodegradation (biosparging).

45
46 Keywords: 002.3 Bioelectrokinetics, 019 Land Contamination, 019.5 Remediation
47 Techniques

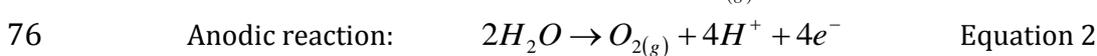
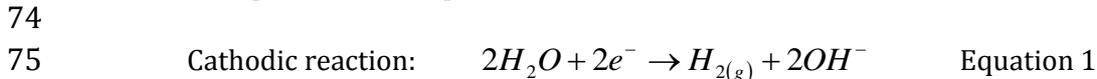
48

49 **Introduction**

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

59
60 The use of electrokinetic phenomena to enhance bioremediation has included increasing
61 contaminant availability through mass transfer of contaminants (Harbottle *et al.*, 2009;
62 Luo *et al.*, 2006) or microorganisms (Deflaun & Condee, 1997; Harms & Wick, 2006),
63 and delivering limiting nutrients (Xu *et al.*, 2010). A low intensity electric field can
64 directly and indirectly stimulate microbial activity in aqueous systems (Thrash & Coates,
65 2008; Jackman *et al.*, 1999; Friman *et al.*, 2012) although impacts on microbial
66 communities in soil can be reduced (Lear *et al.*, 2004 & 2007) and at higher intensity
67 generation of antimicrobial chemical species, as well as the field itself, may negate any
68 positive effects (Martínez-Huitle & Brillas, 2008).

69
70 Lack of availability of oxygen often limits microbial growth and contaminant
71 biodegradation in sub-surface environments and its supply will often enhance biological
72 activity. Application of an electric field to an aqueous system can cause electrolysis of
73 water leading to electrode-specific reactions as follows:



77
78 These reactions have a significant impact on electrokinetic remediation processes in
79 soils. For example, generation of a pH gradient can directly affect mobility and removal
80 of certain contaminants. However, the generation of oxygen gas through reduction of
81 water at the anode has the potential to supply oxygen directly to subsurface processes.

82
83 Fadlalla & Alshawabkeh (2006) presented evidence for significant increases of dissolved
84 oxygen in clay soils through application of a horizontal electric field. Dissolved oxygen
85 generated at the anode moved into the soil through electroosmotic water flow, and
86 elevated levels were maintained over many weeks, particularly near the anode. The use
87 of electrolysis to generate oxygen in bioreactors has been found to be equivalent to
88 standard aeration techniques, with generation of fine bubbles allowing rapid mass
89 transfer between gaseous and liquid phases (Sadoff *et al.*, 1956; Thrash & Coates, 2008).
90 The presence of a gaseous phase can also lead to abiotic mass transfer and remediation
91 of volatile organic compounds (VOCs). Sparging or biosparging of soils or groundwater
92 through direct injection of air or oxygen below the phreatic surface are established
93 methods of removing VOCs through either volatilisation or stimulating microbial
94 activity (Johnson *et al.*, 1993).

95
96 The work reported in this paper has investigated the potential for electrolytic
97 generation of oxygen in aqueous solution and saturated, coarse-grained soils to
98 stimulate microorganisms and abiotically sparge VOCs from solution, using a vertically
99 oriented electrokinetic cell. The vertical orientation allows generation of a bubble
100 column, the extent of which is controlled by electrode dimensions, which would be

101 particularly applicable for treating plumes of mobile pollution in groundwater. The
102 ability of electrokinetics to move ions in the groundwater can also deliver other, ionic,
103 nutrients such as nitrates, or assist in treating mixed contamination, by removing metals
104 through electromigration and so stimulating microbial activity in the oxygen enhanced
105 zone by reducing overall toxicity. A conceptual model illustrating this is presented in
106 Figure 1. Previous work by Wang *et al.* (2007) demonstrated the use of a vertical
107 system for abiotic transport of both metals and organic contaminants.

109 **Methodology**

110 Apparatus

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

121 Bacterium

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

129 Sampling and Analysis

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

147 Experimental structure

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

153 Set A: the effect of a vertical electric field on growth of *P. putida* in 1 L nutrient solution
154 without toluene (in g/L deionised water: glucose - 20; (NH₄)₂SO₄ - 2; K₂HPO₄ - 6; KH₂PO₄
155 - 3; NaCl - 3; MgCl₂ - 0.093; CaCl₂ - 0.011; trace metals solution [CaSO₄·2H₂O - 0.2;
156 FeSO₄·7H₂O - 0.2; ZnSO₄·7H₂O - 0.02; MnSO₄·H₂O - 0.02; CuSO₄·5H₂O - 0.02; CoSO₄·7H₂O -
157 0.01; Na₂B₄O₇ - 0.005; (NH₄)₆Mo₇O₂₄·4H₂O - 0.005] - 1 ml per L [based on Heydorn *et al.*
158 (2000)]) was explored. Two microcosms were used, one with and one without an
159 applied electric field. Three experiments were performed, one in aqueous solution and a
160 10 mA current (A1), one in aqueous solution with a 20 mA current (A2), and one in
161 saturated particulate medium (gravel - 2-9 mm), again with 20 mA current (A3).
162 Experiments continued for 48 hours.

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

168 Set C: the possibility of combined sparging and biosparging was assessed in 1 L aqueous
169 artificial groundwater (g/L in tap water: CaCl₂·2H₂O - 0.526; MgSO₄·7H₂O - 0.184;
170 KH₂PO₄ - 0.0085; K₂HPO₄ - 0.02175; Na₂HPO₄ - 0.0177; KNO₃ - 0.133 [Lutterodt *et al.*,
171 2009]) containing *P. putida* (prepared as above) and toluene (200 and 400 mg/L). Four
172 cylinders were used in each experiment, comprising controls and electrokinetic
173 specimens (both with/without bacteria), and experiments continued for 33 hours. A
174 constant current of 50 mA was applied. Two experiments were performed in Perspex
175 cylinders (experiment C1 with 200 mg/L and C2 with 400 mg/L toluene) and two in
176 amber glass bottles (experiment C3 otherwise identical to C2, and C4, which was
177 deaired prior to addition of contaminants and bacteria). The use of amber glass allowed
178 establishment of whether photochemical and sorption losses were significant, whilst
179 deairing would help to establish the true impact of oxygenation by electrolytic means,
180 without being obscured by dissolved oxygen in the system. In experiment C1, an
181 additional cylinder was included with oxygen bubbles only (the cylinder was partially
182 submerged in a much larger container of water; bubbles were supplied by a horizontally
183 aligned pair of electrodes beneath the cylinder, with oxygen from the anode captured
184 and funnelled to the base of the cylinder). This was designed to avoid pH, temperature
185 or kinetic effects associated with the electric field itself, which would arise outside the
186 cylinder and be diluted by the large volume of water in the surrounding container.
187

188 Results

189 Both cell count and contaminant recovery data are presented as a percentage of the
190 count or contaminant recovery obtained at the start of the experiments. In all
191 experiments where the anode was visible, small bubbles (typically approximately 1mm
192 in diameter) were observed forming on the anode surface before rising up through the
193 water column. In some cases the electric field caused slight discolouration of the water.
194 A preliminary experiment to determine pH changes in aqueous solution as a result of
195 the electric field was performed. The greatest effects were seen close to the electrodes,
196 with maximum and minimum (at cathode and anode respectively) of 8.97 and 6.30
197 observed, compared to an initial pH of around 7.2, although the majority of data were
198 between 6.50 and 8.50. The maximum effect on temperature was observed to be an
199 increase of 0.2°C.
200

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

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

206 400% relative to controls; experiment A2), although there was significant variability in
207 the data from electrokinetic specimens and this effect was not maintained, with no
208 further growth after 16 hours. The presence of gravel appeared to hinder increases in
209 cell numbers; experiments A2 and A3 had the same applied current, but the latter had a
210 similar response to that seen in experiment A1, with a lower current.

211 212 Effect of vertical electric field on abiotic removal of toluene (experiment set B, Figure 213 3).

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

226 227 Combined electrokinetically enhanced sparging and biodegradation of toluene 228 (experiment set C, Figure 4).

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

233
234 In all four experiments, toluene losses in the presence of either bacteria or electric field
235 were faster than in the respective controls. In most cases, the presence of either bacteria
236 or electric field (or both) led to complete removal within the experiment (i.e. by a
237 maximum of 33 hours); with no bacteria or field, this was usually not the case. Removal
238 was considerably faster with a lower concentration of toluene (experiment C1; 200
239 mg/L).

240
241 Experiments C2 and C3 were nominally identical apart from the latter was carried out in
242 amber glass bottles rather than Perspex cylinders. Results indicate relatively little
243 difference between them, although in C2 both bacterial specimens reached zero
244 concentration of toluene by 24 hours, ahead of non-bacterial specimens, whereas in C3
245 both electrokinetic specimens were lower than non-electrokinetic controls. When the
246 specimens were deaired (C4), background losses were apparently reduced. In amber
247 glass specimens, the electric field caused the most significant toluene losses, with little
248 noticeable effect from bacteria in these specimens. Data from C1 demonstrated little
249 difference between the recovery of toluene when an electric field was applied and when
250 a supply of oxygen bubbles only was supplied.

251 252 **Discussion**

253 The presence of an electric field stimulated an increase in microbial cell counts in
254 nutrient broth. In purely aqueous conditions, this was linked to the magnitude of the
255 electric field. The exact cause of enhanced growth is unclear, but significant quantities of
256 gas generation were observed and the current applied in these experiments was low
257 compared to other studies (Martínez-Huitle & Brillas, 2008; maximum approximately 2
258 mA/cm²) and so unlikely to have significant negative effects. Sadoff *et al.*, (1956) applied

259 up to 430 mA and achieved an increase in cells of more than a factor of five (by dry
260 weight). It is possible that both direct stimulation of cells by the field and the presence
261 of oxygen may have contributed to this effect. In addition, heating effects due to the field
262 are sometimes seen which would stimulate growth, but very little effect was observed
263 here. Changes in pH in the region where samples were obtained were not expected to be
264 substantial based on the outcomes of the preliminary experiment. Error bars presented
265 on Figure 2 show variation in cell number between images observed, and so are a
266 measure of accuracy in analysis rather than in sampling. However, the larger errors
267 noted in experiment A2 are indicative of clumping of cells in the observed samples. The
268 presence of particulate solid media (A3) appeared to hinder cell proliferation
269 (compared to A2), although there was still a small increase over the control specimen.
270 This reduced effect may be linked to a reduction in the area through which current
271 passed and through which oxygen bubbles flowed, and may be strongly affected by
272 heterogeneity in the system – enhancement of activity and growth may be limited to
273 certain areas due to the preferential current and oxygen flow.

274
275 Toluene recovery from the majority of specimens in experiment sets B and C, including
276 controls, decreased substantially with time. There is also variability between
277 experiments, indicated by comparison of control specimens. However, the net effects of
278 the field and bacteria in individual experiments can be determined by differences when
279 compared with their respective control samples. Losses from controls are attributed
280 primarily to volatilisation; comparing control data from experiments C2 and C3, in
281 Perspex and glass containers respectively, does not provide evidence for significant
282 sorption to Perspex. Some sorption to microorganisms and electrodes may also occur,
283 but is accounted for in controls. Volatilisation rates will be determined partly by
284 laboratory temperature, and fluctuations may account for a portion of the variability in
285 control data observed.

286
287 Sparging experiments (Set B) demonstrated the effect of oxygen generation on abiotic
288 removal of toluene, with a positive link between voltage level and removal efficiency.
289 The presence of particulate media was found to have a direct impact, with a reduced
290 effect when solids were present (comparing 10 V specimens from experiments B1 and
291 B2). This again may be due to the solids limiting the routes through which oxygen
292 bubbles may travel by encouraging preferential flow. Decreasing grain size decreased
293 the removal of toluene relative to controls, most likely due to the decreasing pore size
294 and consequent difficulty that oxygen bubbles would encounter in travelling unimpeded
295 through the pore space. This is likely to restrict bubble flow to a limited number of
296 preferential flow paths within the medium, such that the majority of the pore fluid
297 would not be exposed to gaseous flow. In traditional air sparging, preferential flow also
298 occurs due to heterogeneity in the ground but flow usually takes the form of air
299 channels rather than bubble flow (Johnson *et al.*, 1993). The ability to generate bubbles
300 of a relatively small size may mean that finer grained materials are treatable – this
301 would require further investigation.

302
303 Although experiments in set C indicate that combining biotic and abiotic phenomena has
304 a beneficial effect on toluene removal the relative extent to which they occur is unclear.
305 The presence of an electric field appears to be the better predictor of enhanced removal,
306 particularly in experiments C3 and C4 where amber glass bottles were used. The
307 potential for an electric resistive heating effect exists as the current was higher than in
308 the preliminary experiment. However, the maximum applied power was low, at 1.5 W
309 (maximum 30 V, 50 mA) to a 1 litre specimen, with power input less than this for the
310 majority of the experiments. In addition, it was shown in experiment C1 that the effect of
311 electrokinetics was very similar to that of the oxygen bubble supply alone, suggesting
312 that additional effects of the electric field (heating, kinetics) did not have a significant

313 effect. In practice, for longer periods, heating may become more significant, but this will
314 only have a beneficial effect through increasing volatilisation of any VOCs, as well as
315 stimulating microbial activity.

316
317 The relatively high levels of toluene used in the experiment set C are likely to have had a
318 negative impact on survival and degradative activity of the *P. putida*, and the use of an
319 artificial groundwater rather than nutrient broth is likely to have reduced activity. Choi
320 *et al.* (2008) found that toluene concentrations of 250 mg/L entirely prevented growth
321 of a related organism although below this growth did occur. The situation in these
322 experiments was different as the bacteria were inoculated rather than grown *in situ*, and
323 so biodegradation may still be possible with these larger numbers. In addition, loss of
324 toluene through volatilisation (either naturally or through sparging) quickly reduced the
325 concentration present, which would quickly bring it to a level where degradation could
326 occur. This may contribute to differences between specimens with and without bacteria
327 tending to be more pronounced later in the experiment.

328
329 These experiments demonstrate the ability of the electric field to stimulate microbial
330 growth and to remove toluene abiotically. The combination of biotic and abiotic effects
331 has enhanced toluene removal also, although evidence suggests that electrokinetic
332 effects may play a large role in this and the extent to which bacteria are able to remove
333 the contamination in conjunction with the electric field is uncertain. Nevertheless, the
334 positive effects seen on both abiotic removal and stimulation of bacteria suggest that
335 with further exploration of the test conditions a combined treatment method may be
336 successful.

337

338 **Practical Relevance and Applications**

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

349

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406

407 **Figure captions**

408

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

411

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

415

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

418

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

424

425 **Table caption**

426

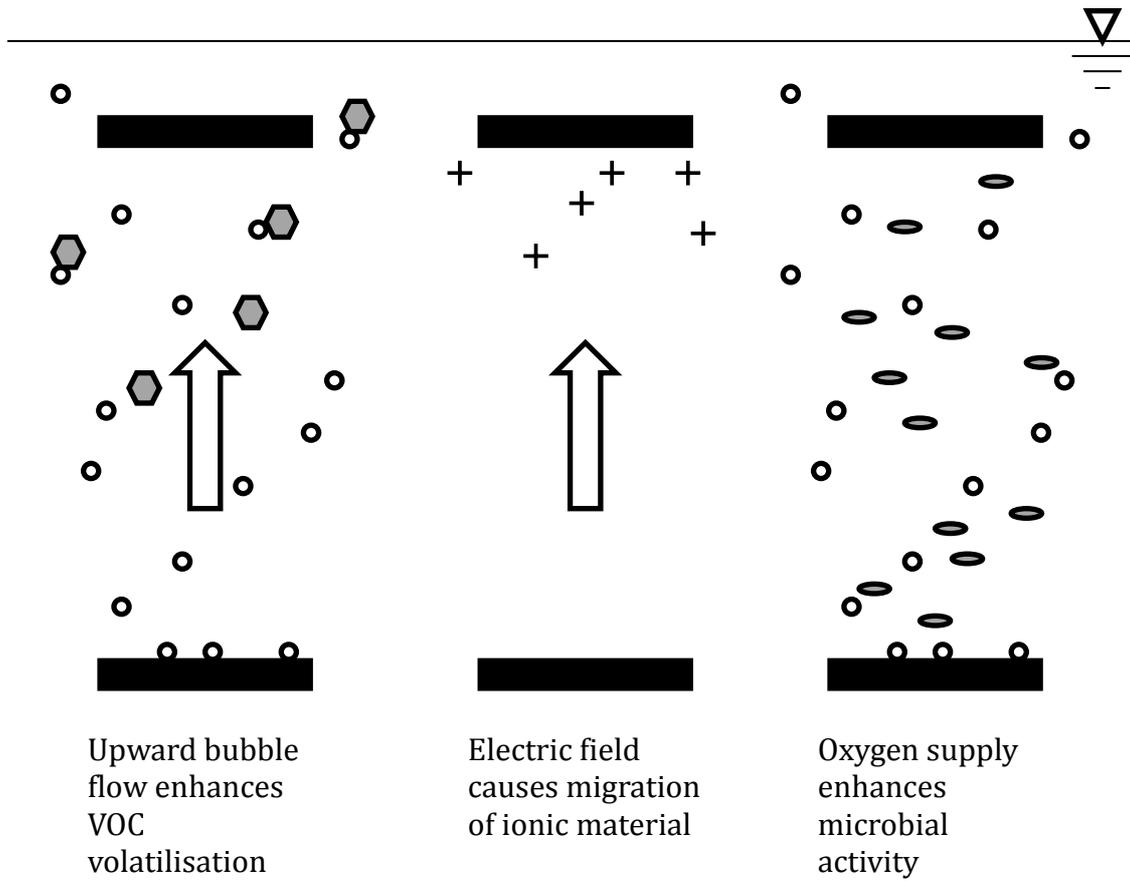
427 Table 1. Experimental structure (cc – constant current; cv – constant voltage).

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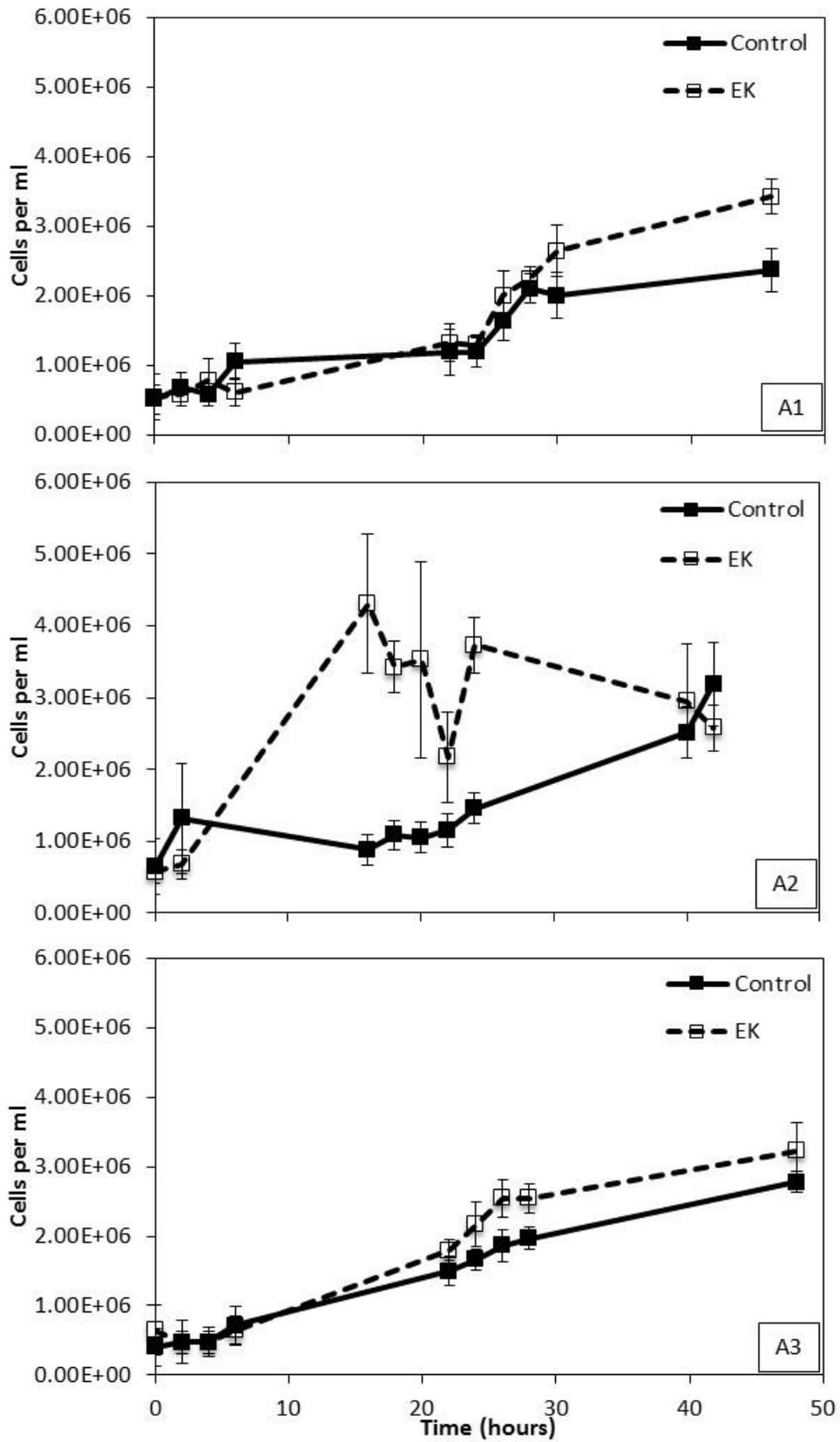
Figure 1



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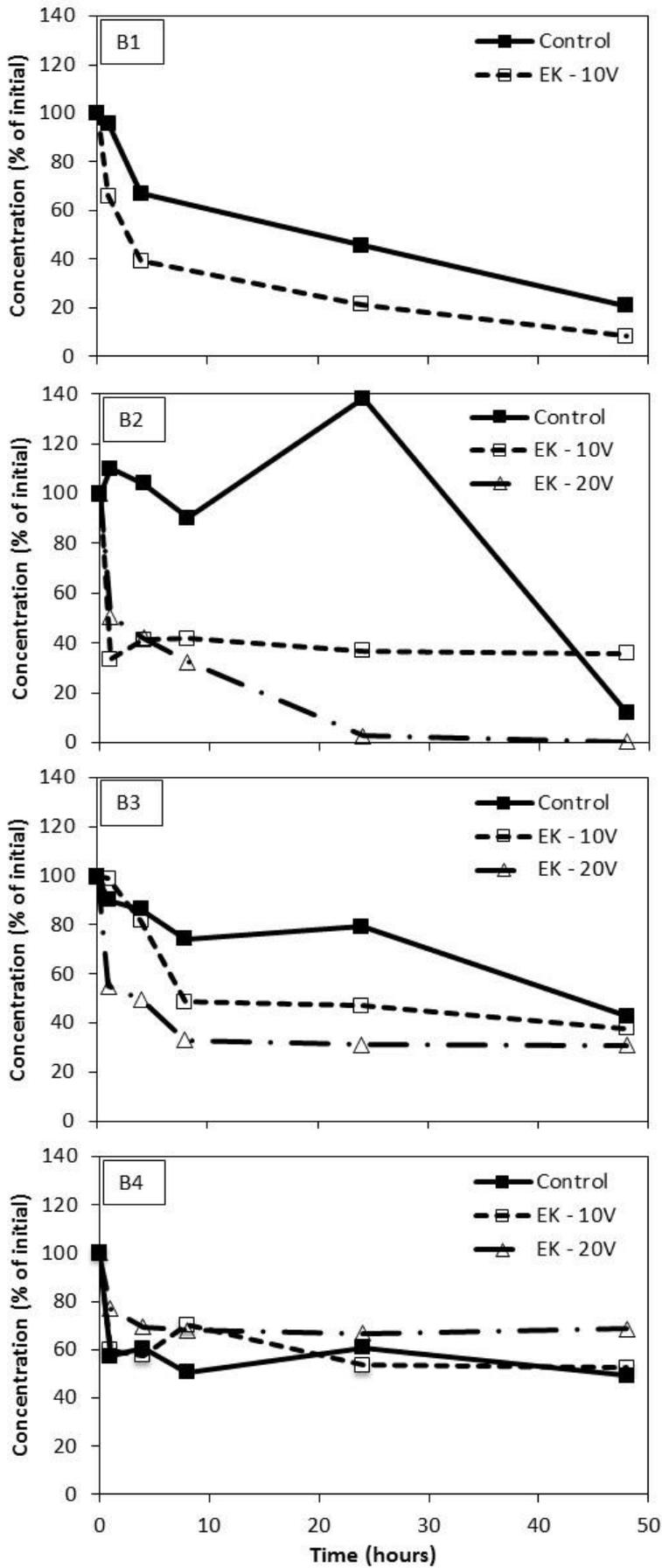
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Figure 2



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439 **Figure 3**



441 **Figure 4**

