Short Communication

Electrochemical Evaluation of Extremely-Low Frequency Magnetic Field Effects on Sulphate-Reducing Bacteria

(bacterial EMF effect / low-frequency magnetic fields / graphite electrode / SRB)

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Abstract. The effects of 50 Hz magnetic fields on sulphate-reducing bacteria viability were studied electrochemically. Two types of graphite electrodes (pyrolytic and glassy carbon) covered with whole bacterial cells behind a dialysis membrane were used for electrochemical measurements. We found about 15% decrease of reduction peak current density (which indicates desulphurization activity of the bacterial cells – their metabolic activity) on cyclic voltammograms after magnetic field exposure compared to the control samples. We suppose that the magnetic field does not influence the metabolic activity (desulphurization) of sulphate-reducing bacteria but most probably causes bacterial death.

Introduction

In the latest years, living cells and microorganisms started to be used in electrochemistry. The main reason for their use is cheaper control of environmental pollutants and/or analytical detection (Takayama et al., 1996a, b), water cleaning (Rabaey et al., 2006; Zhao et al., 2008) and bacterial viability detection (Fojt et al., 2007). We introduce an electrochemical method to monitor and quantify the viability of sulphate-reducing bacteria (SRB). We used this innovative method for detection of low frequency and intensity electromagnetic field (EMF) effects on SRB viability.

Different biological effects of EMF have been widely studied. DNA, as one of the most important biological molecules, belongs to the main studied objects. The focus is on DNA strand breaks after 50 Hz magnetic field exposure (Ivancsits et al., 2003a, b) or microwave exposure (Lai and Singh, 1996; Paulraj and Behari, 2006). Contrary to these results, McNamee et al. (2002) did not find any relevant DNA strand breaks after 60 Hz magnetic field exposure. Gene transcription, changes in synthesis and transcription of DNA have been studied (Repacholi and Greenebaum, 1999; Blank and Goodman, 2001, Strašák et al., 2009). Again, there is controversy in the obtained results. Hone et al. (2006) did not find any damage to chromatids in human leukocytes after 50 Hz magnetic field exposure.

The effects of magnetic fields on different enzymatic processes have been studied as well. Ornithine decarboxylase activity (Mullins et al., 1998; Höytö et al., 2006), denitrification activity (Fojt et al., 2007), glucose-stimulated insulin secretion (Malyapa et al., 1998) were monitored, again with controversial results. There are numerous studies about the effects of low-intensity 50 Hz EMF on structures and functions of different kinds of cell lines (Grassi et al., 2004; Sul et al., 2006; Kroupová et al., 2007). So far, no convincing data have been obtained.

Also unicellular organisms such as bacteria (Berg, 1999; Strašák et al., 2002; Babushkina et al., 2005; Strašák et al., 2005) and yeasts (Ruiz-Gómez et al., 2004; Novák et al., 2007) have served as targets for potential EMF effects. In this context, SRB are ecologically important anaerobes in numerous ecosystems, including most extreme environments like saline, alkaline and thermal habitats. The metabolism of SRB is connected with anaerobic respiration. In general, organic substrates are oxidized via hydrogen transfer and electron transfer to the oxygen groups in inorganic molecules. Actual sulphate reduction is an independent metabolic process, strictly anaerobic. It is of practical importance that SRB cause microbial corrosion (Melchers and Wells, 2006; Kuang et al., 2007) and that SRB are used for removing heavy metals from acid mine

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Abbreviations: bcE – bacteria-covered electrode, CFU – colony-forming unit, CV – cyclic voltammetry, EMF – electromagnetic field, GCE – glassy carbon electrode, PBS – phosphate-buffered solution, PGEb – pyrolytic graphite electrode in basal orientation, SRB – sulphate-reducing bacteria, VBNC – viable but nonculturable state.

drainage (Luptáková and Kušnierová, 2005; Zagury et al., 2006).

The aim of this work was to find out possible effects of 50 Hz magnetic field on SRB by electrochemistry. SRB were chosen for their well-known resistivity to external physicochemical factors and for their shape – rod-like and spherical bacterial strains were studied before (Fojt et al., 2004, 2007, 2009).

Material and Methods

Instrumentation

Alternating magnetic field was generated in a cylindrical coil (Institute of Scientific Instruments, AS CR, Brno, Czech Republic) powered by an autotransformer. The maximum current amplitude at 50 Hz in the coil was I_m = 1.9 A, corresponding to a maximum achievable magnetic field amplitude of B_m = 10 mT. The current was checked during the measurements using an HC3500-T Multimeter (HC, Seoul, South Korea) and the frequency by oscilloscope PM 3266 (Philips, Eindhoven, The Netherlands). Parameters of the coil are given in Table 1 and the distribution of the magnetic field induction inside the coil is shown in Fig. 1. For exposure, the samples were placed on a nonconductive plate in the centre of the coil where uniformity of the magnetic field is optimal (see Fig. 1). Magnetic induction was measured using Hall probe (Institute of Scientific Instruments). The temperature inside the coil was measured with a thermometer and was maintained by airflow at laboratory value (both samples, control and exposed, were at the same temperature of 24–26 °C). Background magnetic field at 50 Hz was determined as 570 nT.

Table 1. Parameters of the coil

Outer diameter	235 mm
Inner diameter	205 mm
Height	210 mm
Number of threads	880
Diameter of wires	2 mm
Weight	5.7 kg

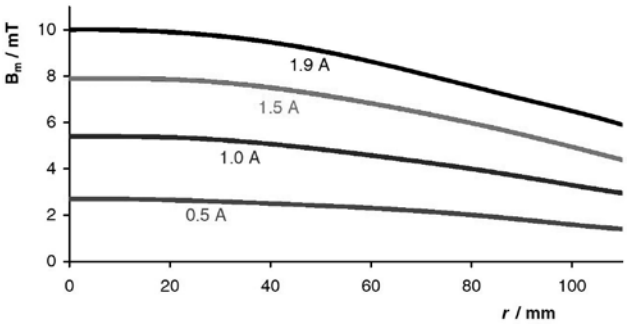

Fig. 1. Magnetic field distribution for magnetic field amplitude B_m inside the coil for different currents. Position r = 0 mm denotes the centre of the coil.

For the electrochemical measurements, a three-electrode system was used. Working electrodes were two types of carbon electrodes. A platinum wire (diameter of 2 mm) served as a counter electrode, Ag|AgCl saturated with 3 M KCl was the reference electrode. The cyclic voltammetry (CV) measurements were performed with the EcoTribo Polarograph (Polaro-Sensors, Prague, Czech Republic). The scan rate of the CV was adjusted to v = 50 mV.s^{-1}

Bacteria and chemicals

Sulphate-reducing bacteria (mixture of genera *Desulfovibrio* and *Desulfotomaculum* in ratio 2 : 1 – the ratio in which the SRB were isolated) were isolated from a mixed culture obtained from the waste water collection tank used (as washing machinery) in a metallurgical plant. We obtained these bacteria from the Institute of Geotechnologies of the Slovak Academy of Sciences in Košice. Postgate's medium C was used for the preparation and cultivation of SRB cultures. pH was adjusted to 7.5 by addition of 5 M NaOH. The medium and growth conditions for bacteria were prepared according to Luptáková and Kušnierová (2005).

For the electrochemical measurements, bacteria were incubated for 48 h at 30 °C in an incubator and then centrifuged at 5000 *g* for 5 min. The bacterial growth was checked spectrophotometrically using a Libra S22 spectrophotometer (Biochrom, Cambridge, UK) according to previously measured growth curves. The harvested pellets were washed two times in phosphate-buffered solution (PBS, pH 7.3) and then used for electrode surface modification.

Preparation of electrodes and measurement

Electrochemical measurements were performed with glassy carbon electrode (GCE, surface area 3.14 mm^2, Metrohm, Herisau, Switzerland) and pyrolytic graphite electrode with basal orientation (PGEb, surface area 15 mm^2, GE-Advanced Ceramics, Huntersville, NC, USA).

Torr-seal (Varian, Palo Alto, CA) epoxy resin was used for electrodes mounting. Electrodes were prepared for measurements as follows. The GCE was mechanically polished with silicon carbide papers. The minimum diameter of the polishing particles was 5 μm (SiC polishing papers, Struers, Copenhagen, Denmark). Diamond particles in spray (1 μm dimension) on Lecloth B polishing cloth (Leco, St. Joseph, MI) were used for final polishing of GCE. The PGEb surface was renewed using adhesive tape. The electrodes were sonicated for 5 min just before use.

The electrode surfaces were modified with living bacteria. Harvested bacteria were diluted in basal PBS. A 20 μl solution containing 10^5 SRB per 10 mm^2 (checked spectrophotometrically according to calibration measurements) was placed on the electrode surface and the solvent was allowed to evaporate. As the final step, the electrode was "clothed" by a dialysis membrane, physically attached to the electrode with nylon net.

All measurements were performed in 3 ml volume (supporting electrolyte PBS) at room temperature (24–26 °C). For each measurement, a newly prepared bacteria-covered electrode (bcE) was used. The PBS was continuously stirred at 3600 rpm and the electrochemical cell was permanently flushed with argon gas to maintain anaerobic conditions.

All measurements (exposure of SRB to magnetic field, electrochemical measurements) were performed at given room temperature. There was no difference between experiments done in the thermostat (exposed and control samples were placed in an incubator at 30 °C during the entire experiments, except for the inoculation).

For statistical analysis of the results, the Student's *t*-test was used. Differences were considered significant when $P < 0.05$. The electrochemical experiments were repeated 10 times.

Results and Discussion

The effects of low-frequency magnetic field (B_m = 10 mT, f = 50 Hz, 24 min magnetic field exposure) on SRB were studied using the CV electrochemical method for viability monitoring. We used two types of carbon-based working electrodes with different surface roughness. This experimental setting originates from the fact that electrode roughness influences the electrochemical signals (Fojt et al., 2006). The electrode surface properties could also be important for magnetic field effects. There exist theories proposing amplification of electromagnetic field effects on larger amounts of cells (Fitzsimmons et al., 1994).

The CV curves of surface-modified electrodes in PBS show a characteristic reduction peak (P_{red}) at the electrode potential around E = -310 mV. The oxidation peak (P_{ox}, position around E = 100 mV) is evolved poorly (Fig. 2A and 2B, curves 1 and 2), which indicates irreversibility of the involved processes. This fact could be caused by indirect observation of the anaerobic respiration process of SRB, desulphurization (it is an anaerobic process of biological reduction of sulphates which could be summarized as (Luptáková et al., 2002): $SO_4^{2-} + 4H_2 + H^+ \rightarrow HS^- + 4H_2O$). As in our previous work with *Paracoccus denitrificans* (Fojt et al., 2007), it is well possible that we observed oxidoreduction of an electron mediator involved in SRB respiration. In such case, the electron mediator would be oxidized in bacteria and reduced electrochemically – this fact may explain the difference between peak heights and the reduction peak being much higher than the oxidation one.

Both electrodes were tested for stability. The value of the peak current density j_p (height of the peak in the position of P_{ox} or P_{red}) was not changed after 30 min of keeping the whole system at rest without any measurement or manipulation. No peaks on CV were observed when the electrode surfaces were covered with dead bacteria (curve 3, Fig. 2A and 2B). From these findings it was concluded that j_p is related to desulphurization in living bacteria. When exposed to 50 Hz magnetic fields for 24 min, bcE showed a decrease of j_p of about 13 ± 4 % for bcGCE and of about 18 ± 5 % for bcPGEb at B_m = 10 mT compared with the non-exposed (B_m = 0) bcE (curve 1, Fig. 2A, 2B – unexposed bcE, curve 2, Fig. 2A, 2B – exposed bcE). The electrodes, either uncovered or covered only with dialysis membrane, both showed no changes of j_p after exposure to magnetic field, thus serving as a control.

The experimental results obtained with the electrochemical approach may have different explanations. The decrease of the j_p after magnetic field exposure could be caused by SRB metabolic activity decrease, by a decrease in the total number of active bacteria in the exposed culture, or by entering of SRB to the viable but nonculturable state (VBNC) (Oliver, 2005). This problem could be solved by results obtained in our previous research on SRB (Fojt et al., 2010). Using colony-forming units (CFU) counting we found a 15 ± 5 % decrease

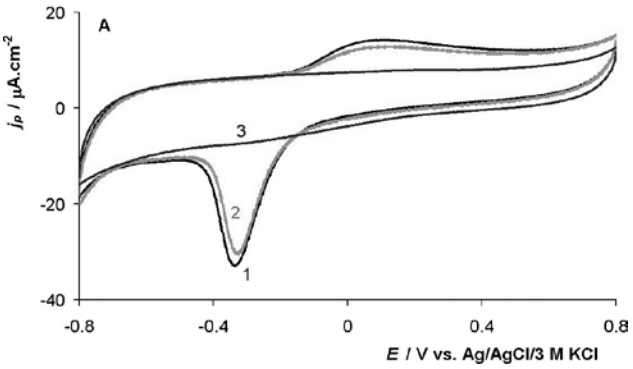

Fig. 2A. Cyclic voltammogram of living bacteria-covered glassy carbon electrode (bcGCE). Scan rate was adjusted to v = 50 mV.s^{-1}, 5th scans. Curve 1 – bcGCE in electrolyte (PBS). Two peaks (oxidation peak P_{ox} and reduction peak P_{red}) can be observed. Curve 2 – system after 24 min at B_m = 10 mT. Curve 3 – electrode covered with a layer of dead SRB.

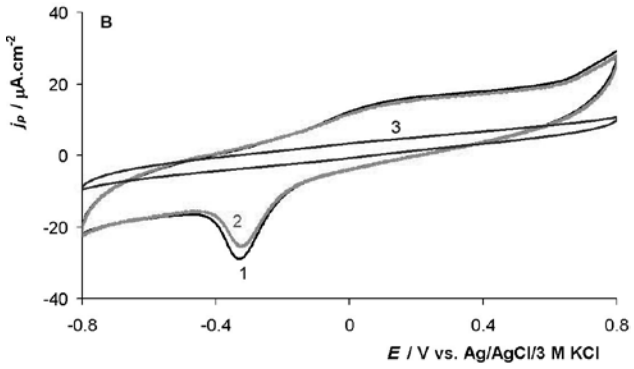

Fig. 2B. Cyclic voltammogram of living bacteria-covered pyrolytic carbon electrode in basal orientation (bcPGEb). Scan rate was adjusted to v = 50 mV.s^{-1}, 5th scans. Curve 1 – bcPGEb in electrolyte (PBS). Two peaks (oxidation peak P_{ox} and reduction peak P_{red}) can be observed. Curve 2 – system after 24 min at B_m = 10 mT. Curve 3 – electrode covered with a layer of dead SRB.

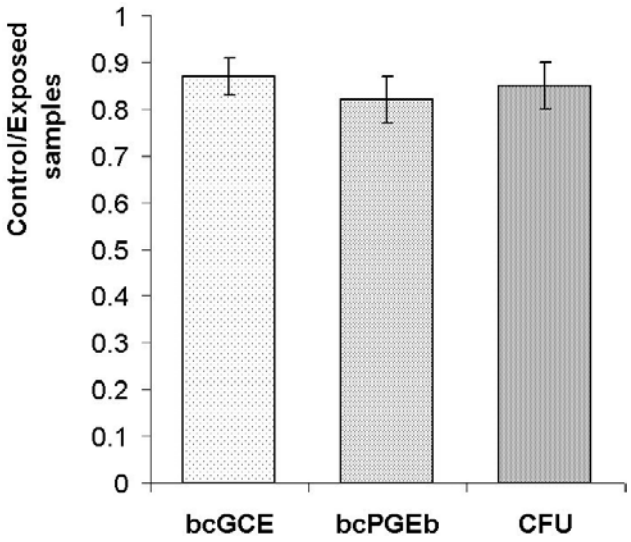

Fig. 3. Plot graph summarizing the effect of EMF (B_m = 10 mT, f = 50 Hz, t = 24 min) on the SRB. Vertical axis represents the ratio control/exposed sample. This relative number denotes, in case of bcPGEb and bcGCE, the ratio between j_p (and thus effects of EMF on desulphurization activity of SRB) and in case of CFU the ratio between the CFU number of control/exposed sample (has a meaning of survived bacteria after magnetic field exposure).

in CFU number after B_m = 10 mT, 24 min magnetic field exposure. We supposed that the decrease in CFU number corresponds to bacterial death (bactericidal effect of magnetic field). The decrease in j_p is statistically undistinguishable from CFU decrease in the same experimental conditions (Fig. 3). This leads us to the assumption that the decrease in metabolic activity is caused by bacterial death. The VBNC state of bacteria may lead to similar conclusion. The bacteria enter the VBNC state after encountering improper environmental conditions (Oliver, 2005). Thus, the EMF will cause response of the bacterium to the unacceptable external environmental conditions. Neverthelles, the SRB are not known to enter the VBNC state (Oliver, 2005). Our results are in good agreement with our previous work on other bacterial oxidoreduction systems. We have detected metabolic activity either electrochemically or spectrophotometrically (Fojt et al., 2004; 2007). Metabolic activity measured in these ways decreased identically as CFU numbers after magnetic field exposure. This fact supports our assumption that the decrease in metabolic activity is caused by bacterial death.

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