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Hydraulic continuity and biological effects of low strength very low frequency electromagnetic waves: Case of microbial biofilm growth in water treatment

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ABSTRACT

This study aims to elucidate the interactions between water, subjected to electromagnetic waves of very low frequency (VLF) (kHz) with low strength electromagnetic fields (3.5 mT inside the coils), and the development of microbial biofilms in this exposed water. Experimental results demonstrate that in water exposed to VLF electromagnetic waves, the biomass of biofilm is limited if hydraulic continuity is achieved between the electromagnetic generator and the biofilm media. The measured amount of the biofilm's biomass is approximately a factor two lower for exposed biofilm than the non-exposed biofilm. Measurements of electromagnetic fields in the air and simulations exhibit very low intensities of fields (<10 nT and 2 V/m) in the biofilm-exposed region at a distance of 1 m from the electromagnetic generator. Exposure to electric and magnetic fields of the quoted intensities cannot explain thermal and ionizing effects on the biofilm. A variable electrical potential with a magnitude close to 20 mV was detected in the tank in hydraulic continuity with the electromagnetic generator. The application of quantum field theory may help to explain the observed effects in this case.

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1. Introduction

Although a number of studies have been conducted over many years, the effect of very low frequency (VLF) electric and magnetic fields (EMF) on living organisms is still insufficiently understood, particularly at low intensity. Radio frequencies (RF) in the range of 3 kHz–30 kHz and wavelenghts from 1 to 10 km are considered to be in the VLF regime. A decrease in biofilm has been empirically noted after electromagnetic treatment of limescale formation in water supply lines of poultry farms and irrigation systems using VLF electromagnetic commercial systems. Treatment of seawater with the same electromagnetic system enabled the partial removal of a mature biofilm adhering to the inner surfaces of the tubes of a heat exchanger-condenser (Trueba et al., 2014). Therefore, it is interesting to investigate the effect of VLF-EMF on biofilm formation in water, through a multidisciplinary approach, under controlled conditions.

Generally speaking, biofilms are defined as a complex community of bacteria, fungi, protozoa and macroinvertebrates with several trophic levels as observed for wastewater biofilms (Tsuneda et al., 2003). Biofilm development on a medium is achieved in several steps (O'Toole et al., 2000): initiation, adhesion, growth, maturation, and detachment. Many factors affect the various steps and involve the physicochemical properties of the substrate, biotic factors and/or environmental factors (Mueller, 1996). Organization of microorganisms in biofilm enables optimal use of available substrates and protects the microorganisms from adverse conditions and stress (Jefferson, 2004). Also, 70–90% of the dry mass of biofilms consists of extracellular polymeric substances (Tsuneda et al., 2003) which gives the biofilm the properties of a gel with a highly hydrophilic porous structure containing up to 95% H_2O. Although in certain cases the presence of biofilms is good (e.g.

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bioprocesses case), in many cases, this presence can have a negative effect due to clogging or health problems; and various techniques are subsequently used to limit or eradicate the unwanted biofilm (Costerton et al., 1999).

Biological effects due to RF exposure; including VLF waves, are classified as thermal effects and non-thermal effects in lower energy fields. Non-thermal effects of electromagnetic waves are the most difficult to identify due to the low exposure energies. Interaction of EMFs with matter can be modeled from the microscopic level to the macroscopic level (Baker-Jarvis and Sung, 2012). In theory, the electromagnetic field interactions with biological matter may be modeled on a microscopic scale by applying quantum mechanics and by using Maxwell's equations at macroscopic level. It is challenging to model interactions in the mesoscopic scale (10^{-7} m–10^{-4} m) since classical analysis begins to be modified by quantum mechanics. According to the quasi-static nature of EMF at low frequencies, such RF electric and magnetic fields act independently of one another (Habash Riadh, 2006) and bioelectric or biomagnetic phenomena are commonly modeled as quasi-static cases in which electric and magnetic fields can be studied separately (Zhou and Uesaka, 2006). Dielectric response of biological materials is related to membrane and cell boundaries, molecular dipoles, together with associated ionic fluids and counterions (Baker-Jarvis and Sung, 2012). At a molecular level RF-EMF interact with moving charges, similar to electrons in cytochrome oxidase (Blank and Soo, 2001).

To the best of our knowledge excluding the work of Trueba et al. (2014), the only studies published on the effects of EMFs on biofilms concern extremely low frequency EMF. Although VLF electromagnetic waves are slightly more energetic than extremely low frequencies (ELF) electromagnetic waves, the nature of observed non-thermal effects could be similar to ELF electromagnetic waves. For prokaryotic systems, exposure to electromagnetic fields produce effects of stress, causing phenotypic and genetic perturbations of planktonic cells (free cells) that may affect the adhesion of these cells and their organization into a biofilm (Cellini et al., 2008; Chua and Yea, 2005; Del re et al., 2004). Bacterial cultures of *Helicobacter pylori ATCC 43629* were exposed to a low frequency magnetic field (50 Hz, 1 mT intensity) for two days (Di Campli et al., 2010). The magnetic field acted on the bacterial population during the formation of the biofilm and after the maturation phase by decreasing cell viability and cell mass when compared to the control biofilm. Based on the work of Pickering et al. (Pickering et al., 2003) when Staphylococcus epidermidis biofilms aged 5 days and incubated 12 h with various concentrations of antibiotics (vancomycin or gentamicin) at 37 °C and 5% CO_2, are exposed to ELF-EMF (72 Hz), the efficiency of gentamicin against biofilms increases by at least 50%. It was also found that electrical fields with low intensity (1.5–20 V cm^{-1}) and current densities ranging from 15 μA cm^{-2} to 2.1 mA cm^{-2} can decrease the inherent resistance of bacterial biofilms to biocides (Blenkinsopp et al., 1992) and antibiotics (Costerton et al., 1994). This bioelectric effect reduces by a factor 1.5 to 4.0 the required concentrations of these antibacterial agents to kill the biofilm bacteria.

In this study we investigated the formation and development of biofilms on glass slides exposed to water subjected to VLF electromagnetic waves. This study represents only part of the ongoing work to characterize the effects of electromagnetic fields at very low frequencies and low intensities on the growth of microorganisms in aqueous media. This work corresponds to a first approach to verify and quantify empirical observations found *in situ*.

Compared with previous studies done with ELF-EMF, the biofilm in our study was not exposed in the heart of the generator but at a distance of one meter, where the field strengths in the air were extremely weak. Both the presence and lack of hydraulic continuity was investigated to assess its role in the transmission of EMF and their possible effects between the generator and the biofilm on glass supports. The assumption would be that the high sensitivity of liquid water to electromagnetic waves was due to its organization in coherence domains (CD), as calculated with quantum field theory (Bono et al., 2012). Under this assumption liquid water is organized in domains by a stacking of coherence overlapping with each other and holding cold vortices of quasi-free electrons (Marchettini et al., 2010), making it sensitive to electromagnetic fields on the order of a few kHz. Intracellular water maintaining its coherence would also be sensitive to electromagnetic waves of very low frequencies, resulting in a disturbance of the metabolic activities (Del Guidice et al., 2010).

2. Materials and methods

2.1. Experimental device (Fig. 1)

Glass slides were placed in glass tanks fed by a nutrient solution that had previously passed through an electromagnetic wave generator (Aqua-4D 60E Pro® from Planet Technologies Horizons, Switzerland). The use of a synthetic nutrient solution enabled control of the operating conditions, particularly the applied organic load: starch 0.05 g/L; glucose 0.05 g/L; KH_2PO_4 1.25 mg/L; $(NH_4)_2SO_4$ 2.5 mg/L; CH_3CO_2Na 2.5 mg/L; peptones 2.5 mg/L. The chemical oxygen demand (COD) was close to 100 mg/L, the electrical conductivity was 0.36 ± 0.027 mS cm^{-1} and the pH of the nutrient solution was 7.2. These physicochemical parameter values correspond to eutrophic-hypertrophic conditions (European Water Framework Directive, 2000) which promote the development of biofilms on the glass slides.

The first tank (C1) arranged downstream from the generator at a distance of one meter was in hydraulic continuity with the generator; while the second tank (C2) was located downstream from C1 at a distance of 0.20 m from C1 and supplied by a dropwise, ensuring hydraulic discontinuity. The biofilm media were glass slides (76 × 26 mm^2). Twenty slides were placed in tanks C1 and C2 and positioned on supports. C1 tank was of cylindrical shape with a diameter of 0.16 m and a water height of 0.15 m. C2 tank was a rectangular parallelepiped having a width of 0.10 m, a length of 0.30 m and a water height of 0.10 m. Each tank had a volume of 3 L of nutrient solution. The facility had a total storage volume of

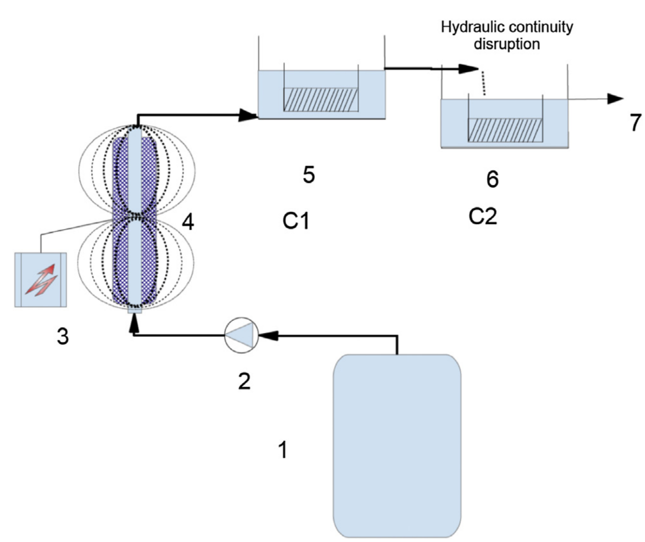

Fig. 1. Schematic representation of one line of the experimental device (1: feeding tank; 2: peristaltic pump; 3 and 4: generator and tube Aqua-4D 60E; 5: tank 1 and glass-slides; 6: tank 2 with glass-slides; 7: outlet).

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