

# The effects of electric, magnetic and electromagnetic fields on microorganisms in the perspective of bioremediation

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**Abstract** Some studies show how exposure to fields can enhance or reduce cell activity, with possible applicative consequences in the field of biotechnology, including biological techniques for depollution. In order to identify full-scale conditions that are suitable and potentially applicable for use in electromagnetic fields to stimulate and accelerate bioremediation processes, this paper offers an examination of the scientific literature that is available on the effects of fields on microorganisms, and a critical analysis of it. The biological effects at times contrast with each other.

**Keywords** Electric field · Magnetic field · Electromagnetic field · Bioremediation

## 1 Introduction

The first studies on the influence of electromagnetism on organisms dates back to the end of the nineteenth century, to then intensify in the following decades after global electrification and the diffusion of

telecommunication. The massive introduction into daily life of technologies that emit electric, magnetic and electromagnetic fields in an enormous range of frequencies and intensities led institutions and the scientific community to question itself about the effects on public health and the environment. Pertinent scientific literature is very vast and includes studies that vary greatly on the type of field, intensity, exposure duration, long-/short-term effects and considered biological targets (cell, tissue, organ and organism).

In contrast to the studies indicated above, the research that investigates the field effects on microorganisms is very limited. Model microorganisms, well characterised with genetic markers, were used in medical-health research to better understand the field action mechanisms. Some of these works showed how, in some situations, exposure to electromagnetic fields tends to enhance rather than reduce cell activity, with possible applicative consequences in the field of biotechnology, including biological techniques for depollution.

In order to identify full-scale conditions that are suitable and potentially applicable for use in electromagnetic fields to stimulate and accelerate bioremediation processes, this paper offers an examination of the scientific literature that is available on the effects of fields applied on microorganisms, and a critical analysis of it. In consideration of the objective of this document, aimed at environmental bioremediation, the effects of electromagnetic fields applied on cells,

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bacterial cells in particular, are focused on. The information obtained from the literature that was consulted is summarised in Tables 1, 2 and 3, respectively on nominal exposure to electrostatic fields/fields generated by direct current (DC), magnetic and electromagnetic currents/fields generated by alternate current (AC). The decision was made to treat electrostatic field applications (typically generated while maintaining a constant voltage between pairs of electrodes) together with fields generated by direct current because when the sources are applied to dielectric means (soil, wastewater, etc.) they produce similar effects. These effects are, in fact, so similar that even in most of the literature that was analysed, they are treated simultaneously, without any distinction between the two situations.

In consideration of the manuscript objectives, the effects indicated in the literature were divided into four different categories (“positive”, “negative”, “undefinable” or “null”) according to their possible implications on environmental bioremediation:

1. “positive” (+) effects: stimulation of the degradation of contaminants, increased denitrification/nitrification activity, acceleration of the substratum consumption kinetics, increase in the resistance to pollutants, increase of the biomass, increase in metabolic activity or in the activity of specific enzymes (e.g. dehydrogenases);
2. “negative” (–) effects: reduction of the degradation of pollutants and/or substratum consumption, inhibition of bacterial growth, reduction in metabolic activity or in the respiration rate, damage to the cellular membrane. At times, these effects are not tied to the direct action of the field/current on the cells, but rather to modifications in the environmental conditions (e.g. extreme pH values, electrochemical production of toxic species, radicals, etc.) (“indirect negative”);
3. undefinable (“×”): in the absence of effects (1) or (2), modifications in the activity of enzymes that are not involved in the degradative metabolism, variation in the concentration of ATP, modifications to the microbial community (structure/diversity/genotype/morphotype), effects of mutagenicity, alterations to the cell proteome, synthesis alterations of the DNA/RNA and correlated activities, variations in the transposition and production of secondary metabolites, modifications in the cell form and the characteristics of the cell wall and its electrostatic charge, increased cell hydrophobicity, increased adhesion between bacterial cells, increased or reduced resistance to antibiotics;
4. “null” (=): absence of significant effects on the aspects indicated above.

## 2 Electrostatic fields and fields generated by direct current

The first experiences in using fields that are electrostatic or generated by DC current to favour microbial growth date back to more than 50 years ago. They were based on the use of water hydrolysis to produce O<sub>2</sub> electrochemically, as a replacement to other aeration systems, in order to grow *Pseudomonas fluorescens* (Sadoff et al. 1956 in Thrash and Coates 2008) or the combined production of O<sub>2</sub> and H<sub>2</sub> to grow the hydrogen-reducing aerobic microorganism *Ralstonia eutropha H16* (Schlegel et al. 1965 in Thrash and Coates 2008).

### 2.1 Studies on microorganisms

Regarding the direct effects on microorganisms of the field that is electrostatic/generated by direct current, the literature indicates possible modifications in the physiology and form of the cells, the chemical–physical characteristics of the cellular membrane (Zimmermann et al. 1973) and membrane permeability and potential, with repercussions on its ability to exchange with the external environment, cell metabolism and mobility (Luo et al. 2005a; Golzio et al. 2004). The entity of these phenomena is a function of the species, but generally proportional to the intensity of the field/current applied and the duration of exposure. The results are stimulation of bacterial activity and increased cell mobility, observed in light intensity fields, as well as irreversible damage to the microorganisms, with a loss of membrane integrity, in the case of exposure to more intense electric fields (Sakakibara and Kuroda 1993; Satoshi et al. 1997; Chen et al. 2002; Diao et al. 2004; Zituni et al. 2014).

Even though different species present different levels of sensitivity, high intensity electric fields, for example of 1000 kV/m, or circulating direct currents of 1 A damage the cells irreversibly and cause the

**Table 1** Summary of the data of the consulted literature, in order of author, on the effects of the application of direct currents and/or electrostatic fields

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
Aerobic or anaerobic sludge	10–40 mA		12–24 h	Direct current applied to a reactor of 0.5 l through two carbon electrodes (approx. 50 cm <sup>2</sup> each)	Inhibition of phenol degradation, both in aerobic and anaerobic conditions; reduced biomass growth (– 40%); reduced ATP levels. Effects due to pH lowering. No variation was seen at 5 mA, even in the pH value	Ailijiang et al. (2016)
Activated sludge		0 V/m, 0.28 V/m, 0.57 V/m, 1.14 V/m	50 h	Aerated and mixed reactor, 2 l, with electrodes	At 28 V/m there were no effects on COD removal At 57 and 114 V/m stimulation of biological activity At 114 V/m, after 24 h, decrease in the COD removal rate 28–114 V/m was the optimal “window” for biomass stimulation	Alshawabkeh et al. (2004)
<i>Escherichia coli</i> , <i>Listeria innocua</i> , <i>Leuconostoc mesenteroides</i>		2500–3500 kV/m	20–40 pulses of 2–4 μs, at frequency of 250 pulses/s	Continuous system, with six tubular treatment chambers (Ø 0.22 cm) in serial. Electrodes mounted externally on each chamber and distant 0.23 cm from each other	Microorganisms’ inactivation increasing with the electric field intensity, duration and number of applied pulses. Even though with negative effects on all the species examined, a different susceptibility was noted, with <i>L. innocua</i> and <i>L. mesenteroides</i> being more resistant than <i>E. coli</i>	Aronsson et al. (2001)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
<i>Escherichia coli</i> , <i>Listeria innocua</i>		500–1000– 2000–2500– 3000 kV/m	20–40 pulses of 2–4 $\mu$ s, at frequency of 250 pulses/s	Continuous system, with six tubular treatment chambers ( $\varnothing$ 0.22 cm) in serial. Electrodes mounted externally on each chamber and distant 0.23 cm from each other	No significant effect on <i>L. innocua</i> at all intensities with pulses of 2 $\mu$ s, reduction by one order of magnitude of the vital cells with 4 $\mu$ s and fields > 2000 kV/m, with effects increasing as the field intensity increased. <i>E. coli</i> had negative effects in all the tested conditions, with a reduction of 5 sizes in the number of vital cells when exposed to fields $\geq$ 1000 kV/m and pulses of 4 $\mu$ s or $\geq$ 1500 kV/m and 2 $\mu$ s	Aronsson et al. (2005)
Denitrifying mixed culture	1 mA		70 h	Current applied to a digester of 0.75 l by way of graphite, steel or copper electrodes	Stimulation of denitrification with graphite and steel electrodes, inhibition with copper electrodes	Cast and Flora (1998)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
<i>P. aeruginosa</i>		500 V/m with polarity reversal every 64 s	50 h	Flow chamber with 5 flat parallel steel electrodes, two of which (at the entry and exit points of the chamber) connected to each other, and the central one connected to the voltage generator	The electric field caused a temporary reduction in the biomass of about 40% during the first 24 h of exposure; in the following 24 h biomass growth was observed, but remained about 2 orders of magnitude smaller than the control at the end of the test. The electric field also reinforced the bactericidal effect of antibiotics	Costerton et al. (1994)
<i>Escherichia coli</i>	16–24 mA/cm <sup>2</sup> (320–480 mA)		0, 5 min a 24 mA/cm <sup>2</sup>	Reactor (6 × 4 × 5 cm <sup>3</sup> ) with 5 flat parallel titanium electrodes (5 × 4 cm <sup>2</sup> ) placed 1 cm from each other	99.8% inactivation at 320 mA and exposure duration 2 min	Diao et al. (2004)
			2 min a 16 mA/cm <sup>2</sup>		Inactivation increased as the current increased, even with shorter exposure durations	

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
Mixed culture	Variable in the range 5.7–21.3 mA	100 V/m with polarity reversal every 6–12 h	15 days	Two electrokinetic cells, (a) (24 × 12 × 7 cm) and (b) (22 × 22 × 10 cm), containing soil contaminated by 2,4 dichlorophenol. Constant voltage applied, without and with cyclic inversion of the polarity, through cylindrical graphite electrodes (∅ 0.5 cm, 6 cm), in (a) 2 electrodes that distance 20 cm from each other, or (b) 7 electrodes positioned in a hexagonal manner and with one at the centre (10 cm between the perimeter electrodes and the central one)	Inhibition of bacterial activity with unidirectional field because of pH variations  With polarity inversion every 6 h, good control of the pH and pollutant removal three times higher  In the hexagonal configuration, the field resulted in a uniform distribution of the residual contaminant in the soil, but with lower degradation efficiencies when compared with the configuration with parallel electrodes  With rotational field, removal higher in the central portion of the system and lower at the edges, with uneven residual pollutant distribution	Fan et al. (2007)
				(b-1) voltage applied between the central electrode and the perimeter ones, connected in parallel with each other, with polarity inversion every 6–12 h (b-2) voltage applied between the central electrode and one of those at the perimeter, with a change every 6–12 h		

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
Denitrifying mixed culture	0–10 mA		520 days	Reactor with amorphous carbon anode and steel cathode surrounded by polyurethane foam colonised by a denitrifying biofilm	Nitrate removal efficiency between 0 and 100%, proportional to the applied current	Feleke et al. (1998)
Nitrifying mixed culture	1.25–2.5 mA/cm <sup>2</sup> (20–50 mA)		40 days	Moving bed batch reactor (365 ml) with flat electrodes (6.18 × 6.35 cm <sup>2</sup> , distant 1.4 cm from each other), titanium anode (thickness 1.4 mm) and steel cathode (0.7 mm)	Denitrification supported exclusively by the electrochemical production of H <sub>2</sub> O <sub>2</sub> generated electrochemically used in nitrification	Goel and Flora (2005a)
Hydrocarbon-degrading mixed culture		100 V/m with polarity reversal every 5 min	100 days	Electrokinetic cell (100 × 100 × 25 cm <sup>3</sup> ) filled with 100 kg of soil contaminated by hydrocarbons (450,000 mg/kg). 25 electrodes of cylindrical graphite (Ø 1 cm, 20 cm) positioned in rows, each row having 5 connected in parallel. Constant potential difference between one row of electrodes and the next one, with periodic inversion of the polarity	Positive correlation between hydrocarbon degradation and electric field intensity	Cheng et al. (2014)
		2D field of 2–50 V/m			Modification in the soil microbe community	

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
<i>Sphingobium</i> sp. UG30, pentachlorophenol-degrader	3.14 A/m <sup>2</sup> (10 mA) constant or with periodic reversal	100 V/m	36–95 days	Soil microcosm (0.5 kg; cell $13 \times 5.9 \times 5.4 \text{ cm}^3$ ) contaminated by pentachlorophenol (100 mg/kg). Graphite electrodes ( $5 \times 5 \times 0.8 \text{ cm}^3$ )	Better results on pentachlorophenol degradation with periodic field inversion	Harbottle et al. (2009)
				Tests (1) and (2) with electrodes in cathode chambers, separated by the soil with an ionic exchange membrane; purified water as the electrolyte; pH control at the cathode with acid dose (test 1) or with anolyte and catholyte mix (test 2)	With mono-directional field, scarce control of the pH and humidity. Biomass inhibition	
				Test (3) with electrodes directly in the soil; pH control with daily inversion of the current		
Denitrifying mixed culture in soil	5–20 mA		5–9 days	Electrodes of steel ( $\varnothing$ 0.6 cm, 15 cm) and graphite ( $\varnothing$ 1.4 cm, 20 cm), distant about 45 cm from each other, inserted by 6.4 cm into columns ( $\varnothing$ 9.7 cm, 60 cm) of sandy soil	Stimulation of denitrification proportional to the circulating current. pH needs to be controlled	Hayes et al. (1998)
Mixed culture in soil		100 V/m, with or without periodic polarity reversal	50 days	Electrokinetic cell ( $26 \times 14 \times 8 \text{ cm}^3$ ) filled with about 1 kg of soil contaminated by pyrene. Two pairs of cylindrical graphite electrodes ( $\varnothing$ 1 cm, 14 cm)	Degradation of pyrene and biomass at the end of the test higher than in the control system without electric field. Better results with periodic polarity inversion thanks to better control of the pH	Huang et al. (2012)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
Sulfur-oxidizing bacteria ( <i>Thiobacillus ferrooxidans</i> and mixed culture), <i>Acidiphilium</i> SJH	200 mA/cm <sup>2</sup> (20 mA)	150 V/m	28–80 h (liquid phase test)	Flat platinum bioelectrodes inserted into cylindrical cones of plastic material and positioned on an orbital mixer	Inactivation of <i>T. ferrooxidans</i> and <i>Acidiphilium</i> SJH at low cellular density in the liquid broth. At high optic densities, the effect of the current was low and did not influence the <i>Acidiphilium</i> SJH	Jackman et al. (1999)
			240–540 h (slurry test)		In a water-soil slurry (with silt at 5, 10, 30% by weight), the sulphur-oxidants showed an increase in metabolic activity (greater production of sulphates). <i>Acidiphilium</i> SJH in a 10% and 30% slurry showed an increased consumption of glucose. No increase in the metabolic activity of the ferro-oxidans	
<i>Burkholderia</i> spp., 2,4 dichloroacetic acid degrader	0.89 A/m <sup>2</sup> (8 mA)	max 47.3 V/m		Electrokinetic cell (22 × 7 × 4 cm <sup>3</sup> ) filled with soil contaminated by 2,4-dichlorophenoxyacetic acid. Graphite fiber electrodes and steel mesh positioned at the ends of the cell	Positive effects on pollutant biodegradation because of electromigration	Jackman et al. (2001)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
<i>Thiobacillus ferrooxidans</i>	20, 30, 40, 80, 120 mA		84 h	Direct current applied to two graphite electrodes positioned in a reactor with biomass and growth soil	Bacterial growth stimulation; better results with currents of 30 mA	Ji et al. (2010)
Mixed culture from soil contaminated by diesel	0.63 mA/cm <sup>2</sup> (10 mA)		25 days	Soil (4 × 4 × 20 cm <sup>3</sup> ) with implanted graphite electrodes	No significant effect at 80 mA and 120 mA The modifications in the pH during electrokinetic treatment reduced the total number of microorganisms and the biodiversity. The use of EDTA as an electrolyte caused toxic effects on the biomass in the cathodic region, while dehydrogenasis increased near the anode and hydrocarbon biodegradation reached 60%. These effects were related with the variation in the pH value and the effect of the electric field	Kim et al. (2010)
<i>Staphylococcus aureus</i> and <i>Yersinia enterocolitica</i>	10–20–30 mA		7 days	Current applied using titanium electrodes coated with platinum (∅ 2 mm), immersed in culture gel on Petri dishes with bacteria	Inhibition of growth in both bacterial strains in all the tested conditions. Growing concentrations of NaCl in the culture gel increased the inhibiting effect of the current	Król and Jammoluk (2014)
Mixed culture from soil	3.14 A/m <sup>2</sup> (1 mA)		27 days	DC applied using graphite plate electrodes (5 × 5 × 0.8 cm <sup>3</sup> ) in an electrokinetic cell (5.9 × 5, 4 × 13 cm <sup>3</sup> ) filled with approximately 0.5 kg of soil	Composition and diversity of the bacterial community only minimally influenced by electrokinetic treatment. Significant variations at the end of the experiments, but only	Lear et al. (2004)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
<i>Sphingobium</i> sp. UG30	3.14 A/m <sup>2</sup> (1 mA)		36 days	DC applied using graphite plate electrodes (5 × 5 × 0.8 cm <sup>3</sup> ) in an electrokinetic cell (5.9 × 5, 4 × 13 cm <sup>3</sup> ) filled with soil contaminated by pentachlorophenol (100 mg/kg)	near the anode, and ascribed to the variations in pH At the end of the test, decrease in the microbial respiration rate and in the use of soil substrata, especially at the anode where the pentachlorophenol accumulated and the pH was more acidic	Lear et al. (2007)
Activated sludge	1.98 A/m <sup>2</sup> (a 17, 7 V/m)	0–5, 9–11, 8–17, 7–29, 4–59 V/m	32–65 h	Aerated bioelectroreactor (21 × 30 × 20 cm <sup>3</sup> ), with polypropylene elements as the supporting material for the bacterial biofilm and a pair of flat steel electrodes (20 × 15 cm <sup>2</sup> ) positioned 17 cm from each other. Reactor supplied continually with synthetic wastewater containing phenol (1600–2800 mg/l)	At an intensity of 5.9 V/m, no significant effects were recorded in phenol removal, while stimulation of the biological activity could be seen between 11.8 and 17.7 V/m. Better conditions (removal + 30%) observed at 17.7 V/m. Higher fields resulted in a reduction in the degradation capacity of the phenol compared with the control, with almost complete inhibition of the biomass at 59 V/m	Li et al. (2006)
<i>Staphylococcus epidermidis</i> and <i>Staphylococcus aureus</i>	0.01–0.1 mA		16 h	Electrodes in a Petri dish with culture gel and microorganisms	Antibacterial effects caused by the formation and accumulation of H <sub>2</sub> O <sub>2</sub> and Cl <sub>2</sub> because of anodic and cathodic reactions	Liu et al. (1997)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
Phenol-degrading bacteria	5–40 mA		9–12 h	Graphite electrodes immersed in a closed mixed beaker (250 ml), containing biomass in mineral medium and phenol as the only source of carbon	Currents < 20 mA did not induce significant modifications in the cellular walls; at 20 mA increase in the hydrophobicity and flattening of the cell form; at 40 mA increase in the negative electrostatic charge and reduction of adhesion to the surfaces, exudates increase (cellular membrane damaged) Currents of 20 mA was the limit not to induce negative effects in the tested bacterial community	Luo et al. (2005a)
Phenol-degrading mixed culture	12 mA (max)	100 V/m monodirectional or with periodic polarity reversal every 1.5–3 or 12 h	10 days	Electrokinetic cell ( $24 \times 12 \times 10 \text{ cm}^3$ ) with cylindrical graphite electrodes ( $\varnothing 0.5 \text{ cm}$ , 12 cm) with silty sand contaminated by phenol (200 mg/kg)	With the monodirectional field, phenol removed by about 20% in 10 days; 46% removal with periodic polarity inversion (4 times higher than the control), which made it possible to contain variations in the pH and soil humidity, with positive effects on biodegradation	Luo et al. (2005b)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
Phenol-degrading mixed culture	100 V/m with periodic polarity reversal every 1.5–3 or 12 h	100 V/m with periodic polarity reversal every 1.5–3 or 12 h	10 days	Electrokinetic cell ( $24 \times 12 \times 10 \text{ cm}^3$ ) with 1–4 pairs of cylindrical graphite electrodes ( $\varnothing$ 0.5 cm, 12 cm) supplied in rotation	Periodic polarity inversion at intervals of 1.5, 3 and 12 h increased the removal of phenol, with abatement respectively of 68%, 60% and 49% in the central portion of the system. The removal of phenol was relatively uniform for inversions every 1.5 and 3 h, while for intervals of 12 h it accumulated near both electrodes	Luo et al. (2006)
Diesel-degrading mixed culture	20–40–60–200 V/m	20–40–60–200 V/m	2–7 days	Flat Ti electrodes ( $2 \times 12 \text{ cm}^2$ ) immersed in a batch reactor of 2 l containing the biomass in mineral medium and diesel/glucose	Increased respiration rate in all the test conditions. The applied field seemed to help degradation of the most recalcitrant fractions of the hydrocarbon mixture	Mena et al. (2014)
Mixed culture	50; 100; 150 V/m with periodic polarity reversal every 24 h	50; 100; 150 V/m with periodic polarity reversal every 24 h	14 days	Electrokinetic cell with clay contaminated by hydrocarbons (10,000 mg/kg)	The degradation of pollutants with application of the electric field was equal or better than the control in all the tests carried out, with better results for fields of 150 V/m. In these conditions, however, there was local inhibition of bacterial activity near the electrodes, probably because of the variations in the pH	Mena et al. (2016a)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
<i>Novosphingobium</i> sp. LH128		50–60 V/m for the first 77 h, then 20–30 V/m till the end of the test	14 days	Electrokinetic cell containing 0.7 kg of soil contaminated by PAH, inoculated with <i>Novosphingobium</i> . Field applied through a pair of steel electrodes fixed in the soil	When compared to the controls (abiotic with electric field and biotic without electric field), greater degradation of phenanthrene in the inoculated cell, above all near the cathode and in the central portion of the cell. Results ascribed mainly to electroosmosis	Niqui-Arroyo et al. (2006)
Seawater bacteria	0.5–2 A		100–2000 ms	To avoid excessive pH variations, recirculation of buffer solution Electrochemical cell with flat Pt electrodes ( $1 \times 8 \text{ cm}^2$ ), distance 1 cm	Reduction in the number of vital cells with substantial damage at cellular level. The effects were amplified by the high concentration of dissolved salts	Park et al. (2003)
Mixed culture from soil polluted with diesel		100–200 V/m	15 days	Cylindrical electrokinetic cell ( $\varnothing$ 120 cm, 30 cm) containing soil contaminated by diesel (20,000 mg/kg) in the central part and sand/gravel in the anodic and cathodic compartments, where graphite electrodes are fixed. To limit the pH variations, citric acid and a buffer solution were added to the electrolyte solution	Greater removal of pollutants with higher intensity field, however with marked reduction in the respiration rate and biodiversity	Pazos et al. (2012)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
Mixed culture	Up to 20 mA	666–1500 V/m	22–100 days	Cylindrical cell (∅ 6 cm, 4.5–7.5 cm) containing 100–400 g of soil contaminated by organochlorine compounds. Platinum or steel electrodes, mounted on the covers of the cell; continuous circulation of water	Removal of 80% of hexachlorobutadiene, ascribable to electrochemical reactions, variations in pH and redox potential	+ Rahner et al. (2002)
<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Micrococcus lysodeikticus</i> , <i>Sarcina lutea</i> , <i>Bacillus subtilis</i> , <i>B. cereus</i> , <i>B. megaterium</i> , <i>Clostridium welchii</i>	800–6100 mA/cm <sup>2</sup>	490–2500 kV/m	10 pulses of 20 s each	Flat graphite electrodes and cell for holding the bacteria in soil/culture gel. Apparatus equipped with water cooling system, to keep the temperature variation within 10 °C	High intensity electric fields caused irreversible damage to the cells and killed the bacteria. The bacterial death rate increased in proportion with the intensity of the applied field and the overall duration of exposure, even though the different species had different sensitivities. Duration and number of pulses were not significant parameters. Effects related to increases in temperature and electrolysis were excluded	– Sale and Hamilton (1967)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
<i>Enterobacter dissolvens</i>	5, 10, 20, 100 mA		25 h	Batch reactors (100 ml) containing biomass in growth medium with glucose as a carbon source. DC applied through platinum electrodes (wires $\varnothing$ 0.3 mm) or saline bridges (KCl in agar)	In the case of electrodes with saline bridge, very limited effects (< 5%) on biomass growth or on the enzymatic activity of the bacteria, at least up to currents of 20 mA (reactions of electrolysis at the electrodes were not observed and the small differences compared to the control were ascribed to the increase in temperature of 0.5 °C in the DC system)  With Pt electrodes, stimulation of the bacterial growth and dehydrogenase activity during the exponential growth phase, ascribed to H <sub>2</sub> and O <sub>2</sub> produced by electrolysis at 10 mA, while in the stationary phase there was an increase in the bacterial death rate, probably related to the accumulation of intermediate radicals (OH <sup>•</sup> and O <sub>2</sub> <sup>•-</sup> ) of the anodic reactions  Currents of 20 and 100 mA inhibited bacterial growth	She et al. (2006)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
<i>Sphingomonas</i> sp. LB126	10.2 mA/cm <sup>2</sup> (240 mA)	100 V/m	40 min	Titanium-iridium electrodes in cuvettes (8 × 8 × 3 cm <sup>3</sup> ) containing biomass suspended in the culture broth	Increase (+ 40%) in the levels of ATP, without other significant effects at a cellular level (membrane integrity, chemical–physical properties of the cell surface, cultivability and fluorene biodegradation rate)	Shi et al. (2008)
Mixed culture		46 V/m	90 days	Six anodes positioned in a circle around the cathode (anode–cathode distance: 2.5 m)	Stimulation of PAH biodegradation, mainly because of increased soil temperature	Suni et al. (2007)
<i>Escherichia coli</i> , <i>Bacillus cereus</i>	5, 10, 20, 40 mA		72–192 h	Direct current with polarity inversion every 60 s, applied through a pair of graphite or copper electrodes in a cylindrical beaker containing microorganisms in the culture broth	With copper electrodes, inhibition in all the tested conditions. With graphite electrodes, no significant effects on <i>E. coli</i> growth at 5–10 mA; at 20–40 mA, inhibition of bacterial activity with a reduction in the ATP and enzymatic activity. At low currents, no effects on <i>B. cereus</i> growth and on the metabolic activity; at 40 mA, stimulation of growth, increase of ATP and stimulation of the activity of some enzymes	Valle et al. (2007)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
<i>Aspergillus niger</i>	Up to 0.42 mA/cm <sup>2</sup> (8 mA)	35 V/m	24 h	Direct current applied to electrodes coated in titanium oxide and covered with 15 g of perlite	The degradation of the hexadecane, after 8 days, was greater in comparison with the control; however, a reduction (– 52%) in the cell growth rate was observed	Velasco-Alvarez et al. (2011)
Heterotrophic bacteria from membrane of a MBR system	3.7–24.7 A/m <sup>2</sup> (30–200 mA)		4 h	Direct current applied to two aluminium electrodes	The % of death was not significant at 3.7 and 6.2 A/m <sup>2</sup> , was 15% at 12.3 A/m <sup>2</sup> and 29% at 24.7 A/m <sup>2</sup> For values above 12.3 A/m <sup>2</sup> , the pH increased to 10, with potential negative effects on vitality	Wei et al. (2011)
<i>Mycobacterium frederiksbergense</i> LB501T e <i>Sphingomonas</i> sp. L138		200 V/m	60 min	Titanium-iridium electrodes (10 × 4 cm <sup>2</sup> , thickness 1.5 mm) immersed in electrode chambers (2 × 7 × 3.5 cm <sup>3</sup> ) located at the ends of a chamber (35.5 × 4 × 3.5 cm <sup>3</sup> ) filled with saturate soil. By-pass channel (35.5 × 2 × 3.5 cm <sup>3</sup> ) hydraulically connected with the electrode chambers	No effect on the anthracene biodegradation rate	Wick et al. (2004)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
<i>Pseudomonas putida</i> PpG7 (NAH7) and mixed culture	1 mA/cm <sup>2</sup> (16 mA)	140 V/m	34 days	Constant voltage applied through titanium-iridium electrodes located in a mesocosm (40 cm × 4 cm × 4 cm), made up of two electrode chambers at the ends and a central treatment cell filled with water-saturated soil	No significant effect on the cellular membrane of <i>P. putida</i> . No significant effect on the physiology and composition of the bacterial community of the soil, except for the areas near the electrodes, where the effects were ascribed to variations in the pH	Wick et al. (2010)
Hydrocarbon-degrading mixed culture		130 V/m constant or with polarity reversal	25 days	Electrochemical cell with soil contaminated by cyclododecane (1000 mg/kg). Constant intensity field, also with periodic polarity inversion	The cyclohexane degradation pathway did not change. The degradation rate increased, in particular in tests with polarity inversion; at the end of the test with electric field, degradation was 79.9% and 87.0% without and with polarity inversion, respectively, in comparison with 61.5% without electric field	Yuan et al. (2013)
Mixed culture		130 V/m	42 days	Microcosms with soil	Stimulation of biodegradation and increase (+ 20%) in the biomass in comparison with the control	Yuan et al. (2013)

Table 1 continued

Culture	Current	Field	Exposure duration	Setup and field source	Biological effect	References
Nitrifying or denitrifying activated sludge	4.4–14 mA	1.2–2.5 V/m	36 days as 4 periods of 9 days each, the first and the third of which without electric field	Electrochemical cell ( $15 \times 5.5 \times 17 \text{ cm}^3$ ) filled with glass balls and graphite grains (1.5 in volume) and 0.5 l of wastewater; current applied through flat graphite electrodes ( $8 \times 15 \text{ cm}^2$ ) colonised by nitrifying and denitrifying biomass	Nitrification and denitrification were simultaneously observed. Removal rates higher for voltages of 0.4 V. Controlling the dissolved oxygen was critical for process efficiency	Zhan et al. (2012)
<i>S. aureus</i> , <i>E. coli</i>	0.1–0.5 mA	< 3; 4–27 V/m	24 h	Two pairs of gold electrodes positioned perpendicularly to Petri dishes containing biomass and connected or not to a DC generator	No significant effect on <i>E. coli</i> , reduction of growth of <i>S. aureus</i> in all the tested conditions (alterations in the cellular morphology, membrane breakage and loss of cellular organisation). For fields > 10 V/m, marked reduction in the number of cells around the anode	Zituni et al. (2014)

Effect: – negative; + positive; = null

**Table 2** Summary of the data in the literature, reported in alphabetical order, on the effects of magnetostatic fields (MSF)

Culture	Field	Exposure duration	Setup and field source	Biological effect	References
<i>Pseudomonas</i> and <i>Enterobacter</i>	0 (annulment of the geomagnetic field)	6 days	Helmholtz coils to cancel the geomagnetic field	Resistance to antibiotics both increased and reduced, according to the strain and the antibiotic	+/-/= Creanga et al. (2004)
<i>Streptomyces marinensis</i>	3, 7, 9, 10, 11, 15 mT	144 h	Permanent magnets	Half of the strains that were tested did not result as being sensitive to MSF In all conditions, increase in growth and synthesis of neomycin (secondary metabolite) in comparison with the control	+ Eilaiah et al. (2003)
<i>Shewanella oneidensis</i>	14,100 mT	1.5–12 h	Permanent magnets	No significant effect on growth during the exponential growth phase, 21 genes over- and 44 under-regulated	= Gao et al. (2005)
<i>E. coli</i> , <i>S. aureus</i>	500–4000 mT	2 h	Superconducting magnet	No effect on growth and resistance to antibiotics	= Grosman et al. (1992)
<i>E. coli</i>	2000–5000 mT	48 h	Superconducting magnet	Effects of mutagenicity	? Ikehata et al. (1999)
<i>E. coli</i>	7000 mT (constant in space); 5200–6100 mT (variable in space)	24–60 h	Superconducting magnet	Modifications in the activity of enzymes not involved in the attack of organic substrata (increase in the ratio between vital cells and total cells in the exposed samples in comparison with the control) with 60 h exposure; no effect up to 20 h	?/= Ishizaki et al. (2001)
<i>Pseudomonas stutzeri</i>	0.6–1.3 mT	10 h (2 h in aerobiosis, 8 h in anaerobiosis)	Helmholtz coils (∅ 18 cm, 0.15 Ω)	No significant effect during the aerobic phase	=/+ Hönes et al. (1998)
<i>E. coli</i>	7000 mT (constant in space); 5200–6100 mT (variable in space)	60 h	Superconducting magnet	During the anaerobic phase, stimulation of growth at 1.3 mT (+ 25–30% in comparison with the control) and 0.6 mT (+ 7%). Cellular replication accelerated, no increase in the specific production of nitrate-reductase Modifications in the activity of enzymes not involved in the attack of organic substrata	? Horiuchi et al. (2001, 2002)

Table 2 continued

Culture	Field	Exposure duration	Setup and field source	Biological effect	References
<i>E. coli</i>	45, 450, 1200, 1800, 3500 mT	30–60 min	Permanent Nd–Fe–B magnets	Reduced bacterial cell vitality during the exponential growth phase in all the examined conditions; the effect grew, as the field intensity, the exposure duration and temperature increased. In relation to the variations in the applied field intensity, inhibition did not progress monotonously (local min and max)	Ji et al. (2009)
Activated sludge	5, 20, 200, 500 mT	Up to 60 h	Permanent magnet	Magnetic fields of 5 or 20 mT had a positive effect on microorganism growth. Higher values had the opposite effect	Ji et al. (2010)
<i>Streptococcus mutans</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i>	30, 60, 80, 100 mT	48 h	Permanent ferrite magnet	Decreased growth rate in anaerobic conditions	Kohno et al. (2000)
<i>Bacillus circulans</i> , <i>Escherichia coli</i> , <i>Micrococcus luteus</i> , <i>Pseudomonas fluorescens</i> , <i>Salmonella enteritidis</i> , <i>Serratia marcescens</i> , <i>Staphylococcus aureus</i>	160 mT (constant in space); field variable in space:	24 h	Permanent ferrite magnets	No effect in aerobic conditions No significant effect	László and Kutasi (2010)
Activated sludge	(1) max 477 mT, lateral gradient of 47.7 T/m (2) max 12.0 mT, lateral gradient of 1.2 T/m (3) max 2.8 mT, lateral gradient of 0.3 T/m	24 h	Reactor positioned inside a magnetostatic device	Increase in substratum removal	Łebkowska et al. (2011)
<i>Microbacterium maritipicum</i>	50, 100, 200 mT	5 days	Pair of cylindrical coils powered by transformer	Increase in the degradation of benzo(a)pyrene	Mansouri et al. (2017)

Table 2 continued

Culture	Field	Exposure duration	Setup and field source	Biological effect	References
<i>Bacillus licheniformis</i>	10 mT	30 h	Solenoid and DC generator	Increased bacterial growth under controlled pH conditions. Increased bacitracin synthesis both in conditions with uncontrolled (+ 36%) and controlled (+ 89%) pH	Mohtasham et al. (2016)
<i>Bacillus subtilis</i>	7000 mT (constant in space); 5200–6100 mT (variable in space)	72 h	Superconducting magnet	No effect with uniform field at 7000 mT. For field variable in space, 50% reduction in the bacterial decay rate of the cells when the stationary growth phase was reached; spore formation inhibited	==/+/? Nakamura et al. (1997)
Activated sludge	13 mT	12 h	Two parallel magnetic plates	Increase in substratum removal	Niu et al. (2013)
<i>E. coli</i>	7000 mT (constant in space); 5200–6100 mT (variable in space)	24 h	Superconducting magnet	In the stationary phase, significant reduction (40–80%) of the bacterial decay rate in the samples exposed to a uniform field, 2.7–3.6 times higher in the samples exposed to a non-uniform field	+ Okuda et al. (1995)
<i>E. coli</i>	7000 mT (constant in space); 5200–6100 mT (variable in space)	12 days	Superconducting magnet	Reduced bacterial decay rate during the stationary phase; the effect was temporary and terminated at the end of exposure	+ Okuno et al. (2001)
<i>Serratia marcescens</i>	8 mT	24–48 h	Permanent magnets	Growth inhibition	– Piatti et al. (2002)
<i>Rhodococcus erythropolis</i>	50 mT	4 h	Electromagnet supplied with constant current	Cell growth and phenol degradation stimulation	+ Pospíšilová et al. (2015)
<i>E. coli</i>	200–250 mT (variable in space)	12 h	Neodymium disc magnets	Increased adhesion between cells No effect in vivo	==/? Potenza et al. (2004)
				Punctual alterations of DNA in the in vitro tests	

Table 2 continued

Culture	Field	Exposure duration	Setup and field source	Biological effect	References
<i>Geotrichum</i> sp.	7, 17, 33 mT	24 h	Permanent magnet	7.0 mT promoted the growth of <i>Geotrichum</i> sp.; no effect at 17 and 33 mT	Qu et al. (2018)
Biofilters	30, 60, 130 mT	185 days	Two magnets placed at variable distances from the reactor	Increase of trichloroethylene biodegradation at 30 and 60 mT; opposite effect at 130 mT	Quan et al. (2017)
<i>E. coli</i>	8–60 mT	90 min	Permanent magnet of Fe oxides and ceramic	<i>Acinetobacter</i> , <i>Chryseobacterium</i> and <i>Acidovorax</i> significantly more abundant in the exposed systems Increase in antibiotic resistance	Stansell et al. (2001)
Activated sludge	40 mT	20 days	Permanent magnet	Improved nitrification	Tomska and Wolny (2008)
<i>E. coli</i>	7000 mT (constant in space)	30 h	Superconducting magnet	During the first 6 h approximately (start of the log growth phase), slight reduction in the growth rate. During the stationary phase, significant reduction in the bacterial decay rate (at 30 h, number of cells in the exposed samples 2–3 times greater than the control). Effect more pronounced with exposure to non-uniform than to uniform fields	Tsuchiya et al. (1996, 1999)
<i>Rhodobacter sphaeroides</i> (anaerobic conditions)	5200–6100 mT (variable in space) 3200–6700 mT (variable in space)	24 h	Permanent magnets	Slight reduction of growth and increase in the extracellular production of porphyrin (secondary metabolites)	Utsumiya et al. (2003)
Anaerobic sludge, main species: <i>Brevibacillus</i> sp. and <i>Bacillus</i> sp. (Cr(VI)-reducing bacteria)	2.4, 6, 10, 17.4 mT	24 h	Addition of Fe <sub>3</sub> O <sub>4</sub> in suspension	Increased biomass in all the tested fields. Better effects at 6 mT	Xu and Sun (2008)
Anaerobic sludge, main species: <i>Brevibacillus</i> sp. and <i>Bacillus</i> sp. (Cr(VI)-reducing bacteria)	4–40 mT	10 h	Pair of permanent magnets positioned inside or outside the sludge line	No problem at all the tested field intensities	Xu et al. (2009)

Table 2 continued

Culture	Field	Exposure duration	Setup and field source	Biological effect	References
Activated sludge	6–46.6 mT	40 h	Solenoid	The 4 mT field resulted as being the most efficient for producing methane (70.7% more than the control) Increased substratum removal in comparison with the control reactor, for a field from 8.9 to 17.8 mT. Opposite effect with higher field values	Yavuz and Çelebi (2000)
Biofilm at the anode of a fuel cell	20, 120, 220, 360 mT	800 h	Square magnet positioned outside the microbial cell near the anode	Biofilm more active up to 220 mT; opposite effect at 360 mT	Zhao et al. (2016)
Activated sludge	8.1 mT	24 h	Magnetic actuator made up of a ring containing a ceramic frit, permanently magnetised	Increased nitrification. Biomass growth stimulation (+ 14%)	Zielinski et al. (2017)

Effect: – negative; + positive; = null; ? undefinable

**Table 3** Summary of the data in the literature, reported in alphabetical order, on the effects of electromagnetic fields

Culture	Frequency	Intensity	Exposure duration	Setup and field source	Biological effect	References
<i>Escherichia coli</i>	16–50 Hz (square wave)	0–22 mT	2–3 h	E-shaped AC electromagnet	Increased growth rate	+/= Aarholt et al. (1981)
<i>E. coli</i>	50 Hz	0.2–0.66 mT	2–3 h	E-shaped AC electromagnet	At 50 Hz the effect became evident at intensities between 0.48 and 0.8 mT; at 16.66 Hz between 0.8 and 1.5 mT Alterations in the synthesis of $\beta$ -galactosidase, with a “window” reply (decrease from 0.27 to 0.3 mT; increase until up to about 0.56 mT; decrease beyond 0.56 mT). The intensity of the alterations seemed to depend also on the number of cells that were present, with more marked effects for lower cell concentrations	? Aarholt (1982)
<i>Staphylococcus aureus</i>	2–500 Hz	0.5, 1.0, 1.5, 2.0, 2.5 mT	90 min	Two pairs of Helmholtz coils	Reduction in cell vitality in all the irradiated samples, with a reduction of at least 20% in all the magnetic flux density values tested for frequencies above 200 Hz The maximum CFU was recorded at 1.5 mT 300 Hz	– Ahmed et al. (2013)
<i>E. coli</i>	2–500 Hz	0.5, 1.0, 1.5, 2.0, 2.5 mT	< 60–90 min	Two pairs of Helmholtz coils	No effect with exposure lasting less than 1 h. For exposures of 90 min, reduction in the growth rate in all the irradiated samples. The effect grew exponentially as both the magnetic flux density and the frequencies increased. The maximum reduction of CFU (77%) was recorded at 2.5 mT 500 Hz	=/– Ahmed et al. (2015)
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	< 20 Hz (square wave)	5–20 mT	4–12 h	Three pairs of prismatic-shaped magnets positioned on the recirculation circuit of a fermenter	Increase in the production of nisin (secondary bacterial metabolite) for exposures at 20 mT for 4 h, without an increase in bacterial growth	? Alvarez et al. (2006)
<i>E. coli</i>	50 Hz	0.5–1–2 mT	20 min	Pair of coils	Growth inhibition only at 2 mT	=/– Aslanimehr et al. (2013)

Table 3 continued

Culture	Frequency	Intensity	Exposure duration	Setup and field source	Biological effect	References
<i>Staphylococcus aureus</i>	50 Hz	0.5–1–2 mT	20 min	Pair of coils	Significant reduction (up to 65%) of the growth rate at 0.5 and 2 mT; increase in growth at 1 mT for exposure of 20 min	+/-
<i>Chromobacterium violaceum</i>	50 Hz	0.66 $\mu$ T	7 h	Electric line 5000 V	Slight alteration of the cellular proteome in the exposed samples The field probably acted as a stress factor	? Baraúna et al. (2015)
<i>E. coli</i> and <i>S. aureus</i>	20, 40, 50 Hz	2–4 mT	1–2–4–6 h	Electromagnetic dipoles (inductance 60 mH, resistance 0.45 $\Omega$ )	Significant CFU reduction in the exposed samples, especially for prolonged exposure duration. Maximum inhibition (95.2% for <i>S. aureus</i> , 85% for <i>E. coli</i> ) at 4 mT 20 Hz for 6 h. Under the same magnetic flux density, <i>S. aureus</i> was inhibited even at lower values in comparison with <i>E. coli</i>	- Bayır et al. (2015)
<i>E. coli</i> and <i>Linfociti umani</i>	2–24 Hz	0.2 mT	15 min	Helmholtz coils (1200-turn, $\varnothing$ 17.6 cm, 384 $\Omega$ each, inductance 251 mH)	Different mutations of the genotype according to the frequencies. DNA–protein complex alteration, in correspondence with the frequency window (9 and 16 Hz), specific for the different species	? Belyaev and Alipov (2001)
<i>E. coli</i>	9 Hz	0.03 mT	15 min	Helmholtz coils (384 $\Omega$ , inductance 251 mH)	Temporary effects of DNA–protein complex alteration observed until 2 h after exposure to the field, evident only for initial densities $\geq 4 \times 10^8$ cell/ml (negligible effects for lower cellular densities)	? Belyaev et al. (1998)
<i>E. coli</i>	60 Hz	1.1 mT	15 min	Helmholtz coils	Increased DNA transduction activity	? Cairo (1998)
<i>E. coli</i>	50 Hz	0.1, 0.5, 1 mT	20–120 min	Copper cylindrical solenoid ( $\varnothing$ 170 mm, length 450 mm, 180 turn)	In the 24 h following exposure, greater vital/death cell ratio in relation to the control. Temporary morphotype change (higher number of spherical cells during exposure and partial return of the bacillus form in the following 24 h) The magnetic field was a stress condition	+/? Cellini et al. (2008)

Table 3 continued

Culture	Frequency	Intensity	Exposure duration	Setup and field source	Biological effect	References
<i>E. coli</i>	50 Hz	0.1, 0.4, 0.8, 1.2 mT	1 h	Helmholtz coils	Increased transduction activity at 1.2 mT Increase in the efficiency of the DNA repair processes with exposure at 0.4, 0.8 and 1.2 mT (+ 20% in comparison with the control)	? Chow and Tung (2000)
<i>E. coli</i>	50 Hz	0.1, 0.2, 0.5, 1 mT	58 h	Helmholtz coils	Reduced transposition activity; cell vitality stimulated. No cell proliferation or morphological variations	? Del Re et al. (2003)
<i>E. coli</i>	50 Hz (sinusoidal wave)	0.05, 0.1, 0.2, 0.5, 1 (max dB/ dt = 0.66 T/s @ 1 mT)	58 h	Helmholtz coils	No effect at 0.05 mT	=/? Del Re et al. (2004)
<i>E. coli</i>	50 Hz (square wave)	0.05, 0.1, 0.2, 0.5, 1 (max dB/ dt = 0.14 T/s @ 1 mT)	58 h	Helmholtz coils	At higher intensities, stimulated transposition activity and reduced cell vitality, with effects that increased in a linear manner with the field intensity No cell proliferation or morphological variations No effect at 0.05 mT	=/?
					Reduced transposition at higher intensities; stimulated cell vitality in the stationary phase No cell proliferation or morphological variations	

Table 3 continued

Culture	Frequency	Intensity	Exposure duration	Setup and field source	Biological effect	References
<i>E. coli</i> , <i>S. aureus</i>	1, 5, 25, 50 Hz	22, 25, 29, 34 mT	1 h	Stator and three-phase squirrel-cage induction motor with glass beaker for housing the samples	Increase in the growth and metabolic activity of <i>E. coli</i> and <i>S. aureus</i> cells, with greater effects on <i>E. coli</i> than <i>S. aureus</i>  Increase in the formation of bacterial biofilm, both for <i>E. coli</i> and <i>S. aureus</i> , at 25 Hz > 29 mT and 50 Hz 34 mT	+  Fijalkowski et al. (2013)
Cocci ( <i>S.aureus</i> , <i>S. xyloso</i> , <i>S. mutans</i> ); Bacilli ( <i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. marcescens</i> etc.); <i>Coccobacilli</i> ( <i>A. baumannii</i> )	0.5–60 Hz	25, 34 mT	1 h	Stator and three-phase squirrel-cage induction motor with glass beaker for housing the samples	Increase in the growth and metabolic activity of <i>E. coli</i> , <i>S. aureus</i> , <i>S. marcescens</i> , <i>S. mutans</i> , <i>C. sakazakii</i> , <i>K. oxytoca</i> and <i>S. xyloso</i> cells; inhibition of <i>A. baumannii</i> and <i>P. aeruginosa</i>	+/-  Fijalkowski et al. (2015)
<i>Gluconacetobacter xylinus</i>	50 Hz	34 mT	144 h	Stator and three-phase squirrel-cage induction motor with glass beaker for housing the samples	Increase in the production of bacterial cellulose in the samples exposed to the field, without degradation of the characteristics of the cellulose produced in comparison with the control	+  Fijalkowski et al. (2016)
<i>E. coli</i> , <i>Leclercia adecarboxylata</i> and <i>S. aureus</i>	50 Hz	10 mT	(a) 72 h B = 34 mT + 72 h B = 0 mT (b) 72 h B = 0 mT + 72 h B = 34 mT (c) 144 h B = 34 mT < 30 min; 1 h	Cylindrical coil	Decrease in the number of colonies in all the exposed samples  Greater effects on <i>E. coli</i> , while <i>S. aureus</i> was the most resistant species No morphological alteration	-  Fojt et al. (2004, 2009)
<i>E. coli</i>	50 Hz	1 mT (6 mV/m)	8 min; 2.5–15 h	Helmholtz coils	No effect on bacterial growth and cell morphology	=  Huwiler et al. (2012)

Table 3 continued

Culture	Frequency	Intensity	Exposure duration	Setup and field source	Biological effect	References
<i>Staphylococcus epidermidis</i> , <i>S. aureus</i> , <i>Enterococcus faecalis</i> , <i>E. coli</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i>	50 Hz	0.5 mT	6 h	Helmholtz coils	Reduction in the growth rate during exponential growth that persisted even after exposure, except for <i>Klebsiella</i> , until the stationary conditions	Inhan-Garip et al. (2011)
<i>E. coli</i>	< 300 Hz	10–100 mT	1–12 h	Solenoid (on the recirculation line of a fermenter)	Alterations in the cellular morphology in all species Contrasting results from the various experiments: proliferation or bacterial growth inhibition. Effects also on the cellular yield coefficient (biomass growth for unit of substratum removed)	Justo et al. (2006)
<i>Corynebacterium glutamicum</i>	15 Hz	2.4, 3, 3.4, 3.8, 4.2, 9 mT	8 h	Helmholtz coils	Increased ATP levels at between 2.5 mT and 4.4 mT (with max difference at 3.4 mT). Lower or higher intensities induced a drop in ATP	Lei and Berg (1998)
<i>Corynebacterium glutamicum</i>	10–70 Hz	3.4 mT	8 h	Helmholtz coils	Increase in the levels of ATP at 15–20 Hz (max + 20% in comparison with the control) At 3.4 mT > 30 Hz, reduced levels of ATP (– 40% at 70 Hz) Stimulation of bacterial growth after 4 h	+
<i>Corynebacterium glutamicum</i>	50 Hz	2.4, 4.2, 4.9, 6 mT	6 h	Helmholtz coils	ATP levels increased at 2.4–5.5 mT (max + 30% at 4.9 mT)	?
<i>Corynebacterium glutamicum</i>	30–70 Hz	4.9 mT	6 h	Helmholtz coils	ATP levels increased at 4.9 mT at 45–60 Hz (max + 40% at 50 Hz)	?
<i>Corynebacterium glutamicum</i>	50 Hz	4.9 mT	8 h	Helmholtz coils	ATP levels increased (+ 30%)	?
<i>Escherichia coli</i> , <i>Proteus vulgaris</i> , <i>Photobacterium phosphoreum</i> , <i>Photobacterium fischeri</i>	2–50 Hz	1–10 mT	7.5–15 h	Solenoid	Reduction (3–4%) of growth in the exponential phase at 4 mT 50 Hz for 7.5 h and 2 mT 50 Hz for 15 h	Mittenzwey et al. (1996)

Table 3 continued

Culture	Frequency	Intensity	Exposure duration	Setup and field source	Biological effect	References
<i>E. coli</i>	60 Hz	0.05 mT	8 h	Coil	Growth stimulation (decrease of the lag phase) and increase in the consumption of glucose	Nasciminto et al. (2003)
<i>Bacillus subtilis</i>	800 Hz (2 s on/2 s off)	0.8, 1.6, 2.5 mT	30 h	Helmholtz coils at a distance of 7.5 cm (∅) 160 mm, width 16 mm, 250 coils, inductance 15 mH)	Growth stimulation	Ramon et al. (1987)
	1000 Hz (2 s on/2 s off)				Alterations in cell morphology and loss of intercellular cohesion	
<i>E. coli, Pseudomonas aeruginosa</i>	50 Hz	2 mT	24 h	Helmholtz coils at a distance of 7.5 cm (∅) 130 mm, section 2 mm <sup>2</sup> , 800 coils, inductance 39 mH, resistance 2.4 Ω)	No significant effect on growth	Segatore et al. (2012)
<i>E. coli</i>	50 Hz	2.7–10 mT	12 min	Cylindrical coil, powered by current generator (50 Hz, 1.9 A effective value)	Reduced number of colonies, proportionally to magnetic flux density and exposure duration during the exponential growth phase	Strašák et al. (2002)
					The redox activity decreased proportionally to the number of cells that were present (bactericide effect of the field but no effects on the bacterial metabolism)	
<i>E. coli, L. adecarboxylata, S. aureus, P. denitrificans, S. paucimobilis, R. erythropolis</i>	50 Hz	10 mT	24 min	Cylindrical coil, powered by current generator (50 Hz, 1.9 A effective value)	Reduction in the number of cells	Strašák (2005)
<i>Staphylococcus epidermidis, Staphylococcus aureus, Serratia marcescens and Escherichia coli</i>	6–25 Hz variable in time (“Thomas pattern”)	2.1–5.1 μT	12 h	Resonator (cylindrical neodymium magnets rotating at 2000 rpm)	Increase in the growth rate of <i>Staphylococcus epidermidis</i> , <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> , reduced growth of <i>Serratia marcescens</i>	+/- Tessaro et al. (2015)

Table 3 continued

Culture	Frequency	Intensity	Exposure duration	Setup and field source	Biological effect	References
<i>Monascus purpureus</i>	50 Hz	0–2.5 mT	2–4–6–8 days	Five pairs of cylindrical coils powered by an AC generator (0–2 mA)	Exposure did not cause alterations in growth  Reduction in the production of citrinin (toxic metabolite) at 1.2–1.6 mT; increased at 2 mT; no effect at 0.5–0.9 mT. The production of citrinin can increase, reduce or remain constant according to magnetic flux density, duration and exposure period; inhibition more evident with exposure during the initial growth phase	= Wan et al. (2017)
<i>Salmonella typhimurium</i>	60 Hz	14.6	4 h (5 min on, 10 min off)	Solenoid (972 copper coils) connected to an AC generator (60 Hz, 12 A)	No effect on DNA	? Williams et al. (2006)
Activated sludge	50 Hz (2 s on/2 s off)	17.8 mT	40 h	Solenoid ( $\varnothing$ 5 cm, 15 cm high)	Greater resistance to stress co-factors (e.g. thermal shock) Not significant effects with exposure to a pulsed field	= Yavuz and Çelebi (2000)
<i>Geobacter</i> spp.	100 Hz	0.005	60 days	Outer coil of a single-chamber microbial fuel cell	Increase in the generated power density; increased abundance of <i>Geobacter</i> spp.	+ Zhou et al. (2017)

Effect: – negative; + positive, = null, ? undefinable

bacteria to die (Sale and Hamilton 1967; Park et al. 2003). Indeed, membranes that are exposed to an electric field become charged, in the same manner as a condenser, and this induces a potential transmembrane that, if greater than 1 V, causes cellular death. The bacterial mortality rate grows proportionally with the intensity of the field that is applied and the overall duration of exposure.

## 2.2 Studies in an environmental setting

Excluding extreme intensity values, a summary is given below of the environmental experiences carried out with modest intensity fields; this determines negative and/or positive effects, even contextual, on the microorganisms.

### 2.2.1 Beneficial effects

**2.2.1.1 Electrochemical reactions** Electrochemical reactions, for example various electro-oxidations and water hydrolysis, can increase the availability of oxygen or hydrogen, respectively favouring aerobic biodegradation processes (Mena Ramírez et al. 2014) and anaerobic biodegradation processes (She et al. 2006).

Bioelectric systems based on water electrolysis were tested, in various configurations and with different operation parameters, for the removal of nitrogen from wastewater, mainly using the production of hydrogen at the cathode to promote denitrification reactions (Cast and Flora 1998; Feleke et al. 1998; Hayes et al. 1998; Mousavi et al. 2010, 2012), or oxygen at the anode to stimulate aerobic nitrification (Goel and Flora 2005a, b), or both processes simultaneously (Kuroda et al. 1996; Zhan et al. 2012). Variations in the pH and the production of H<sub>2</sub> induced near the cathode by a current of 20 mA were used to stimulate the activity of sulphur-oxidising bacteria (Jackman et al. 1999). She et al. (2006), in a bioelectrochemical system with a current of 10 mA, stimulated dehydrogenase activity thanks to the simultaneous production of O<sub>2</sub> and H<sub>2</sub> at the electrodes.

In addition to water hydrolysis, other phenomena tied to electrochemical reactions were recently identified, such as the partial oxidation/reduction of pollutants, the release or removal of ions in solution, the possibility of adjusting redox potential for

activating/stimulating the production of specific enzymes, as well as variations in bacterial metabolism (Aronsson et al. 2001; Li et al. 2006; Huang et al. 2012).

**2.2.1.2 Electron exchange and enzyme production** Some microorganisms were able to use electroactive soluble substances (for example iron and humic substances) (Lovley et al. 1996) or solid electrodes (Bond et al. 2002; Gregory et al. 2004; Aulenta et al. 2009) as donors/acceptors of electrons for substrata oxidation/reduction. Zhang et al. (2013) affirm that in bioelectrochemical tests the electrochemical assistance provided the electrons and accelerated the electron transfer rate in the microbial reduction of 2,4-dichlorophenoxyacetic.

Zhang et al. (2014) observed an increase in the mineralisation efficiency of 2-fluoroaniline by an aerobic culture exposed to a direct current of 10–15 mA, as a result of the increased activity of the catechol dioxygenase and the selection of microorganisms with specific degradative abilities. Velasco-Alvarez et al. (2011), by applying a current of 8 mA for 24 h to a culture of *Aspergillus niger*, observed that the bacterial growth halved but that the hexadecane degraded more, causing the supposition of transition from an assimilative metabolism without the electric field to a non-assimilative one with the electric field.

**2.2.1.3 Electrokinesis** Electrostatic fields generated by constant differences in potential (of the order of 100 V/m) applied between pairs of electrodes and fields generated by direct current are already being applied full-scale for decontamination with electrokinesis, to remove pollutants in sediments or soils (both saturated and unsaturated), especially if the particles are small (Acar et al. 1995).

The benefits of applying electric fields to soils/sediments were initially related to activated transport mechanisms (electroosmosis, electromigration, electrophoresis and dielectrophoresis) (Alshawabkeh and Bricka 2000). Electroosmosis is the movement of the liquids present in the soil pores, generally from the anode to the cathode, under the action of an electric field, which promotes the migration of pollutants towards the cathode by advection (Acar and Alshawabkeh 1993; Acar et al. 1995). Electromigration is the movement of ionic species caused directly by the electric field. Electrophoresis is the transport of solids

with a charged surface, for example bacteria or clay, towards the electrode with the opposite pole, while dielectrophoresis is the movement of neutron solids with a diameter of between 1 and 1000  $\mu\text{m}$  because of induced polarisation (Pohl et al. 1978). These mechanisms determine the movement of organic molecules, nutrients, fluids and bacterial cells (Luo et al. 2005b; Wick et al. 2007) with a faster recovery of pollutants, mass transfers and interactions between pollutants, bacterial cells and nutrients, which is advantageous for the bioremediation processes.

Electrostatic fields can also cause pollutants to degrade partially with an increase in their bioavailability/biodegradability (Wick et al. 2007; Yeung and Gu 2011; Gill et al. 2014; Moghadam et al. 2016). Increases in temperature because of ohmic losses can accelerate the kinetics of bioremediation (Sun et al. 2007).

Experiences of electrokinesis with electric fields having an intensity of 20–200 V/m (Wick et al. 2007; Gill et al. 2014) showed better degradation for various classes of compounds, among which petroleum hydrocarbons due to changes in the microbial community structure (Probst and Hicks 1993; Pazos et al. 2012), polycyclic aromatic hydrocarbons due to the transport of PAH-degrading bacteria in the medium (Pazos et al. 2010), organochlorine compounds due to changes in pollutant mobility (Gomes et al. 2012), and phenols due to variation in bacteria hydrophobicity and pollutant mobility (Luo et al. 2005a, b).

### 2.2.2 Negative effects

On account of the variety of processes induced by the electrostatic fields or generated by DC currents, inhibitive effects on biological activity were reported, mostly related to: (1) important variations in the pH (Fan et al. 2007; Yeung and Gu 2011; Gill et al. 2014; Ailijiang et al. 2016), above all near the electrodes (Lear et al. 2004; She et al. 2006); (2) electrochemical reactions, with the production of reactive species of oxygen, chlorine or metallic ions, according to the species present in the system and the materials used for the electrodes (Liu et al. 1997; Li et al. 2011); (3) excessive heating because of ohmic loss (Palaniappan et al. 1992; Shi et al. 2008). Part of the research on these technologies focuses on investigating operation expedients for optimising the degradation processes

and guaranteeing maintenance of optimal conditions for bioremediation (Jamshidi-Zanjani and Darban 2017). Approaches proposed for controlling the pH include, for example, the continual injection of electrolytes (Kim et al. 2005), anolyte and catholyte mixing (Rabbi et al. 2000), the use of buffer solutions (Niqui-Arroyo et al. 2006), the periodic inversion of the electric field polarity (Luo et al. 2005b; Guo et al. 2014; Mena et al. 2016a, b).

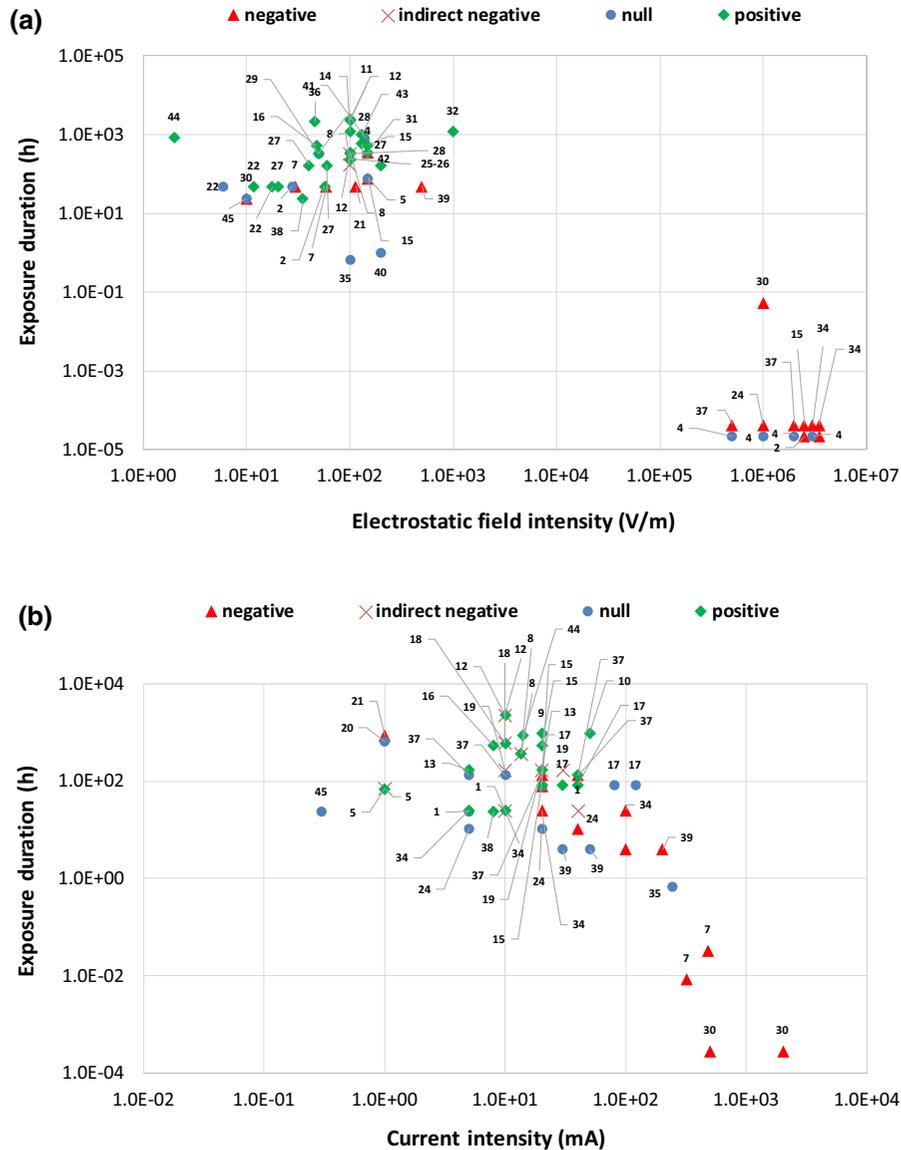
### 2.2.3 No effects

Some experiments with electrostatic fields of 100–200 V/m or applied/induced currents below 20 mA, typically bioelectrochemical and bioelectrokinetic treatments, excluded important field effects on the basis of biotransformation kinetics, evolution of the  $\text{CO}_2$ , microbial charges or enzymatic activity (Jackman et al. 1999; Wick et al. 2004; Harbottle et al. 2009). Lear et al. (2004), for example, do not report any effect on the composition and structure of the microbial community of the soil following the application of direct current at 1 mA for 27 days, and attribute the variations observed near the electrodes only to the variations in pH. In Wick et al. (2004), exposure to DC currents did not cause *Mycobacterium LB501T* to have any effect on the degradation of some polycyclic aromatic hydrocarbons; in relation to the control experiment, the exposed microorganisms did, however, show levels of ATP that were higher by about 50%, even though without repercussions on the development of biomass and on the degradation speed of fluorine. Zanardini et al. (2002) refer to an increase of about 3 times the ATP content in a mixed culture in wastewater, after exposure for 10 days to direct currents of 40–200 mA. Luo et al. (2005a), studying the properties of the cellular membranes of phenol-degrading bacteria exposed to direct currents of different intensities, conclude that currents < 20 mA induce unimportant modifications in hydrophobicity, the electrostatic charge of the membrane and the cell form; on the contrary, currents of about 40 mA caused increases in the extracellular concentrations of cytoplasmic substances and cell flattening. Jackman et al. (1999) observed a temporary reduction in the growth rate of acidophilic bacteria subjected to a current of 20 mA for 80 h, due to bacteria membrane degradation close to the surface of the electrodes. Wei et al. (2011) indicate death rates of heterotrophic bacteria

that were 10% lower than the control when exposed for 4 h to currents lower than 52 mA, and reductions of approximately 15% and 30% respectively for

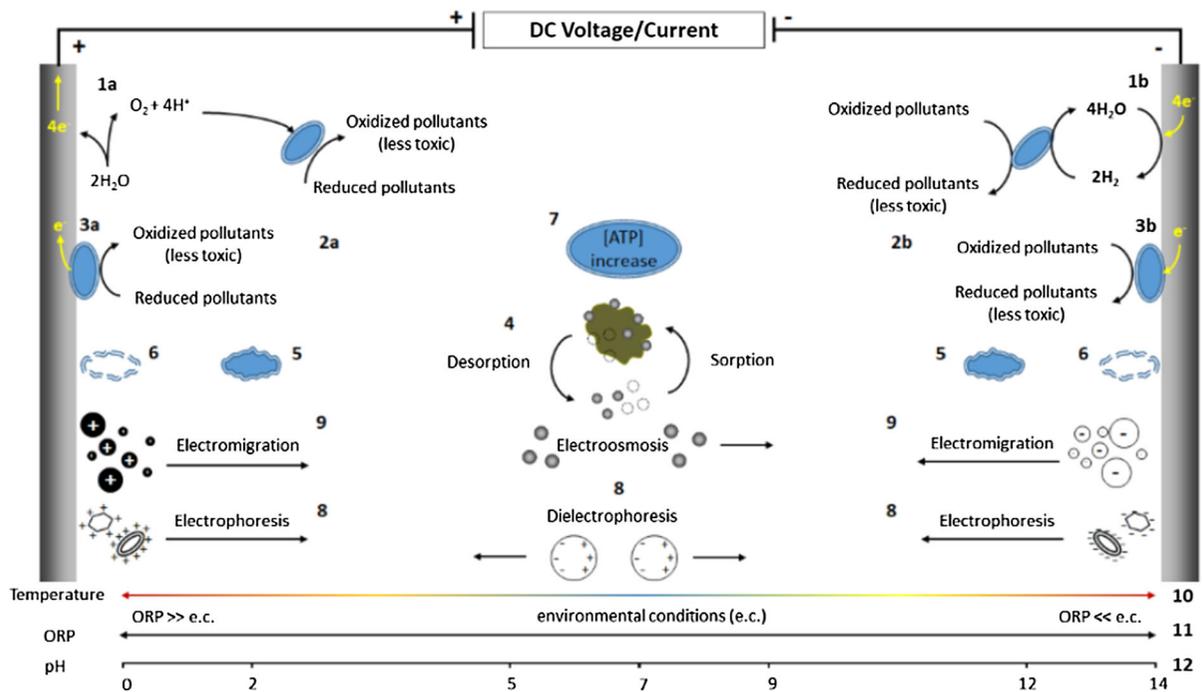
currents of 100 and 200 mA, due to pH variation close to the cathode surface.

Figure 1 sums up the experiences described in the literature and given in Table 1, divided in terms of



**Fig. 1** Type of effects observed according to the intensity of the electrostatic field that was applied (a) or the intensity of the current generated by an electrostatic field (b) and the exposure duration. The numeric label of each point associates it with the reference in Table 1. “Negative” effects (red filled triangle): reduction in the degradation of pollutants and/or substratum consumption, bacterial growth inhibition, reduced metabolic activity or respiration rate, damaged cellular membrane. “Indirect negative” effects (red times symbol): not tied to a direct action of the field/current on the cells, but rather to

modifications in the environmental conditions (e.g. extreme pH levels, electrochemical production of toxic species, radicals, etc.); “Positive” effects (green filled diamond): stimulation of the degradation of contaminants, increased denitrification/nitrification, acceleration in the consumption kinetic of substrata, increased resistance to pollutants, increased biomass, increased metabolic activity or in the activity of specific enzymes (e.g. dehydrogenases); “Null” effect (blue filled circle): no significant effects on the aspects mentioned above. (Color figure online)



**Fig. 2** Scheme of the various phenomena resulting from the application of an electrostatic field or a field generated by direct current: (1) hydrolysis resulting in  $O_2$  production (a) and  $H_2$  production (b); (2) partial oxidation (a)/reduction (b) of pollutants; (3) solid electrodes as electron acceptor (a)/donor (b); (4) increase in pollutant bioavailability; (5) modification in the physiology and morphology of the cell; (6) loss of membrane integrity, with release of cytoplasmic material and cell death; (7)

increase in intracellular ATP concentration; (8) increase in the transport of organic molecules, nutrients, and bacterial cells due to electroosmosis, electrophoresis and dielectrophoresis; (9) transport of dissolved ions due to electromigration; (10) increase in temperature near the electrodes; (11) divergence of the redox potential from the environmental conditions; (12) pH variation close to the electrodes

categories of effects found following exposure to electrostatic fields (Fig. 1a) or fields generated by direct currents (Fig. 1b), even according to the exposure duration. As a result of the numerous experiences that refer to negative effects on microorganisms, not because of the direct action of the electric field but for the variations induced in the environment (for example variations in the pH), they are highlighted in the figures with a different colour, because using suitable expedients (buffer solutions, periodic polarity inversion) this type of undesired phenomenon can be limited and controlled. When considering the intensity of the electrostatic field, all the positive effects occur at values within about 1000 V/m; however, in the same range of values, even with short exposure duration, negative effects are found. With currents up to 10 mA, the effects are above all positive or at most negligible; the few negative effects are all

indirect; for higher current intensities, especially above 150 mA, no positive effects can be seen.

A scheme of the various phenomena resulting from the application of an electrostatic field or a field generated by direct current is shown in Fig. 2.

### 3 Magnetostatic fields

Thanks to the relative simplicity of bacteria, using these organisms as models to examine the fundamental metabolic replies to magnetic fields should make it possible to reduce experimental result interpretation errors to a minimum. In spite of this, the data reported in the literature are often conflicting, and the action mechanisms not clear. A systematic approach, an analysis of the exposure-reply relationship, and physical, biochemical and physiological explanations

(Letuta and Berdinskiy 2017) are missing from the majority of the studies that have been done.

### 3.1 Studies on microorganisms

Potenza et al. (2004) observed an increase in the ability of *Escherichia coli* to form colonies when exposed to a static magnetic flux density of 300 mT, as a function of the incubation medium. In Horiuchi et al. (2001), the number of *E. coli* cells during the stationary growth phase was  $10^5$  times higher when under the effect of a high intensity magnetostatic field (5.2–6.1 T) than when they were exposed only to the geomagnetic field.

Pospíšilová et al. (2015) showed how *Rhodococcus erythropolis* favours the use of phenol under a magnetostatic field of 50 mT.

Some studies demonstrate that the magnetic field can act on DNA stability, interacting with it directly or reinforcing the activity of oxidant radicals (Li and Chow 2001).

Gao et al. (2005) observed how a magnetic flux density of 14.1 T in *Shewanella oneidensis* stimulated the transcription of 21 genes on the one hand, and suppressed the transcription of 44 genes without causing substantial variations in growth on the other.

According to Kohno et al. (2000), static magnetic fields can induce the formation of the hydroxyl radical and amplify the negative effect of nitrogen oxide on the proteins-channels of the cellular membrane.

The mechanism of the radical pair (highly unstable species made up of two radicals) is considered as the most reasonable mechanism of interaction between weak magnetic fields and the biochemical systems (Steiner and Ulrich 1989; Woodward 2002). Each of the two radicals has an unpaired electron; the radicals can therefore be in the singlet state or the triplet state. The mechanism of the radical pair is present in three processes that are of fundamental importance for the cells: the enzymatic synthesis of ATP, the replication of DNA, and the enzymatic phosphorylation of the proteins (Buchachenko 2009, 2014; Buchachenko et al. 2012).

The  $Mg^{2+}$  ion, just like other ions ( $Ca^{2+}$  and  $Zn^{2+}$ ), participates in hundreds of enzymatic processes, many of which involved in fundamental biological mechanisms (Andreini et al. 2008; Rittié and Perbal 2008). The magnetic fields modify the interactions between these ions and the intracellular enzymes, in particular

those involved in ATP synthesis (Buchachenko et al. 2012; Buchachenko 2016; Letuta and Berdinskiy 2017).

### 3.2 Studies in an environmental setting

Experiments were carried out on the application of static magnetic fields for treating wastewater in activated sludge systems, in relation to a potential improvement in solid–liquid separation during the sedimentation step. At times, an increase in the removal rate of the Chemical Oxygen Demand (COD), thanks to the production of more unsaturated fatty acids to stimulate the dehydrogenase activity (Niu et al. 2014), and of other compounds (Zaidi et al. 2014) were observed. In an aerobic activated sludge reactor exposed to a magnetostatic field, the overall content of biomass increased by more than 14% in comparison with a control reactor that was not exposed to the field (Zielinski et al. 2017). In Ji et al. (2010), the acclimation of the activated sludge and the removal of COD under the effect of a magnetic field up to 20 mT were stimulated in comparison with a control system; the same result appeared in Łebkowska et al. (2011) at 7 mT. Also in Yavuz and Çelebi (2000), the biological activity of the sludge was stimulated up to 17.8 mT; opposite effects were observed with higher intensities (46.6 mT). In Tomska and Wolny (2008), periodic exposure to a magnetic flux density of 40 mT increased nitrification.

In Zhao et al. (2016), the use of a magnetic field of 220 mT stimulated the activity of the biofilm at the anode of a fuel cell for treating wastewater, thanks to the production of more extracellular polymeric substance. With a magnetostatic field of 360 mT, instead, the opposite occurred due to harmful effects to microbial growth.

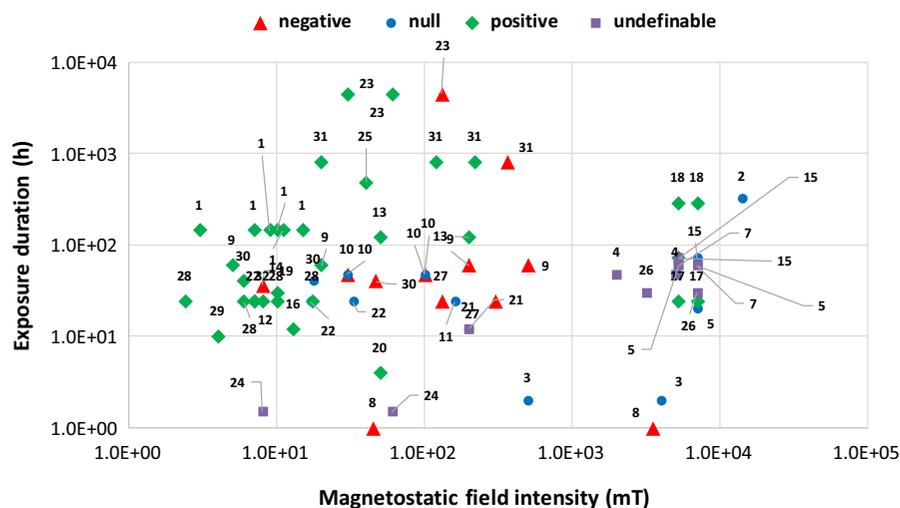
Xu and Sun (2008) presented the effect that magnetostatic fields at different intensities (2.4 mT, 6 mT, 10 mT, 17.4 mT) had on the treatment of wastewater that had been contaminated by Cr(VI), and in particular on *Brevibacillus* sp. and *Bacillus* sp. with Cr-reducing abilities. In all the cases, the quantity of microorganisms in the liquid medium was higher than the control (32–65%), with maximum abatement of Cr(VI) occurring when exposure was at 6 mT. On the sludge line, Xu et al. (2009) found an increase in the production of methane with exposures at 4 mT.

In Xu and Sun (2008), soil exposed to a magnetostatic field of 0.15–0.35 T had a higher respiration rate in comparison with the control. A magnetic flux density field of 7.0 mT instigated both the desorption of Cr(VI) and the growth of *Geotrichum* sp. in a soil column test (Qu et al. 2018).

In Mansouri et al. (2017), *Microbacterium maritopicum*, isolated from a contaminated lagoon, doubled the biodegradation rate of benzo(a)pyrene when exposed to a magnetic flux density of 200 mT.

Using biofilters to degrade trichloroethylene, exposed to magnetostatic fields of 30–60 mT, Quan et al. (2017) recorded more removal (+ 2.4%) than the control. The result was mainly ascribed to the differences in the bacterial community that developed, with relative abundances of *Acinetobacter*, *Chryseobacterium* and *Acidovorax* that were significantly higher in the exposed systems.

Figure 3 summarizes the experiences described in the literature given in Table 2 in relation to exposure to magnetostatic fields, divided into categories of effects found, even according to exposure duration. The effects are classified into “positive”, “negative”, “null” and “undefinable”, as already reported previously. From an analysis of the data, the opportunity of containing the intensity of a magnetostatic field within 10 mT appears to be evident. Even though positive effects were obtained also for exposure to fields of 10 mT and higher, experiences with negative effects were found to be more frequent. In addition, by analysing Fig. 3, it can be seen how negative effects on the microorganisms can result even with low exposure duration; progressive adaptation by the microorganisms cannot, however, be excluded for extended exposure duration.



**Fig. 3** Type of effects observed according to the intensity of the magnetostatic field and the exposure duration. The numeric label reported close to each point associates it with the reference in Table 2. “Negative” effects (red filled triangle): reduction in the degradation of pollutants and/or substratum consumption, bacterial growth inhibition, reduced metabolic activity or respiration rate, damaged cellular membrane. “Positive” effects (green filled diamond): stimulation of the degradation of contaminants, increased denitrification/nitrification, speed up in the consumption kinetic of substrata, increased resistance to pollutants, increased biomass, increased metabolic activity or in the activity of specific enzymes (e.g. dehydrogenases); “Null” effect (blue filled circle): no significant effects on the aspects

mentioned above; “Undefinable” effects (purple filled square): in the absence of positive or negative effects, modifications in the activity of enzymes that are not involved in the degradative metabolism, variation of the ATP concentration, modifications of the microbial community (structure/diversity/genotype/morphotype), effects of mutagenicity, cellular proteome alterations, DNA/RNA synthesis alterations/modifications and related activities, variations in secondary metabolite transposition and production, modifications to the cell form and the characteristics of the cellular wall and its electrostatic charge, increased cell hydrophobicity, increased adhesion between the bacterial cells, increased or reduced resistance to antibiotics. (Color figure online)

## 4 Electromagnetic fields

### 4.1 Studies on microorganisms

The first studies on the effects of pulsed electric fields on microorganisms were carried out by Sale and Hamilton in 1967–1968 (Sale and Hamilton 1967, 1968), who investigated the effects on the vitality of the exposed cells and the lethal effects. The reduction in vitality (up to 99.99%) following exposure to high intensity electric pulses was ascribed to the increase of the external cellular membrane permeability. The lethal effect resulted as being related mainly to field intensity and exposure duration, but was also influenced by the production of toxic substances through electrolysis (Hülshager and Niemann 1980; Hülshager et al. 1981, 1983).

In 1986 it was seen how the electric charges of cells exposed to an alternate electric field separate, with the formation of an oscillating dipole (Hofmann and Evans 1986).

More recent research (Schoenbach et al. 1997, 2000) clarified that the exposure of cells to an electric field causes the accumulation of electric charges on the cell membrane and, as a result, a variation in the potential gradient between the two sides of the membrane. In the case of low intensity electric fields, this causes the tension-dependent channels of the cell membrane to open. As a result, a flow of ions ( $\text{Na}^+$ ,  $\text{K}^+$ ) crosses the channels and modifies the concentrations close to the membrane, causing cellular stress. Stress on short electric signals with a low intensity electric field lasts for a few milliseconds and does not cause irreversible damage. With more intense electric fields, a major potential gradient invests the cell membrane, modifying its permeability until the cell is no longer able to fix its damage, which results in cell death (irreversible breakage). The entity of the voltage that causes the tension-dependent channels to open or cell membrane lysis depends on the cell type and size, and the duration of the pulse. For pulses that vary from tens of microseconds to milliseconds, an electric field intensity of around 10 kV/cm is critical for *E. coli* lysis (Hülshager et al. 1981).

The presence of an alternate magnetic field influences singlet  $\rightleftharpoons$  triplet interconversion in the mechanism of the radical-pair already mentioned in the section on magnetostatic fields (Maeda et al. 2008;

Rodgers 2009) and, as a consequence, the physiological state of the cell and the enzymatic reaction rates can change (Binhi 2001).

Rakoczy et al. (2016) and Fijałkowski et al. (2015) demonstrated that 1 h of exposure to a sinusoidal magnetic field with an effective intensity of 30 mT and frequency 50 Hz increases the growth and cellular metabolic activity of *E. coli* and *Staphylococcus aureus* significantly in comparison with the controls. Furthermore, the authors observed greater stimulation of growth and metabolic activity in cultures of *S. aureus* in comparison with *E. coli*. In Fijałkowski et al. (2015), different results were obtained in cultures of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, where the sinusoidal magnetic field (34 mT, 50 Hz) caused the metabolic activity of the cells to decrease. As proposed by Strašák (2005) and Fijałkowski et al. (2015), the effect observed after exposure to the magnetic field could depend on the form of the exposed bacteria. However, following a comparison of the results of the study carried out on *A. baumannii* and *P. aeruginosa* with those on other rod-shaped bacterial species (*E. coli*, *Serratia marcescens*, *Cronobacter sakazakii*, *Klebsiella oxytoca*), the effect on the microorganisms could depend on the specific species, independently from the cellular form.

The results of an additional study carried out on *E. coli* exposed for 1 h to a sinusoidal magnetic field of intensity 10 mT and frequency 50 Hz, to verify how the field affected cell vitality, were not significantly different from the non-exposed controls (Fojt et al. 2009).

A study carried out on *Salmonella* exposed to a sinusoidal magnetic field (14.6 mT, 60 Hz) demonstrated no direct damage to the DNA; the results, however, supplied evidence that exposure to the field induces the expression of *heat-shock proteins* (Williams et al. 2006), which act as biological indicators of cellular stress and help repair or degrade the proteins that were damaged by thermal shock.

Alternate magnetic fields at moderate intensity (200–660  $\mu\text{T}$ , 50 Hz) alter the transcription speed of the lac operon in *E. coli* (Aarholt 1982). A non-linear dose–effect relationship seems to exist for this type of effect. As an example, while a field intensity of 300  $\mu\text{T}$  suppresses transcription, a field intensity of 550  $\mu\text{T}$  causes a substantial increase.

It was seen that sinusoidal magnetic fields (1.1–1.2 mT, 50–60 Hz) increase the translation

activity of the mRNA in *E. coli* (Goodman et al. 1994; Cairo 1998).

In two consecutive studies, Del Re et al. (2003, 2004) observed the effects on transposition activity in cultures of *E. coli* exposed to two electromagnetic fields with different characteristics. The results of the first study highlighted that *E. coli* cells exposed to a sinusoidal magnetic field (50 Hz, 0.1–1 mT) were significantly less active than those of the controls. Conversely, in the subsequent study, the exposure of *E. coli* cells to a pulsed magnetic field, having the same intensity and frequency, led to significantly greater transposition activity than that of the non-exposed controls being observed. In both studies, transposition was negatively/positively linked to the field intensity with a linear dose–effect relationship. In addition, in both the first and the second study, this phenomenon did not influence bacterial cell proliferation and a significant difference between the amount of colonies exposed or not to the field did not arise. These results suggest that the biological effects depend critically on the physical characteristics of the magnetic signal, in particular the wave shape.

Cellini et al. (2008) exposed cultures of *E. coli* to magnetic fields of frequency 50 Hz and variable intensity (0.1, 0.5, 1.0 mT). During this study, the effects of electromagnetic radiation on different biological parameters were investigated: Colony-forming Units (CFU), cellular vitality state, morphological and transcription profile. According to the results of the experiments, the studied parameters of the irradiated samples did not present significant differences in comparison with the controls, except for increased cellular vitality and change in the morphology of *E. coli*, with the presence of “coccoid” cells even aggregated in clusters.

Another study on the effects of pulsed electromagnetic radiation at extremely low frequency on the growth of the bacteria *S. aureus* showed a decrease in the growth rate. The results evidenced how, in all the tests carried out on cell cultures exposed to fields of intensity within the 0.5–2.5 mT range and frequencies between 2 and 500 Hz, there was a reduction in the number of CFUs in comparison with the non-irradiated controls. In particular, the lowest CFU value was reached after exposure for 90 min at 1.5 mT and 300 Hz (Ahmed et al. 2013).

#### 4.2 Studies in an environmental setting

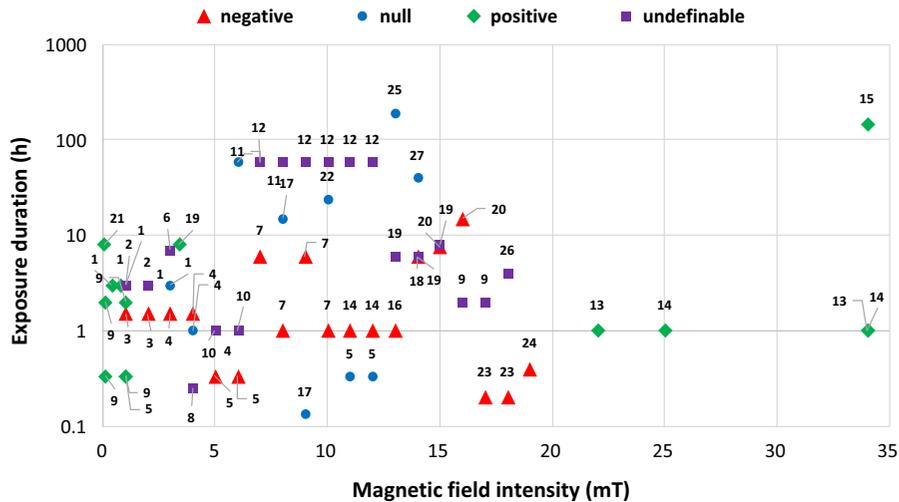
The recent review of Piyadasa et al. (2017) sums up the experiences carried out when controlling precipitation and fouling in inverse-osmosis membrane systems, in particular for desalination. The use of pulsed electromagnetic fields helped speed up clogging of suspended particles and their precipitation.

In the wastewater treatment field, Yavuz and Çelebi (2000) present the effects of an alternate (8.9–46.6 mT, 50 Hz) or pulsed (17.8 mT 2 s on/2 s off) electromagnetic field. In the first case, an increase of 44% in the substratum removal rate was observed, while in the second there were no significant effects.

In Zhou et al. (2017), a pulsed electromagnetic field (square wave with frequency 100 Hz and intensity 5  $\mu$ T) applied to a bioelectrochemical system caused changes in the microbial community at the anode: a relatively greater abundance of *Geobacter* spp. was found (4–8%) than in the control.

Figure 4 shows a summary of the effects on microorganisms (according to the positive, null, negative and undefinable classification) following exposure to sinusoidal electromagnetic fields with frequencies 50–60 Hz, according to the intensity of the magnetic field (given that in most of the manuscripts the induced electric field intensity is not reported) and exposure duration. The choice was made to report the experimental data referred to 50–60 Hz, being typical frequencies of the electric distribution networks, which are most surveyed in the literature.

Most of the experiments took place with magnetic intensity below 20 mT. Even though not numerous, in the case of fields lower than 1 mT the experiments resulted in effects that were mostly positive; on the contrary, with fields that varied between 1 and 10 mT, more studies indicated effects that were negligible, undefinable and negative than those in which positive effects were observed. It should be noted, however, that some authors refer to a field intensity even of a few tens of mT, at which positive effects were observed. The “window” of field intensity at which negligible, undefinable and negative effects were observed varies in relation to the bacterial species. As the mechanisms responsible for bacterial stimulation/inhibition are not currently understood, it seems not possible to forecast the microorganism behaviour to electromagnetic fields of different intensities.



**Fig. 4** Type of effects observed for exposure to electromagnetic fields of frequency 50-60 Hz, according to the intensity of the magnetic field and the exposure duration. The numeric label reported close to each point associates it with the reference in Table 3. “Negative” effects (red filled triangle): reduction in the degradation of pollutants and/or substratum consumption, bacterial growth inhibition, reduced metabolic activity or respiration rate, damaged cellular membrane. “Positive” effects (green filled diamond): stimulation of the degradation of specific contaminants, increased denitrification/nitrification, acceleration in the kinetic consumption of substrata, increased resistance to pollutants, increased biomass, increased metabolic activity in general or in the activity of specific enzymes (e.g. dehydrogenases); “Null” effect (blue filled circle): no significant effects on

the aspects mentioned above; “Undefinable” effects (purple filled square): in the absence of positive or negative effects, modifications in the activity of enzymes that are not involved in the degradative metabolism, variation of the ATP concentration, modifications of the microbial community (structure/diversity/genotype/morphotype), effects of mutagenicity, cellular proteome alterations, DNA/RNA synthesis alterations/modifications and related activities, variations in secondary metabolite transposition and production, modifications to the cell form and the characteristics of the cellular wall and its electrostatic charge, increased cell hydrophobicity, increased adhesion between the bacterial cells, increased or reduced resistance to antibiotics. (Color figure online)

In comparison with electrostatic and magnetostatic fields, the experiments with electromagnetic fields were limited to an exposure duration of a few days at most, and it is not clear if there were microorganism adaptation.

### 5 Conclusions

The scientific literature that was considered refers to biological effects that at times contrast with each other. For some common microorganisms, suitably insulated and exposed to fields, increases in the cell activity were noted, with effects on the biomass growth rate and the metabolic kinetics. In the few experiences carried out in the environmental field, there is also some evidence of positive effects of exposure to fields, even in the biological wastewater treatment or bioremediation.

The most surveyed fields in relation to bioremediation are certainly the electrostatic fields or generated

by continuous currents, already being used today in electrokinesis processes to remove pollutants in sediments and fine grain soils. In some of these processes carried out at full-scale and in situ, biostimulation was also ascribed to the applied electric field. These results are reported in very recent studies and, certainly, further research and experiments must be done to find further evidence using different soils and contaminants, in addition to understanding the mechanisms.

Regarding magnetostatic fields, the most important applications in the environmental field involve wastewater treatment in activated sludge systems. In addition to a potential improvement in solid–liquid separation during the sedimentation step, increases in the degradation kinetics of the organic substance or increased biomass were related to exposure to magnetic fields.

The limited experiences concerning electromagnetic fields in the environmental ambit involve wastewater treatment and in particular the control of fouling.

Even though in the review it was not possible to identify operative conditions that could certainly stimulate biological activity, additional research could be done:

- on the effects of fields that are electrostatic or generated by direct currents until around 100 V/m (or induced currents of about 10 mA), for the bioremediation of contaminated soils or sediments. Some positive laboratory experiences have been reported for treating solid matrixes in bioelectrochemical or electro-bioremediation systems (as a development of the electrokinesis systems). In consideration of these initial results, but also considering the scarce understanding of the phenomena in play and the decisive role of the choice of operative conditions for compensating the negative effects induced by the electric field (extreme variations in pH, etc.), further research on this matter is certainly necessary for developing and scaling up the technologies;
- an assessment of the effects of magnetostatic fields of 1–10 mT on bioremediation in soil; from the literature, this aspect does not seem to have been explored yet. Special attention should be placed on assessing how the applied field interacts with the mineral components of the soil, which could present ferromagnetic properties;
- on electromagnetic fields; even though some research refers to positive effects on bacterial activity, the results as a whole seem to be very contrasting and do not make it possible to identify, from the start, the operative conditions on which to sketch out possible experiments.

#### Compliance with ethical standards

**Conflict of interest** The authors declared that they have no conflict of interest.

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