Original Article

Magnetotactic Bacteria used to Generate Electricity based on Faraday`s Law of Electromagnetic Induction

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Running title: Electromagnetic induction with bacteria

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Significance and Impact of the Study

This study provides proof-of-concept of electromagnetic induction using magnetosomes or magnetotactic bacteria in an experimental setup based on the law of Faraday. The concept of using these bacteria or their biomineralised magnetic nanoparticles as a biological alternative in low voltage electricity generation has the potential to be further explored and developed.

Abstract

Magnetotactic bacteria (MTB) have the unique ability to produce magnetic particles surrounded by a biomembrane to form the magnetosome organelle. Therefore, MTB have novel physical and magnetic properties and have consequently been used in several biotechnological applications. The magnetic properties of these microorganisms and their magnetosomes have, however, never been used for the generation of electricity as described in this letter. Comparisons were made between, firstly, the electricity generated from purified magnetosomes, MTB culture (bacterial cells with magnetosomes) and sterile, liquid growth medium (control). Secondly, the electricity generated by a dilution series of purified magnetosomes were compared. A statistically significant difference was found between the voltage measured from the purified magnetosomes (highest voltage), MTB culture (lower voltage) and liquid growth medium (lowest voltage). In the dilution series, the voltage measured increased as the magnetosome concentration increased, but only up to an optimum concentration (0.0376 mg ml^{-1}). In the current study we have demonstrated that a significantly higher voltage than that of the control could be measured when MTB or purified magnetosomes were pumped through a solenoid by applying Faraday`s law of electromagnetic induction.

Keywords

Magnetotactic bacteria, magnetosomes, electricity, electromagnetic induction, Faraday`s law

Introduction

MTB biomineralise intracellular organelles composed of a phospholipid bilayer membrane with bound magnetite (Fe_3O_4), greigite (Fe_3S_4) or both crystals. These magnetosomes are used during magnetotaxis, in conjunction with chemotaxis, which enable the bacteria to reach optimal growth conditions in aqueous environments (Lefèvre and Bazylinksi 2013). The magnetosome biomineralisation is genetically controlled (Uebe and Schüler 2016) and it has been suggested that magnetosome formation is linked to environmental conditions such as oxygen availability (Bazylinski *et al*. 2013). Individual magnetite crystals in MTB have a diameter of between 35 and 120 nm, thus placing it in the single magnetic domain size range. This gives the magnetosomes the highest possible magnetic moment per unit volume. Usually the magnetosomes are in straight chains to optimize the magnetic dipole moment which gives it the ability to passively align the cell according to the earth`s geomagnetic field lines even when cells are dead (Bazylinski and Frankel 2004; Frankel 1984) .

The potential application of MTB in various fields has made an impact, especially on biomedical research. Most recently, MTB were reported as a possible treatment for malaria (Murugan *et al.* 2017). The use of magnetosomes as a potential antitumour drug carrier has also been successfully applied (Sun *et al.* 2008). MTB are also used for the improved imaging of tumours by magnetic resonance imaging (MRI) (Mériaux *et al*. 2015) and (Boucher *et al.* 2017). Furthermore, it has been suggested that cancers could be treated by using magnetosomes in magnetic hyperthermia (Alphandéry *et al.* 2012). Photothermal therapy using magnetosomes is also considered as potential cancer treatment (Chen *et al.* 2016). Other applications of MTB in biotechnology include biological phosphate removal with *Magnetospirillum gryphiswaldense* (Zhou *et al.* 2017), organophosphate pesticide biodegradation with magnetosomes acting as nanobiocatalysts (Ginet *et al.* 2011), biosorbents for heavy metals (Zhou *et al.* 2012) and bioremediation of cobalt (Tajer-Mohammad-Ghazvini *et al.* 2016). Lastly, it has been shown that magnetosomes can immobilize enzyme activity

such as uricase and glucose oxidase. However, magnetosomes proved to be 40 times more effective in immobilizing these enzymes compared to artificial magnetic particles and 100 times more effective compared to magnetic iron-zinc particles (Matsunaga et al. 1996).

Microorganisms have been employed to produce alternative forms of energy as well as recently discovered electricigens. Electricigens are able to oxidize organic compounds to carbon dioxide completely with electrodes being the sole electron acceptor while still maintaining sufficient electron transfer for growth. *Shewanella* and *Geobacter* species have been used as electricigens (Lovley 2006). In the case of *Shewanella*, electron transfer does not take place directly but with electron shuttles, whereas with *Geobacter* electron transfer to the electrodes do take place directly in a microbial fuel cell setup (Reguera *et al.* 2005). Thus, *Geobacter* has an advantage over *Shewanella* as *Shewanella* incompletely oxidises organic acids such as lactate (Lovley 2006). There are other lesser-known electrogenic bacteria used to produce electricity such as *Dietzia* and *Pseudomonas* species. *Dietzia* sp. typically forms biofilms that secrete redox mediators (Sacco *et al.* 2017), *Pseudomonas* species also use mediators for electron transfer (Mavrodi *et al.* 2001). The potential of MTB in the field of energy production has not been explored using electromagnetic induction.

Faraday`s law of electromagnetic induction states that if a magnetic field changes relative to a solenoid, an electromagnetic force is induced on the solenoid (Faraday 1832). The aim of this study was to investigate the possibility that MTB and/or pure magnetosomes could potentially be used to convert mechanical energy into electrical energy, using the principle of electromagnetic induction. A mini (pilot) setup was used to investigate whether a net voltage could be measured when magnetosomes/MTB were pumped through copper coils.

Results and Discussion

The magnetotactic bacterium, *Magnetospirillum magneticum* strain AMB-1 was used in this study to test whether this bacterium or its purified magnetosomes could be used to produce an electromotive force. The bacterium produced an average of 15 magnetosomes cell^{-1} when grown in culture medium (Fig. 1A). Upon investigation of the extracted and purified magnetosomes, it was observed that the magnetosomes remained in suspension, and did not form aggregates (Fig. 1B). The magnetosome membrane was also observed to be still surrounding the magnetic particles (Fig. 1C).

As shown in Figure 2 the experimental setup consisted of a capillary (thin end of a Pasteur pipette) placed between two poles of an electromagnet (0.5 – 0.6T) (Leybold, 56214; powersource: Iso Tech IPS 303DD transformer). Before the pipette was placed between the poles, copper wire (0.1mm diameter and enamelled) was spun around the pipette starting at a distance of 8cm from the tip to create a 3mm X 3mm cylindrical coil (solenoid) around the pipette. The distance between the electromagnet and the solenoid was 0.5cm with solutions passing through the magnetic field first and then the solenoid. The ends of the copper coil were connected to a multimeter (Hewlett Packard 34970A) which captured the data.

A plastic pipe from the peristaltic pump (Watson Marlow 205S) was connected to the large end of the pipette. The experimental solutions and controls were pumped through the pipette, passing through the magnetic field and coil. Solutions were pumped at a flow rate of 4.4 ml min^{-1}.

Voltage measurements were taken every second and the multimeter recorded the average value over each second. With power line frequency synchronisation, the background interferences were suppressed with each measurement. The averages of each second were used to present the results graphically. For representation of the data the same measurements were split into positive (Fig. 3) and negative (Fig. 4) values. This was done in order to calculate the alternating current correctly.

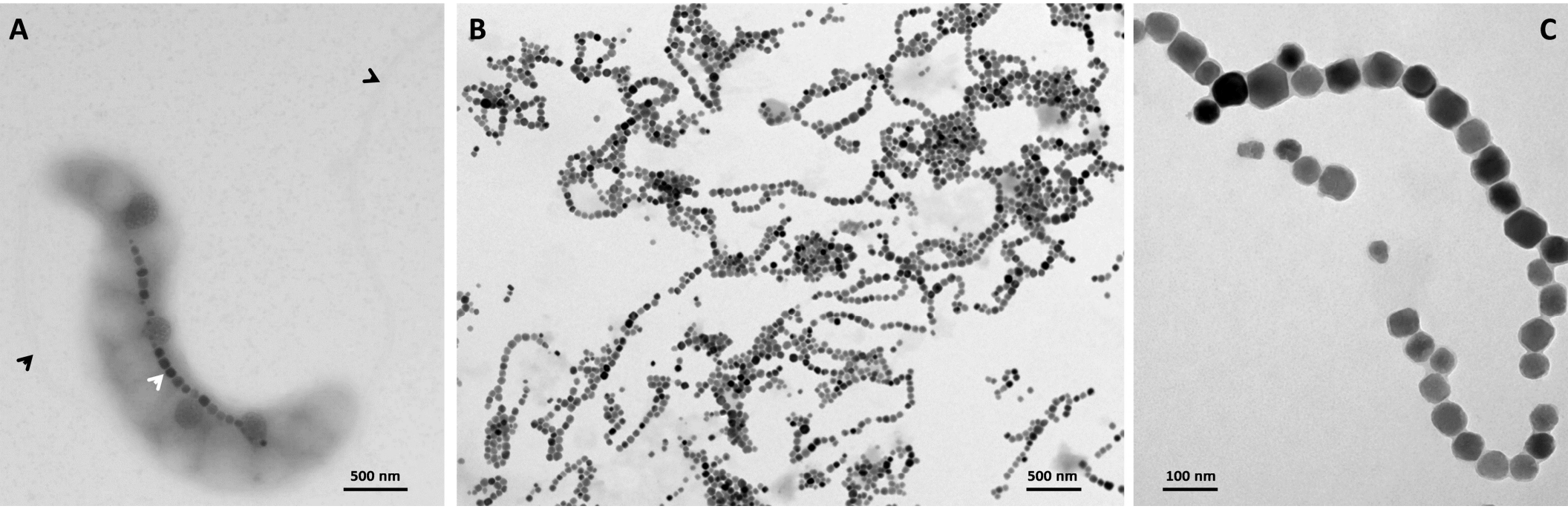

Figure 1. Transmission electron microscope (TEM) images of magnetosomes and the magnetosome membrane. (A) TEM micrograph of a cell of Magnetospirillum magneticum strain AMB-1 deposited onto a Formvar-coated electron microscope grid showing a chain of magnetosomes (white arrowhead) and the two polar flagella of the cell (black arrowhead). (B and C) TEM micrograph of extracted and purified magnetosome chains showing cuboctahedral magnetite crystals surrounded by the magnetosome membrane.

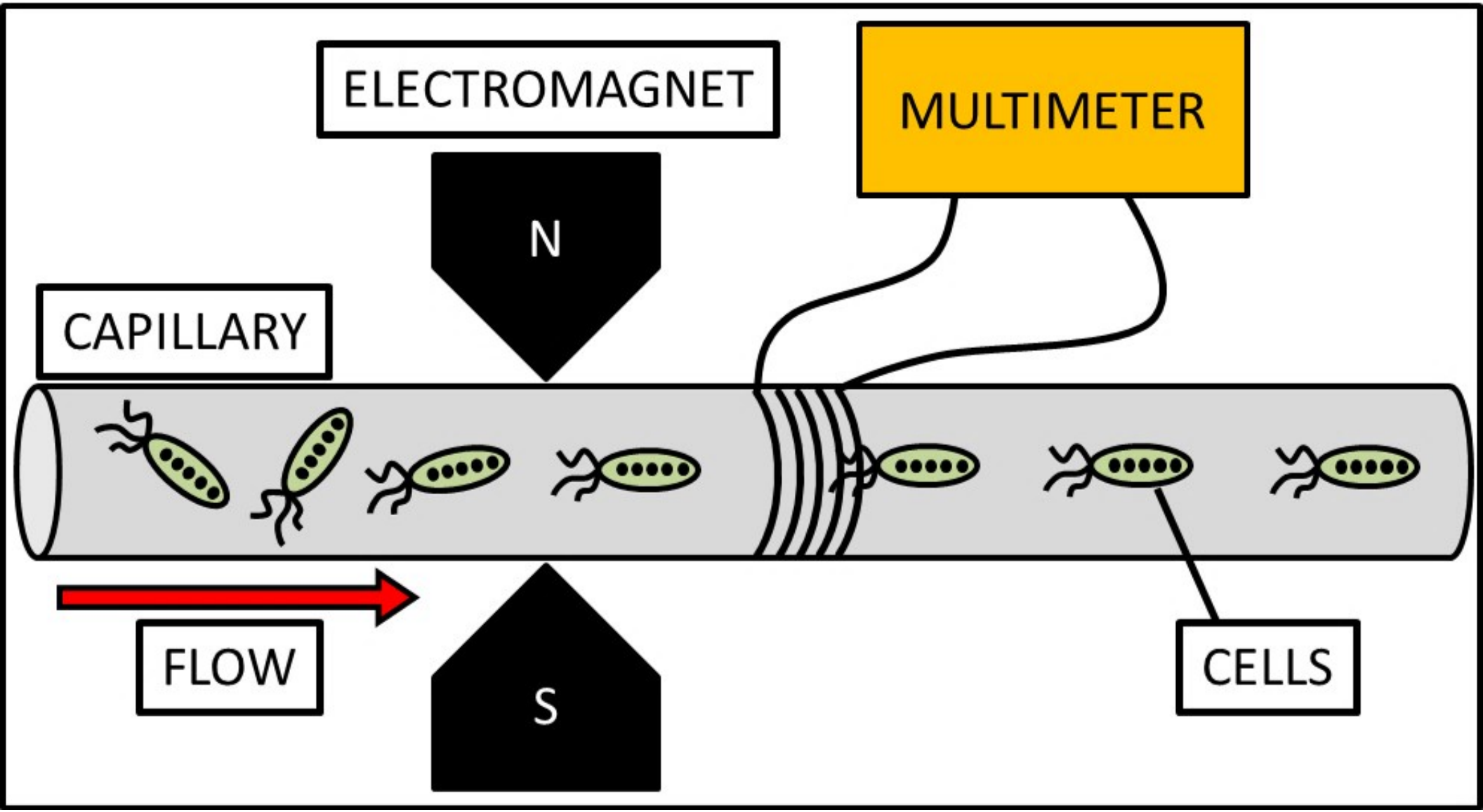

Figure 2. Diagrammatic representation of the experimental setup in which the voltage produced by different materials was tested. The suspensions were pumped from left to right in the capillary and magnetic materials were aligned by the electromagnet if it was needed. Materials then passed through the solenoid and induced an emf on the coil. This voltage was then measured by the multimeter and analysed.

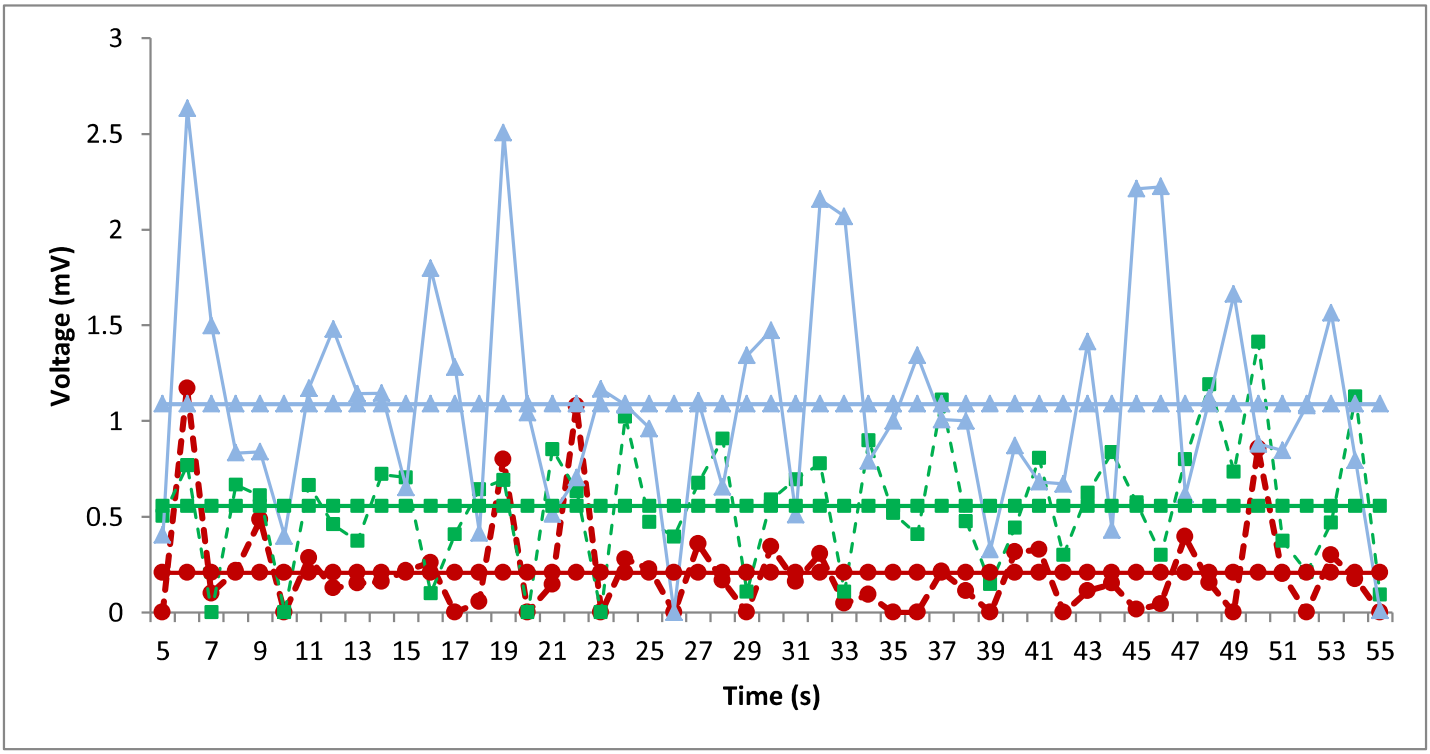

Figure 3. Positive voltage measured from uninoculated growth medium (····●····), liquid AMB-1 culture (- - ■ - -) and magnetosomes (—▲—) suspended in liquid growth medium. Horisontal lines represent the average value of each test over the 55 seconds: magnetosomes 1.09mV, liquid AMB-1 culture 0.56mV and uninoculated growth medium 0.21mV.

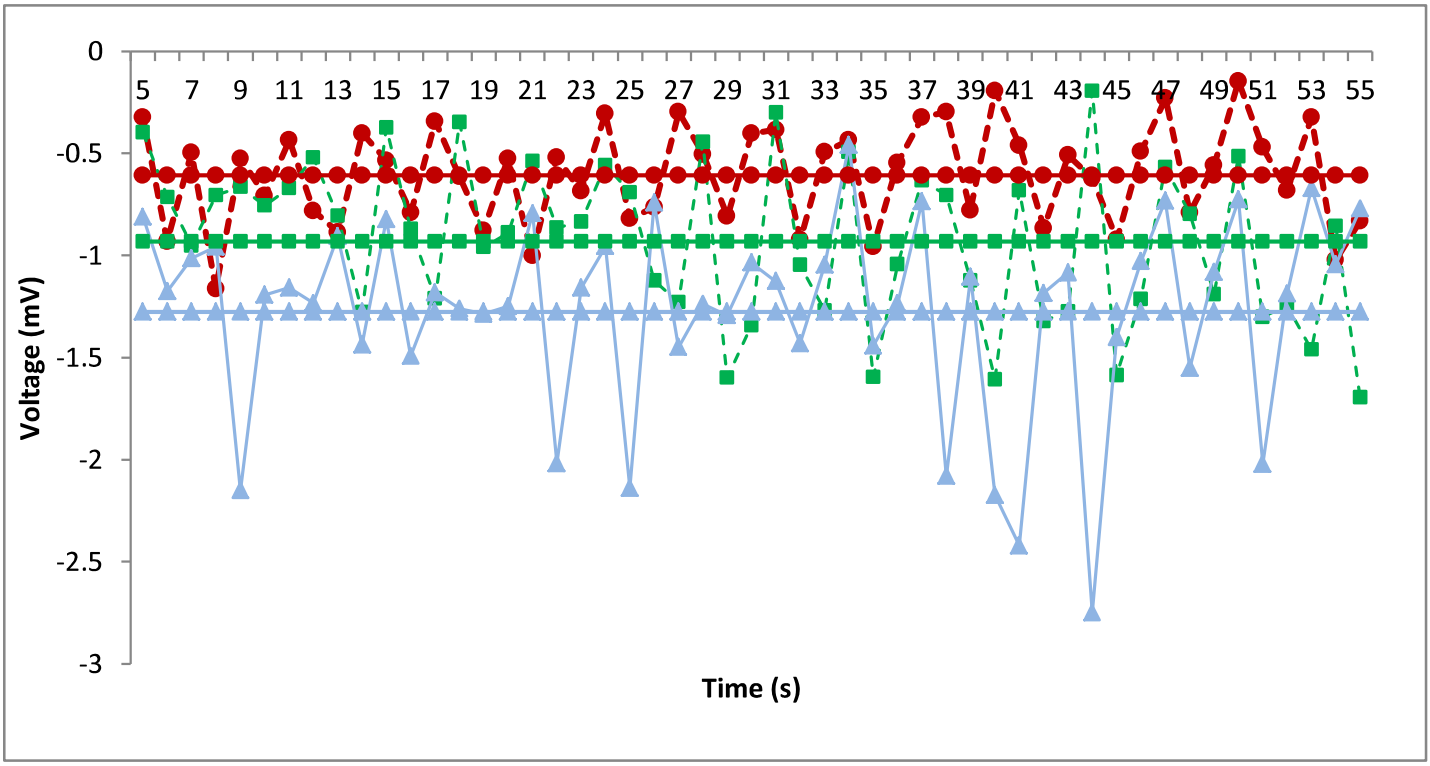

Figure 4. Negative voltage measured from uninoculated growth medium (···●···), liquid AMB-1 culture (- - ■ - -) and magnetosomes (—▲—) suspended in liquid growth medium. Horizontal lines represent the average value of each test over the 55 seconds: magnetosomes -1.28mV, liquid AMB-1 culture -0.93mV and uninoculated growth medium -0.61mV.

Measurements were repeated between three to five times under identical conditions to keep the electrical background interference the same. The first and last five seconds of each series of measurements were discarded as operation of the pump or computer sometimes created interferences. In order to limit the unavoidable background interferences from the surroundings it was also important that the multimetre had the ability to integrate and synchronise the data.

The voltage measurements for the induction experiments are shown as positive and negative values respectively (Figures 3 and 4, Supplementary Table 1 and 2) to demonstrate the observed alternating current. The results clearly indicated that the uninoculated growth medium (negative control) produced the lowest voltage, the AMB-1 culture in the liquid growth medium a higher voltage and the pure magnetosomes suspended in liquid growth medium, the highest voltage. From the dilution series it was observed that the voltage increased as the magnetosome concentration increased, but only up to a certain concentration (0.0376 mg ml^{-1}), where, interestingly, the voltage dropped steeply (results not shown). The analysis of variance was performed on both tests (with null hypotheses $\mu_{medium} = \mu_{culture} = \mu_{magnetosomes}$ and $\mu_{low\ concentration} = \mu_{medium\ concentration} = \mu_{high\ concentration}$) according to D'Agostino *et al.* (2006). The analysis of variance test confirmed that the sets of data displayed were significantly different from each other (Supplementary Table 1 and 2).

Here we have successfully shown the possibility to produce energy with MTB and magnetosomes. The fact that an alternating current has been measured can be ascribed to the magnetic nanoparticles passing completely through the solenoid as separate magnetosomes or magnetosome strings. This observation was also reported for the first time by Faraday (1832) in his original study with magnets and we hereby confirm his findings in this novel approach. Furthermore, if the magnetosomes were inducing a current as separate magnetosomes and acting as a uniform distribution, then an alternating current could also possibly be explained by the peristaltic actions of the pump. As the last roller of the pump squeezed the tube, liquid were pushed forward at a faster

rate for a very brief moment. On the other hand, the reverse could occur: as the flattened tube was released, the liquid could flow for a brief moment in the opposite direction in relation to the coil, which could have caused a negative voltage and thus an alternating current could be generated. Another explanation for an alternating current could be that the magnet held magnetosomes with the magnetic force at the pole until many magnetosomes caused a flow obstruction and were then released as a slightly higher concentration. This might have caused an alternating current as the magnetosome distribution was not uniform, but in effect, a dipole was created.

In the experiment with the magnetosome dilution series, the voltage measured was directly related to the magnetosome concentration. The increase in voltage and sudden drop as the magnetosome concentration increased can possibly be explained by the number of magnetosomes attached to each other because of their magnetism. It can be shown that magnetic balls placed in a straight line touching each other will form a circle as more magnets are attached. We postulate that the same phenomenon occurred as the concentration of magnetosomes increased. The string of magnetosomes formed a circle and the magnetic field lines of the circle were concentrated towards the centre. In the case of a straight string, the magnetic field lines would protrude from the ends, resulting in a higher voltage due to more magnetic field lines moving through the solenoid (Giambatista *et al.* 2013).

A net voltage (millivolts) was measured in the novel manner described in this paper and we have successfully proven this concept. Although the emf was only in the millivolt range, it has the potential for many applications. This study adds knowledge to the field of applications involving MTB, and suggests that MTB and their magnetosomes can potentially be utilised as an alternative source of electrical energy, especially where millivolts are required. We realise that the electrical energy generated is not economical as we used a peristaltic pump to simulate the cells' swimming ability. In further studies the economical output could be addressed as we believe our setup can be improved. Moreover, the electricity production using MTB is not yet competitive compared to what

is produced with *Geobacter* for instance but our proof of concept we present here along with progresses in MTB cultivation (Bond and Lovley 2002) could make our setup competitive in the future. Furthermore, this study could be enhanced by developing a model which can predict the voltage produced in relation to the magnetosome or cell concentration. However, as we are not able to determine exactly in which conformation (rings, individual or strings) the magnetosomes or cells were passing through the coil, a model of this scale is not in the scope of this letter but may be beneficial to a larger paper. In further studies the effect of different magnetosome sizes, amounts and orientations in different species as well as other factors, such as, magnetosome membrane and cell wall thickness and cell size could be measured.

Materials and methods

Growth of MTB and magnetosome purification

Magnetospirillum magneticum (AMB-1) was grown at 28°C in defined media described by Mériaux *et al.* (2015). Cultures in late-exponential growth phase were used in all the experiments. Cells might have died during the course of experimental work, but as we used a constant flow, this would not have had any effect on the induction. Magnetosomes of strain AMB-1 were extracted and purified according to Mériaux *et al.* (2015).

Controls and experiment

AMB-1 liquid culture and AMB-1 magnetite magnetosomes (3.39 mg ml^{-1} suspended in 30 ml of uninoculated liquid culture) were used in the described setup to test for induction. Uninoculated AMB-1 liquid media acted as the negative control. A bar magnet was brought next to the coils to confirm that the experimental setup was functional as seen by the change in voltage. The voltage produced by a dilution series of magnetosomes, suspended in physiological salt water (0.9% sodium chloride), was also measured. The initial concentration of the magnetosome suspension was 0.113 mg ml^{-1}. This suspension was diluted 1:3 and 1:9.

Microscopy of magnetosomes and MTB

For TEM imaging, 10 µL deposits of a culture or diluted magnetosomes suspension were allowed to sediment for one minute onto 200 mesh copper grids covered by a formvar-carbon film. After removal of the liquid with Kimwipe paper, digital electron micrographs were recorded with a Tecnai™ G2 BioTWIN transmission electron microscope (FEI Company) at 100 kV acceleration voltage.

Acknowledgements

We thank Prof. Marla Tuffin (University of the Western Cape), Bernard Genty, Michel Philibert, Michel Péan and Nicolas Ginet (all from the CEA), Prof. Zander Myburg and Prof. Marion Meyer (both from the UP), Ms Carin de Beer (Hoërskool Waterkloof), Mr Paul Moulang, Ms Helena Coetzee, Mr Andries and Ms Hetty Smit. SP and CTL were funded by the French National Research Agency (APP-P2N 2012 and ANR-16-TERC-0025-01).

Conflict of interest

No conflict of interest declared.

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Supporting Information legends

Supplementary Table 1: Voltage measured from the uninoculated medium, MTB culture and purified magnetosomes, respectively.

Supplementary Table 2: Voltage measured from the dilution series of purified magnetosomes