ARTICLE

Galvanic lithotrophy, a new path to fuel bioelectrochemical processes.

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Electron sources for bacterial cell processes are diverse and include water (in phototrophs), organic (in organotrophs) and inorganic compounds (in lithotrophs). All of them share the characteristic of having a low enough oxidation-reduction potential to allow cell energy gaining when coupled to typical cell electron acceptors. While most metals and alloys have a potential low enough to serve as electron donors for bacteria, data about their direct microbial oxidation are very limited. In this work we show that magnesium, a metal with the lowest reduction potential in the galvanic series, cannot be oxidized directly by denitrifying bacterial cells, but can serve as electron donor when galvanically connected to them through graphite. We recognize this as a new way of accessing metal electrons for bacteria which, owing to the requirement of galvanic coupling, we propose to identify as *galvanic lithotrophy*. We exemplify the impact that this process may have, by showing its application to simultaneously remove nitrate, ammonium and phosphate from water, by using a readily scalable approach that allows recover these nutrients, in which an energy input is not required.

Introduction

In nature, microorganisms use a variety of electron sources including water (by phototrophs) and organic compounds (by organotrophs). Inorganic matter also serves as energy source for lithotrophic microorganisms, with hydrogen, carbon monoxide, ammonia, nitrite, sulfur, sulfide, ferrous iron and manganese (II) within known electron donors.[1–3] While most of these donors are soluble, some are solids that cannot enter the cell, denoting the existence of mechanisms for internalizing electrons from the cell exterior. These mechanisms give molecular support to direct interspecies electron transport,[4–6] which is now a recognized way through which bacteria can exchange electrons, and to the use of polarized electrodes as electron donors, which has been described to support life of bacteria such as nitrate and sulfate reducers in bioelectrochemical systems.[7,8]

A prerequisite for a compound to serve as electron donor for bacteria is having a negative enough oxidation-reduction potential to warrant an energy profit for the electron receiving cells. This is the case for most metals and alloys in the galvanic series[9] but information about bacteria directly taking electrons from them is still very limited. Except by the paradigmatic case of iron, which has called exclusive attention in consequence of the deep economic impact of microbial corrosion,[10–13] the absence of data about direct microbial oxidation of metals suggests the existence of physical, chemical or biological constraints for their use as direct electron donors.

We show here that while metallic magnesium cannot function as a direct source of electrons for denitrifying bacteria, it can serve as electron donor when coupled galvanically to cells through a conductive material such as graphite. This exemplifies a concept that can be readily extended to the coupling of virtually any active metal or alloy with electrochemically active bacterial cells able to get electrons from the cell exterior. According to this, there is a pathway for bacteria for getting electrons from otherwise inaccessible inorganic sources, which owing to the need of a galvanic coupling we propose to identify as *galvanic lithotrophy*. This pathway may unravel connections not previously considered in natural nor in man-made environments, influencing not only known bioelectrochemical processes, but also others like microbial corrosion and biogeochemical cycles.

To demonstrate the deep impact that galvanic lithotrophy may have on bioelectrochemical technologies, we applied the concept to promote bioelectro-denitrification[14–17] and found that galvanic coupling to magnesium allows the process to work at the same rate reached when a denitrifying biocathode is polarized with a potentiostat, but without the need of an energy input or sophisticated equipment. Furthermore, we show that upon cathodic alkalinization by virtue of the galvanic current and in the presence of ammonium and phosphate, magnesium ions dissolved from the anodes promoted struvite precipitation, a process of interest for the present societal aim of recovering minerals from rock-independent sources.

Results and discussion

The galvanic nitrogen removal process

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To demonstrate the use of magnesium as electron donor to promote nitrogen removal, we set single compartment bioelectrochemical reactors filled with anaerobic culture medium containing no electron donor and 20 mM $NaNO_3$ as the sole electron acceptor. Ammonium was at a concentration of 16 mM. We equipped the reactors with graphite and magnesium bar electrodes, galvanically coupled through variable external loads (0 to 100 KΩ) resulting in galvanic polarization (i.e.: polarization by means of galvanic coupling). In these arrangements magnesium bars acted as sacrificial anodes, while external loads enabled the adjustment of cathodic polarization on graphite electrodes to -0.5 V versus the Ag/AgCl reference electrode, a condition necessary for promoting microbial denitrification.[14,16–18] Upon inoculating reactors with an denitrifying consortium of known composition[14] in stationary growth, we measured a galvanic current that gradually increased to reach current density value of 0.033 ± 0.008 A m^{-2} (Fig. 1a) that sustained until the end of the assay (ten days). Bacterial activity resulted in the removal of N-NO_3^- at a maximum rate of 0.506 ± 0.065 mM N-NO_3^- per day (Fig. 1b), with a coulombic efficiency of 88%, which was well in the order of the 92% obtained using a potentiostat and auxiliary electrodes for polarization (positive control). When the model strain *Thiobacillus denitrificans* DSMZ 12475 was used, similar removal rate values of 0.55 ± 0.018 mM N- NO_3^- per day were obtained with a coulombic efficiency of 89% (data not shown). These results demonstrate that in the absence of any other electron donor, the bacterial consortium was able to use galvanic current (i.e.: electrons coming from magnesium dissolution) to reduce nitrate with no nitrite accumulation.

As maximum denitrification rate was comparable to that of equivalent cultures in positive control experiments, our results confirm that magnesium sacrificial anodes were able to provide electrons at the full rate required by biological electro-denitrification (Fig. 1a), giving support to the use of this strategy in future technological applications.

The galvanic lithotrophy concept

According to the natural potential value of magnesium (-1.6 V vs Ag/AgCl 3 M NaCl), cells may gain energy from the oxidation of this metal upon direct contact to it. Nevertheless, negligible nitrate reduction rates (Fig. 1b) and low magnesium weight losses (Table 1) were measured in experiments where magnesium anodes and graphite cathodes were not electrically connected. This strongly suggests that the use of magnesium as a direct source of electrons for bacteria is somehow impeded or not possible.

Before anthropogenic introduction, magnesium did not exist in metallic form in nature, possibly explaining the inability of cells to use it directly as an electron donor. Besides, it has a unique crystallographic structure of closest packed hexagonal arrangement[19] to which bacterial surface enzymes may not be adapted. Indeed, the extreme negative potential (and else surface charge) of magnesium may prevent negatively charged cells from contacting the material,[20] while the presence of a surface oxide may even block bacterial action.[21–23](

Notably, when metallic magnesium is electrically coupled to graphite, metal electrons can fuel bacterial denitrification as shown above (Fig. 1). We recognize this as a new kind of lithotrophy enabled by the galvanic connection. Nevertheless, in order to confirm this as a real lithotrophic process and taking into account that most denitrifying bacteria are hydrogenotrophic,[18,24–27] mediation by cathodically produced H_2 in the process of electron transfer to cells needs to be considered.

While the poor catalytic properties of graphite determine the need of strongly negative polarization (i.e. lower than -1.0 V vs Ag/AgCl) for proton reduction to occur at a relevant rate on its surface,[18] biologically mediated electroreduction of nitrate on this material typically occurs at less negative potentials (over -0.4 V vs Ag/AgCl),[14,16–18] as demonstrated here by the appearance of a catalytic reduction wave in cyclic voltammetry analysis of biocathodes when nitrate was present (Fig. S1).

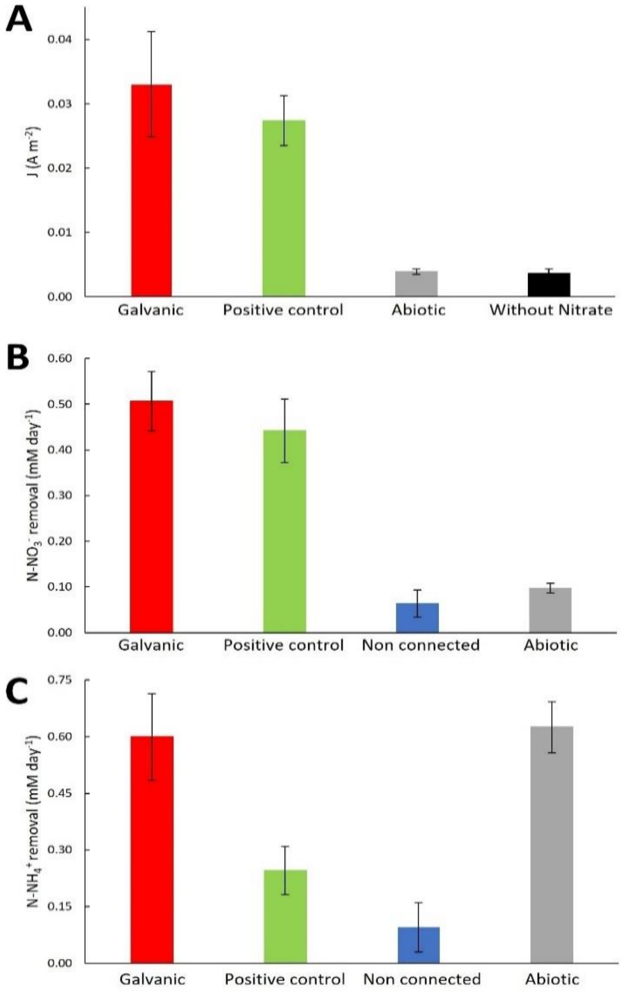

Figure 1: Galvanic current density (A), N-NO_3^- removal rate (B) and N-NH_4^+ removal rate (C) of a denitrifying consortium galvanically coupled to a magnesium anode through a graphite cathode polarized to -0.5 V (Ag/AgCl - 3 M NaCl) (red). Positive control values obtained by polarizing the cathodes with a potentiostat (green) are included, as well as values obtained in negative control experiments where electrodes were not connected (blue), bacteria were not present (grey) or nitrates were not added (black). Cell count was adjusted to 10^6 cells mL^{-1}. Reactors were permanently flushed with N_2:CO_2 mixture 80:20 to ensure anoxic conditions and to maintain pH at 6.8. At least three independent replicates (n=3) were evaluated for each condition.

Table 1: Magnesium sinks during biotic and abiotic galvanic polarization experiments and non-polarized experiments. Anodic mass loss is indicated as directly measured and as calculated from galvanic current, together with theoretical struvite formation as related to dissolved magnesium (estimated from mass loss) and the amount of removed ammonium. All values are expressed in mg except those of mass ratios.

		Treatment		
		Galvanically polarized		non-polarized
		Biotic	Abiotic	Biotic
Magnesium dissolution	Anode weight loss	725 ± 106	530 ± 42	71 ± 26
	Galvanic Current	48.96 ± 8.22	6.87 ± 0.77	0
	galvanic current/mass loss	8.10 ±1.96%	1.30 ± 0.14%	0%
Potential struvite formation	Anode weight loss	7090.5 ± 1036	5183.4 ± 410	694.38 ± 254
	Removed ammonium	247.36 ± 47.25	257.67 ± 28	41.23 ± 26.53
	Removed ammonium / anode weight loss	3.5 ± 2.3%	5.0 ± 1.8%	5.9 ± 4.2%

Taking advantage of this difference, we measured the change of the potential adopted by biocathodes upon the application of nitrate pulses during a chronopotentiometric experiment (i.e.: an experiment performed with a potentiostat under controlled current). As can be seen in figure S2, upon the introduction of a pulse of nitrate to act as the electron acceptor for bacteria, the potential of the biocathode rapidly positioned at about -0.4 V, to lately return to much more negative values of about -1.0 V when nitrate was exhausted and proton reduction became the dominant cathodic reaction.

The observed potential shift implicitly demonstrates that H_2 evolution cannot operate at the rate required to sustain the imposed galvanic current, unless it occurs at very negative potentials, thus confirming that at the stabilization potential of -0.4 V, cells are getting electrons from the electrode in a direct way, without H_2 intermediation.

In clear agreement to these results, the power curves obtained from nitrate respiring biocathodes showed a maximum at about -0.5 V, again pointing to nitrate bio-electroreduction as the controlling process at a potential at which proton reduction is negligible (Fig. S3).

In the absence of hydrogen mediation, presented evidence is in accordance with graphite directly channeling electron flow to fuel cellular metabolism in a process that we propose to identify as *galvanic lithotrophy*. In galvanic lithotrophy, a redox active material which is unable to act as electron donor for a certain microorganism in a direct way, plays this role thanks to the intermediation of a biocompatible conductor material to which it is electrically (galvanically) connected, as schematically represented in Figure 2. It is here shown to occur with magnesium and denitrifying bacteria, but the process might be generalized to the coupling of these and other bacteria to other electronegative materials such as, for example, zinc and aluminium, which have not been reported yet as electron donors for supporting bacterial life.

The combined nutrient removal process

In parallel to nitrate reduction, and as ammonium and phosphate were present in the medium, solubilization of Mg^{2+} ions from the anodes promoted the generation of struvite and the subsequent removal of ammonium (Fig. 1c) and phosphate. Periodic measurements of ammonium levels indicated an ammonium removal rate of about 0.6 mM $N\text{-}NH_4^+$ day^{-1} when polarization was applied with magnesium sacrificial anodes both in the presence and the absence of bacteria (Fig. 1c). The rate was more than 2-fold lower under external polarization with a potentiostat, or in the absence of polarization (Fig. 1c). The mineral was observed to scale as a thin layer over magnesium anodes but, as demonstrated by X-Ray diffraction results, it was also the dominant component of a fine precipitate recovered at the bottom of reactors in which magnesium was present (Fig. S4).

Shown in Table 1 are the results of weight loss determinations on magnesium anodes, together with the mass loss of magnesium as calculated from the integration of the galvanic current and the amount of struvite that can potentially be formed according to removed ammonium, or dissolved magnesium (Table 1).

Magnesium weight loss was one order of magnitude higher on polarized systems (Table 1) but notably, galvanic current accounted only for 8.10% and 1.30% of dissolved magnesium in biotic and abiotic experiments, respectively. This indicates that magnesium oxidation was coupled to an additional reduction process, which was independent of current generation. In this direction it is to be noted that, upon galvanic connection, magnesium anodes were polarized anodically with respect to its open circuit potential; which may have induced the active dissolution of magnesium at the expenses of hydrogen evolution as a local reduction process (i.e.: on the magnesium

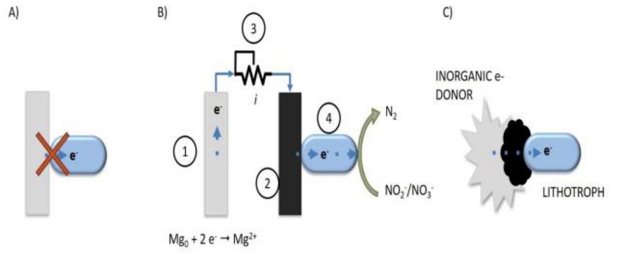

Figure 2: Schematic representation of galvanic lithotrophy as an indirect way for bacteria to access electrons from a metallic donor (in grey). A) bacteria cannot access magnesium electrons in a direct way. B) experimental galvanic contact between a magnesium bar (1) and a graphite electrode (2) allows utilization of electrons coming from magnesium oxidation by denitrifying lithotrophic bacteria (4) to reduce nitrate / nitrite. In this proof of concept, a variable resistance (3) controlled the applied polarization and allowed measurement of galvanic current. In classical electrochemical cell, polarization is applied by a potentiostat using auxiliary electrodes (positive control). C) conceptual process occurring in nature where an inorganic electron donor is galvanically coupled to a lithotrophic bacteria through a conductive biocompatible material.

surface), in parallel to galvanic dissolution.[28–30] If hydrogen produced in the anode was used by bacteria as electron donor for denitrification, this would have influenced the coulombic efficiency of the system yielding higher values than the ones of the positive control. As very similar coulombic efficiencies were obtained in galvanic and positive control systems (with values agreeing with those reported in bibliography)[14,31] it can be assumed that hydrogen contribution to denitrification was negligible.

Struvite is a dibasic phosphate of ammonium and magnesium with equimolar concentrations of each component and according to estimations from removed ammonium, struvite formation consumed only a minor fraction (less than 6%) of Mg^{2+} produced from anode dissolution (Table 1). On the same basis, the mineral formation consumed less than half of initially added ammonium and phosphate amounts, indicating that its formation was controlled by other factors rather than the availability of its components.

It has been largely known that struvite formation (eq. 1) requires alkaline conditions. Although some studies reported its recovery at pH values between 6 and 7.5,[32–34] the optimal pH for struvite crystallization is known to be between 8 and 11.[35–38]

Eq 1. Struvite formation
$Mg^{2+} + NH_4^+ + H_nPO_4^{3-n} + 6H_2O \rightarrow MgNH_4PO_4.6H_2O + nH^+$

Eq 2. Anode reaction
$Mg_0 \rightarrow Mg^{2+} + 2e^-$

Eq 3. Bioelectrochemical cathode reaction
$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O$

Eq 4. Abiotic cathode reaction
$2\ H^+_{(aq)} + 2\ e^- \rightarrow H_{2(g)}$

In the cases here, sacrificial anodes dissolution (eq. 2) was balanced to nitrate bioelectrochemical reduction (eq. 3) when bacteria were present or with the reduction of protons (eq. 4) under abiotic conditions. Both reactions consume protons at the cathode interphase, with an equimolar dependence on galvanic current (eq. 3). Local alkalinization in the vicinity of the cathode, may be displacing struvite formation reaction (eq. 1) to the products side, thus determining struvite precipitation. This scenario is in agreement with the fact that the most of produced mineral was not collected on the anodes, but on precipitated material.

Further analysis by thermogravimetry revealed that the precipitate recovered from galvanic polarization experiments in the presence of bacteria was composed by more than 99% struvite, while that from the abiotic experiments presented a combination of struvite (49%) and magnesium ammonium carbonate (51%) (Fig. S5 and Table S1) (see the electronic supplementary material for calculations). This suggests that the presence of bacteria can increase phosphorous removal by more than 51% as compared to the abiotic counterpart.

Beyond potential improvements and optimization, which could be achieved for example, by exploring different cell configurations or changing the electrodes area ratio, we here demonstrate that the approach of galvanically coupling electrobiological nitrate reduction with magnesium dissolution allows removing nitrate, ammonium and phosphate simultaneously, improving water quality and giving place to the recovery of valuable minerals of agronomic interest, without the need of a direct energy input.

Although magnesium production is energy intensive with an estimated energy requirement varying between 140 to 366 kJ per kg, depending on the production system,[39] by considering the amount of nitrate removed per mg of dissolved magnesium in the galvanically connected system (0.079 ± 0.0016 gram of nitrate per gram of magnesium) an indirect energy input of 3.14 ± 0.64 kJ per g of NO_3^- can be estimated. This is 56% lower than the energy applied by a potentiostat in the positive control system that can be estimated on 5.53 ± 1.16 kJ per g of NO_3^-, considering the average current density (0.0274 ± 0.0039 A m-2), the area of the electrodes (112 cm2), the voltage applied by the potentiostat (0.7 V difference between working and counter electrodes) and the rate of nitrate removal (0.44 ± 0.07 mM day^{-1} with a reactor volume of 0.12 L). This reveals that, despite allowing simultaneous phosphate, nitrate and ammonium removal, the application of magnesium anodes for biocathodes polarization may also allow a reduction of the net energy input of polarized bioelectrochemical systems such as microbial electrolysis cells.

The one shown is a good example to demonstrate that implementing galvanic lithotrophy may clearly have an impact on the improvement of microbial electrochemical technologies, but stressing the concept of galvanic lithotrophy may unravel connections not previously considered and serve, for example, for improving cathodic protection systems[40] that may have been stressed by biological electron consumption, or for reconsidering new approaches that use magnesium as an alloying element to improve corrosion resistance of prosthetic devices.[41–43] At a global scale, it is known that pyrite and other conductive minerals (magnetite, graphite, biochar) confer high conductivity to soils[44] and may be capable of transporting electrons over distances that can reach tents of centimeters[45] allowing the exchange of electrons between microbial species.[46] Through galvanic lithotrophy these minerals may serve also as electron exchange intermediates allowing bacteria to exploit inorganic donors whose use may not be feasible to them through direct contact, due to potential or physicochemical restrictions; galvanic lithotrophy may in this way impact on geochemical processes where minerals abundant in the earth crust as pyrite or chalcopyrite may be available as electron donor to a wider variety of lithotrophic bacteria than previously considered, upon electrical interaction through conductive intermediates as e.g. graphite, which is often found associated to them.[47–51]

Conclusions

In this work we show that metallic magnesium, the material with the lowest oxidation potential in the galvanic series, can serve as a source of electrons for lithotrophic bacteria, but only if coupled galvanically to cells through graphite. We demonstrate that magnesium electrons can promote bioelectro-denitrification at a rate comparable to that sustained by external polarization, exemplifying a way of accessing electrons that may be potentially occurring in nature through galvanic coupling to metals. We define here this process as *galvanic lithotrophy*.

Author Contributions

Rodríguez Simón, Carlos Norberto: Data curation, Formal analysis, Investigation, Visualization, Writing – review and editing. Bonanni, Pablo Sebastian: Data curation, Formal analysis, Funding acquisition, Writing– review and editing. Busalmen, Juan Pablo: Conceptualization, Formal analysis, Investigation, Funding acquisition, Project administration, Supervision, Validation, Writing – original draft.

Conflicts of interest

There are no conflicts to declare.

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