

Electronic Supplementary Material

Galvanic lithotrophy, a new path to fuel bioelectrochemical processes.

Rodríguez Simón, Carlos Norberto^a; Bonanni, Pablo Sebastián^a and Busalmen, Juan Pablo^{a,*}.

Materials and methods

Growth medium and inoculum

A nitrate reducing bacterial consortium was enriched by serial inoculation of hermetic vials containing culture medium, using a sample of conductive material from a bioelectrochemical wetland treating domiciliary wastewater as the initial source of microorganisms. A modified DSMZ 113 medium was used, containing: 2 g KH_2PO_4 ; 2 g KNO_3 ; 1 g NH_4Cl ; 0.8 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 2 mL trace elements^{*}; 1 g NaHCO_3 and 0.002 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per litre. 5 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ per litre were used as the electron donor for the enrichment and no electron donor were supplied for electrochemical assays. The medium was purged with a mixture of 80% N_2 and 20% CO_2 and sterilised in an autoclave. Bacterial growth was followed by measuring optical density at 600 nm (OD_{600}) while nitrate removal was taken as an indication of bacterial activity. Nitrate determinations were made by measuring optical density at 220 and 275 nm and nitrite was determined by colorimetry following standard methods.¹ Ammonia was measured with an ammonia ion selective electrode (HANNA HI 4101) following the procedure provided by the manufacturer. ^{*}Trace elements composition 0.5 g $\text{Na}_2\text{-EDTA}$; 0.2 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 0.1 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; 0.03 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$; 0.3 g H_3BO_3 ; 0.2 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$; 0.01 g $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$; 0.02 g $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and 0.03 g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ per litre adjusted at final pH of 7.

Bioelectrochemical experiments

In galvanic polarisation experiments $28.4 \pm 2 \text{ cm}^2$ magnesium rods were used as sacrificial anodes, while $112 \pm 10 \text{ cm}^2$ effective area graphite bars served as cathodes in single chamber bioelectrochemical reactors of 0.12 L effective volume. They were connected through a 0-100 K Ω variable resistance that was adjusted to polarise the cathodes at -0.5 V versus an Ag/AgCl - 3 M NaCl reference electrode. Before connection, reactors were made anoxic by bubbling a $\text{N}_2\text{:CO}_2$ 80:20 gas mixture and inoculated with the enriched consortium in stationary phase at an initial absorbance at 600 nm (OD_{600}) of 0.1. Galvanic current was measured and registered by using a Fluke 289 meter connected in series with the variable resistance. In negative control experiments reactors were kept sterile. In positive control experiments graphite electrodes were polarised with a potentiostat. A platinum wire served as the counter electrode and an Ag/AgCl - 3 M NaCl electrode as the reference electrode. At least three replicates of each condition were tested. Temperature was maintained at 30 °C and liquid was gently stirred with magnetic bars. Coulombic efficiency was calculated as the ratio between the number of electrons required to produce the observed reduction of NO_3^- to N_2 and the measured current in each individual experiment. Chronopotentiometric experiments were performed on the graphite/Mg couple in inoculated reactors at a fixed current of 0.012 A m^{-2} , using the potentiostat in a two electrodes configuration. Cyclic voltammetry was performed between 0.5 and -0.7 V at a scan rate of 0.005 V s^{-1} , starting cathodically at -0.5 V. The scan rate was selected to minimise the time of the assays, considering that the catalytic response during nitrate reduction on biocathodes can be clearly observed at scan rates lower than 0.020 V s^{-1} , as reported in bibliography.² All electrochemical experiments were performed using a PGSTAT101 potentiostat controlled by Nova 1.6 Software (Autolab, The Netherlands).

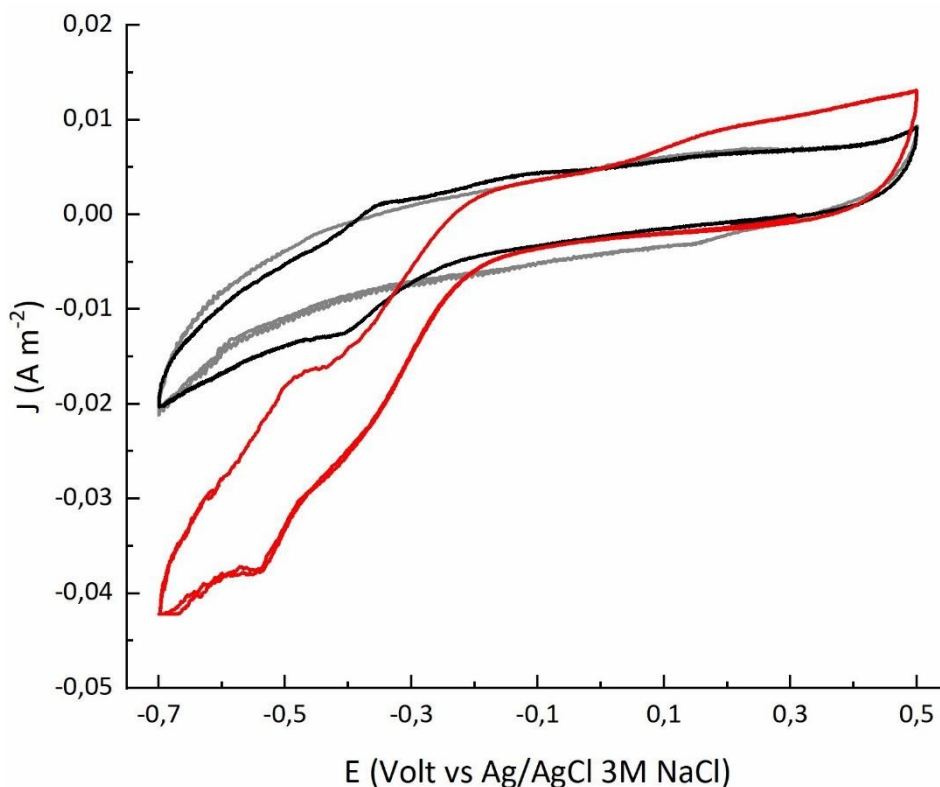
Exposed cathodic area was estimated from capacitive current density measured by cyclic voltammetry of clean electrodes at increasing scan rates and using a capacitance density of 89.14 μFcm^{-2} as a reference.³ Reported anodic area corresponds to geometric exposed area. Mass loss measurements were performed by determining the dry weight of Mg electrodes before and after the experiments in and digital balance. Electrodes were dried at 100 °C in a stove until reaching a constant weight.

X-Ray Diffraction

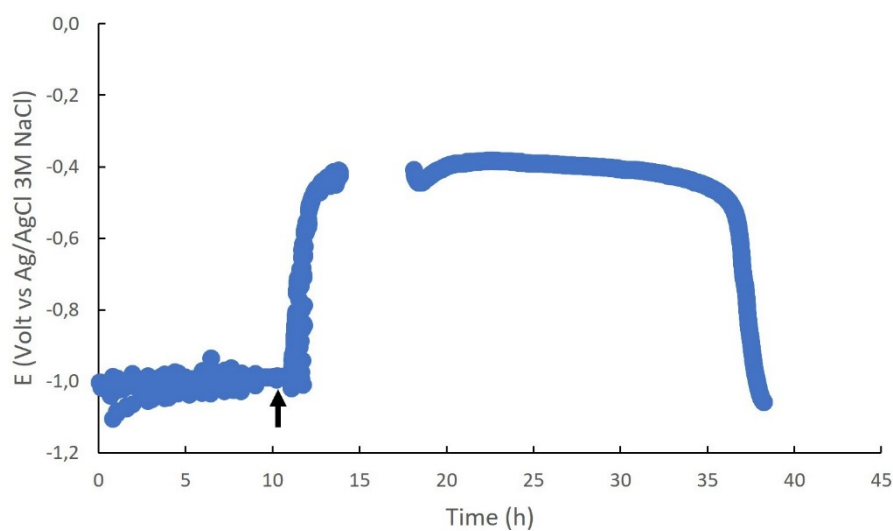
Powder X-ray diffraction (XRD) analyses were carried out on dry precipitates accumulated on the bottom of reactors by using a PANalytical X'Pert Pro diffractometer at 40 kV – 40 mA with Cu K α radiation (1.5418 Å) and a graphite monochromator, it was done over a 10–80° 2 θ angular range (with 0.02° step size and 0.5 s reading time). COD23 (Crystallography Open Database) database was used to identify the crystalline phases with PANalytical X'Pert highscore software. Results are shown in figure S4.

Thermogravimetry – Differential Thermo Gravimetry

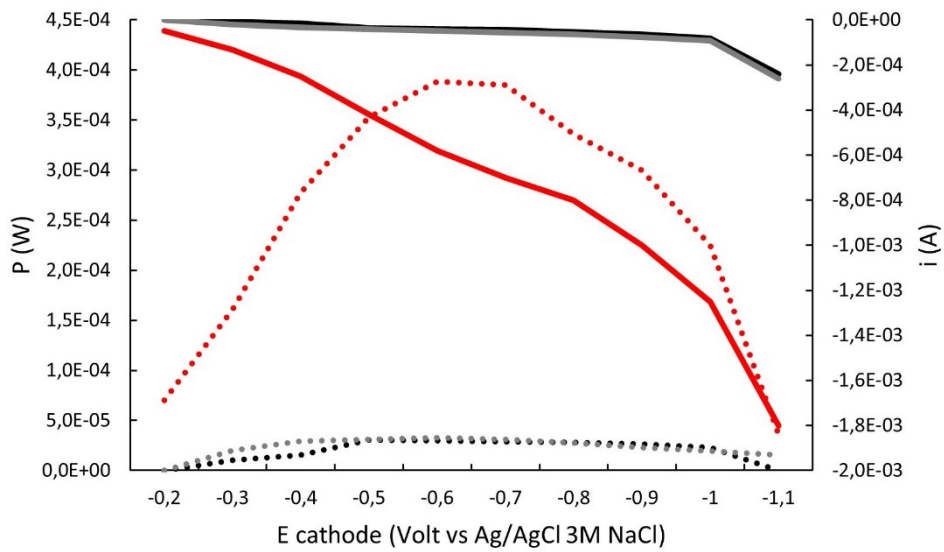
A thermogravimetric analysis (TGA) was performed on dry precipitates accumulated on the bottom of reactors using an Auto MYGA Thermogravimetric Analyzer (TA Instruments brand) and following a ramp from 30 to 800 °C under a N₂ stream, at a flow rate of 10 °C min⁻¹. Results are shown in figure S5 and table S1.



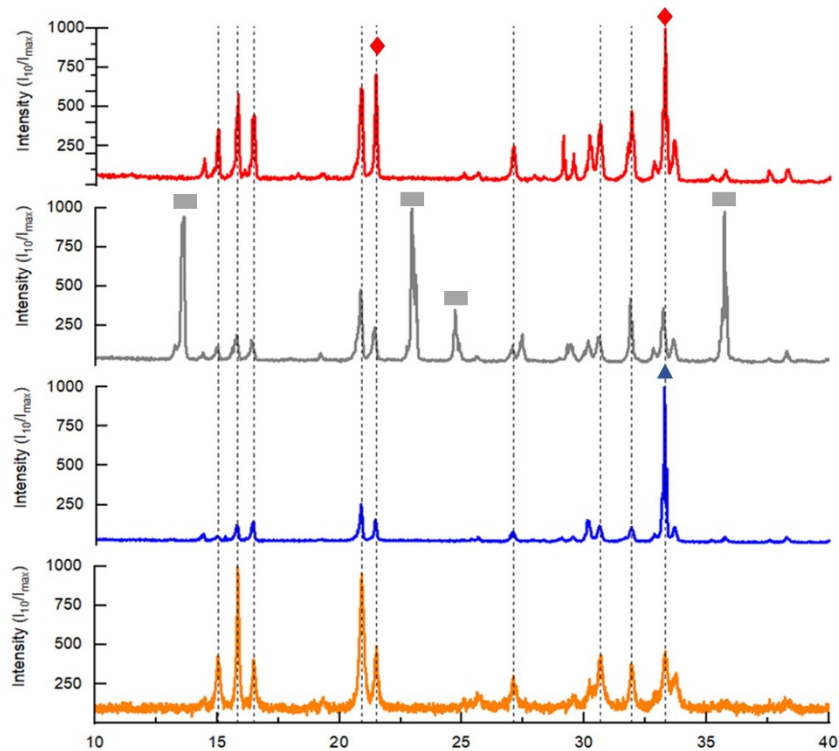
Supplementary figure 1. Cyclic voltammetry profiles obtained at 0.005 V s⁻¹ from a denitrifying bacterial consortium grown over a galvanically connected cathode with 18 mM of NO₃⁻ as the only electron acceptor (red line). A biological control in the absence of NO₃⁻ (black line) and an abiotic control (grey line) are included.



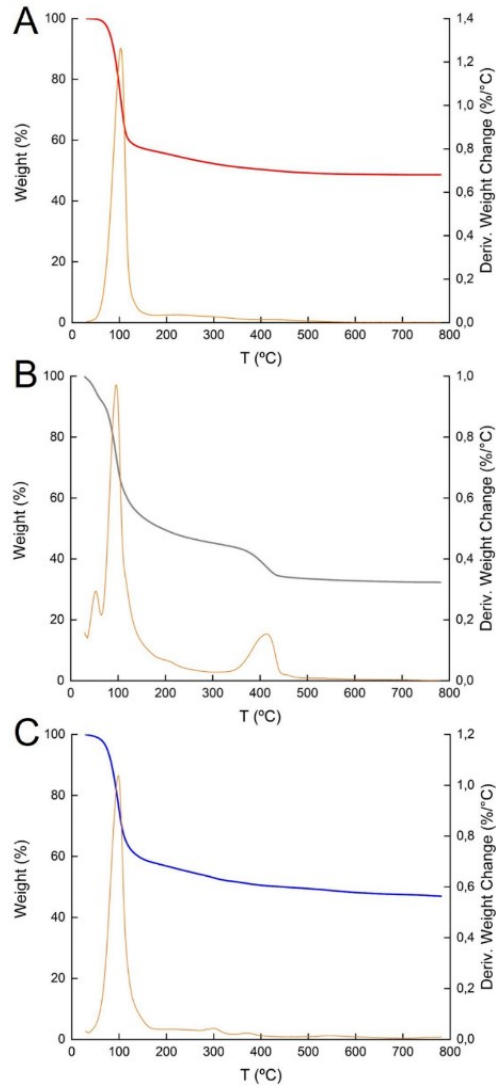
Supplementary figure 2: Evolution of potential of a denitrifying biocathode coupled to a magnesium anode under a fixed galvanic current of 0.012 A/m² upon the application of a pulse of NO₃⁻ to a final concentration of 0.4 mM. Arrow indicates the time of NO₃⁻ addition.



Supplementary figure 3: Power (dotted lines) and current (full lines) plot obtained from galvanic couples composed by graphite cathode and a magnesium anode in the presence of a denitrifying consortium interacting with the cathode with (red) and without (black) NaNO_3 as the electron acceptor. Data obtained in the absence of bacteria (grey) are also included.



Supplementary figure 4: X-ray diffraction patterns of precipitates obtained from reactors with magnesium bars acting as sacrificial anodes connected to graphite cathodes polarised to -0.5 V (Ag/AgCl - 3 M NaCl) in the presence (red) and absence (grey) of denitrifying bacteria, unconnected controls (blue) and struvite XRD pattern (orange). Dotted lines indicate peaks associated with struvite. (◆) calcium phosphate, (◻) magnesium ammonium carbonate and (▲) nitrate.



Supplementary figure 5: TG-DTG curves obtained from precipitates accumulated in reactors with magnesium bars acting as sacrificial anodes connected to graphite cathodes polarised to -0.5 V (Ag/AgCl - 3 M NaCl) in the presence (A) and absence (B) of denitrifying bacteria; those from unconnected controls (C) are also included.

Supplementary table 1: Thermal decomposition data corresponding to plots in figure S5. The percentual (%) weight loss obtained along the indicated temperature ranges is presented for every experimental condition.

Temperature	Treatment		
	Galvanically polarised		non-polarised
	Biotic	Abiotic	Biotic
30 - 129.3 °C	41.32	42.56	38.48
129.3 °C - 189.5 °C	2.81	7.19	4.24
189.5 °C - 305.3 °C	3.75	5.16	4.51
305.3 °C - 476.1 °C	2.72	11.36	3.08
476.1 °C - 600 °C	0.62	0.92	1.52
600 °C - 800 °C	0.19	0.51	1.23
Total 30 °C - 800 °C	51.41	67.7	53.05

Compound percentage estimations.

According to each XRD pattern and each of the assigned compounds weight loss over the analysed range (Struvite (51.42%), Calcium phosphate (6.27%), MAC (83.9%) and Nitratine (62%)), and following previous works,⁴⁻⁹ we could estimate the percentage composition from the TG-DTG assay considering the following equations, where X_n represent the percentage of the X_n compound, ranging between 0 and 1.

$$\text{Eq 1. } \sum X_1 * (X_1 \text{ weight loss\%}) + X_n * (X_n \text{ weight loss\%}) = \text{Sample weight loss\%}$$

$$\text{Eq 2. } \sum X_1 + X_n = 1$$

Biotic galvanically polarised $X_1 * (51.42\%) + X_2 * (6.27\%) = 51.41\%$; $x = >99\%$; $y = <1\%$

Abiotic galvanically polarised $X_1 * (51.42\%) + X_2 * (83.9\%) = 67.7\%$; $x = 0.49\%$; $y = 0.51\%$

Biotic non-polarized $X_1 * (51.42\%) + X_2 * (62\%) = 53.05\%$; $x = 0.85\%$; $y = 0.15\%$

References:

- 1 A. Apha, *American Public Health Association, Washington, DC*.
- 2 K. P. Gregoire, S. M. Glaven, J. Hervey, B. Lin and L. M. Tender, *Journal of The Electrochemical Society*, 2014, **161**, H3049.
- 3 M. Łukaszewski, M. Soszko and A. Czerwiński, *Int. J. Electrochem. Sci*, 2016, **11**, 4442–4469.
- 4 C.-C. Wang, X.-D. Hao, G.-S. Guo and M. Van Loosdrecht, *Chemical Engineering Journal*, 2010, **159**, 280–283.
- 5 G. Stoica, M. Santiago, S. Abelló and J. Pérez-Ramírez, *Solid state sciences*, 2010, **12**, 1822–1830.
- 6 J. Zhu and C. Jia, *Materials Chemistry and Physics*, 2022, **292**, 126787.
- 7 X. Zhang, X. Wang and Y. He, *Inorganic Chemistry Communications*, 2023, **148**, 110289.
- 8 H. Ren, Z. Chen, Y. Wu, M. Yang, J. Chen, H. Hu and J. Liu, *Journal of Thermal Analysis and Calorimetry*, 2014, **115**, 1949–1960.
- 9 C. Kramer and Z. Munir, *ECS Proceedings Volumes*, 1981, **1981**, 494.