Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

Aerobic denitrification with an electrode as sole electron and energy source

Rodríguez Simón, Carlos Norberto; Pedetta, Andrea; Busalmen, Juan Pablo and Bonanni, Sebastian*

Electronic supporting information (ESI)

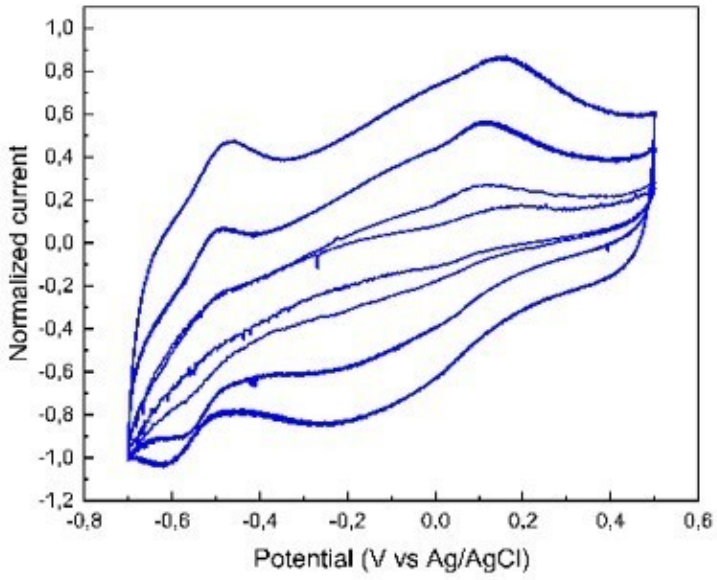

Figure S1. Cyclic voltammetries at different scan rates. Cyclic voltammetries performed on electrodes with fully developed bacteria grown under aerobic conditions. Scan rates of 1, 5, 20 and 50 mV/s were used and the resulting current was normalized by dividing its value with the value obtained at -0.7 V.

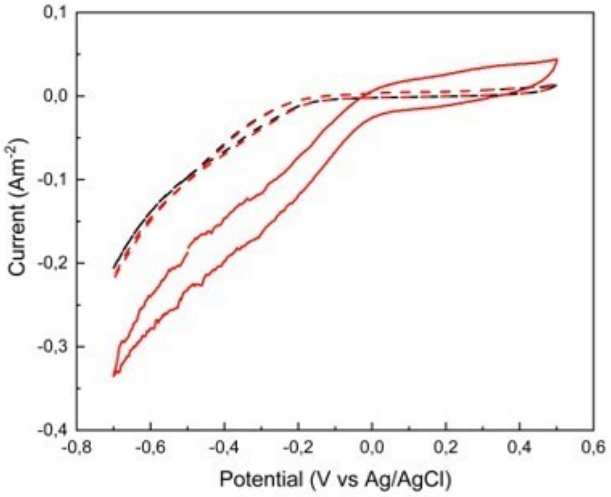

Figure S2. Cyclic voltammetries of control electrode. Cyclic voltammetries performed, to an abiotic control electrode prior to inoculation (black dotted line), to an electrode with fully developed bacteria grown under aerobic conditions (orange line) and on a new (clean) electrode immersed on the same medium as the orange line (orange dotted line). A scan rates of 5 mV/s was used in all cases.

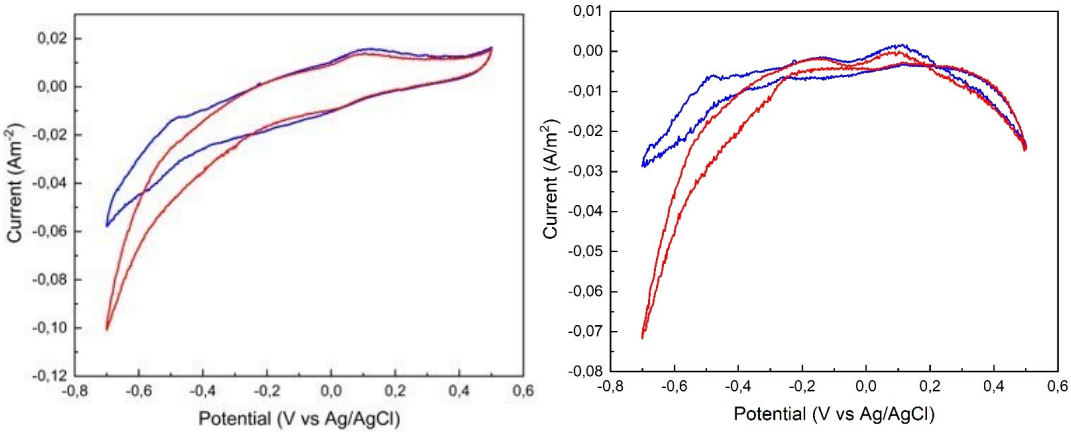

Figure S3. Voltammetries of anaerobic and aerobic grown electrodes deoxygenated. Left: Cyclic voltammetry for aerobic grown consortium deoxygenated (red line) and anaerobic consortium (blue line) at 5 mV/s. Right: same signals signals but obtained by subtracting abiotic CVs in the absence of oxygen.

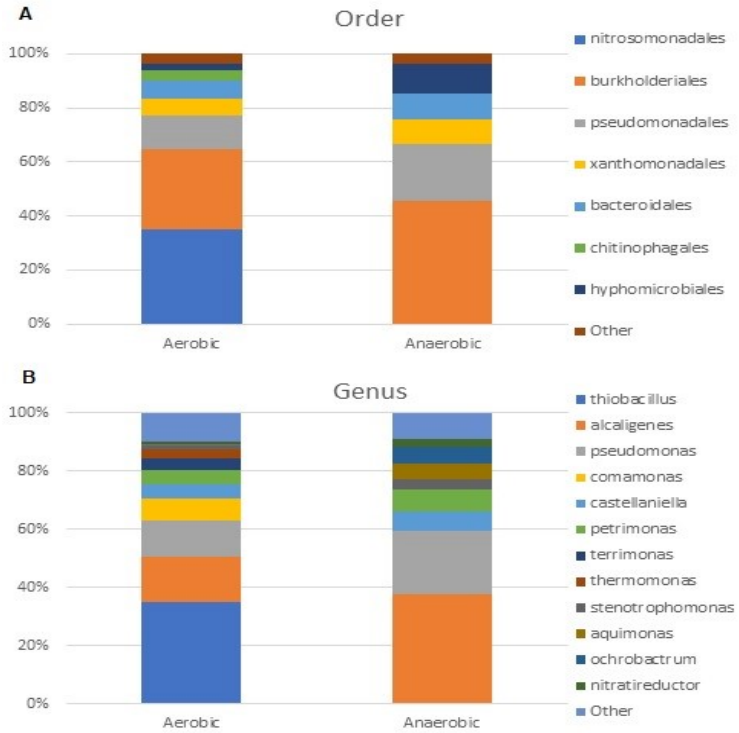

Figure S4. Genomic analysis. Taxonomic distribution at order and genus level from consortia obtained from electrodes grown under aerobic and anaerobic conditions based on 16S gene metagenomics analysis. “Other” includes operational taxonomic units with less than 3 % in both conditions.

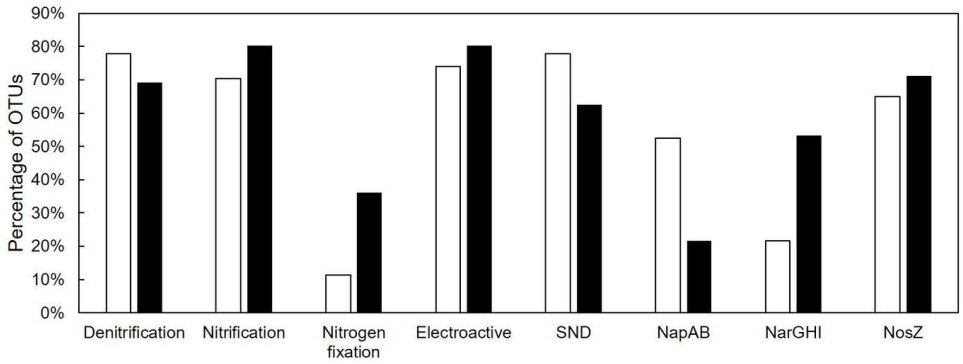

Figure S5. Distribution of the possible metabolic pathways and nitrogen cycle enzymes. Distribution of the possible metabolic pathways and key enzymes present in the operative taxonomic units for aerobic (white bars) and anaerobic (black bars) cathodes, based on species and genus quantification and information available in Prosite (prosite.expasy.org), ncbi (ncbi.nlm.nih.gov/) and Kegg (genome.jp/kegg) databases.

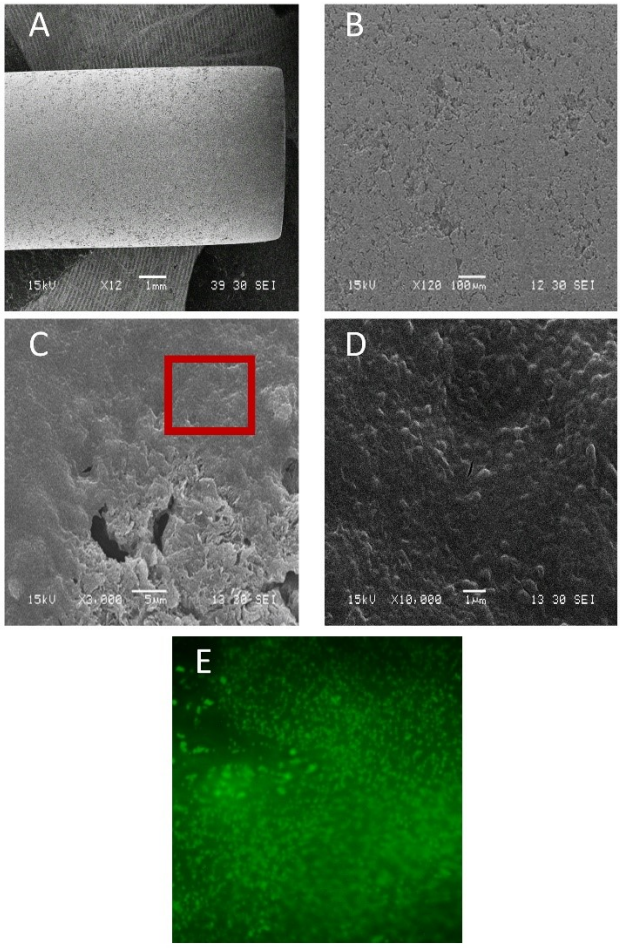

Figure S6. Scanning electron microscopy (SEM) and acridine orange staining images of Bacteria grown on the electrodes for 70 days under aerobic conditions. In images A and B, taken with low magnification, the typical porosity of graphite electrodes can be observed, indicating that a biofilm was not formed on the surface. On image C) a closer look of a region where bacteria were adhered. Image D) shows the portion marked in red on Figure C) at higher magnifications. E) is an image of electrodes stained with acridine orange evaluated under a fluorescence microscope at 500 nm. Analysis of the image with ImageJ software revealed coverages close to 5%.

Estimation of potential denitrification through heterotrophic death cells oxidation

Bacteria death can lead to the availability of organic molecules that could be used by heterotrophic denitrifiers to remove nitrates. In order to analyse the impact that this process could have on nitrate removal process observed in the experimental systems, a theoretical denitrification rate for biomass oxidation can be estimated.

According to bibliography bacteria oxidation requires between 1.14 and 1.66 mgO_2 per mg of biomass.[1] From the mean value of 1.4 mg_{O2} per mg of biomass it can be estimated that the oxidation of bacteria would release 17 Coulomb per mg of biomass.

Considering that the weight of a single cell of *Thiobacillus denitrificans* is $1x10^{-9}$ mg $cell^{-1}$ [2] it can be estimated that the complete oxidation of the biomass on the aerobic electrodes (4.1×10^6 cell cm^{-2}) would potentially release 697 C per m^2 of electrode.

Considering that this charge is released during the time of the nitrate removal assays (8 days), and with the fraction of electrons taken from the electron donor that end up in nitrate reduction according to the estimations presented in the manuscript (38%), it would produce a nitrate reduction rate of 0.096 $\mu g_{N\text{-}NO3}$ cm^{-2} day^{-1}:

$$r_{N-NO3} = \frac{697\frac{C}{m^2}\frac{1m^2}{10000cm^2}\ 0.38\ \ 14\frac{g_{N-NO3}}{mol_{N-NO3}}\frac{1x10^6\mu g_{N-NO3}}{g_{N-NO3}}}{8\ day\ 5\frac{mol_{e-}}{mol_{N-NO3}}96500\frac{C}{mol_{e-}}} = 0.096\frac{\mu g_{N-NO3}}{cm^2 day}$$

This value represents only a minor fraction (0.68%) of the nitrate removal rate observed in the aerobic reactors (14 $\mu g_{N\text{-}NO3}$ cm^{-2} day^{-1}) indicating that, if present, heterotrophic oxidation of bacterial cells played only minor role on nitrate reduction.

References

1 E. M. Contreras, N. C. Bertola, L. Giannuzzi and N. E. Zaritzky, A modified method to determine biomass concentration as COD in pure cultures and in activated sludge systems, *Water Sa*, 2002, **28**, 463–468.

2 C. Trouve, P. M. Chazal, B. Gueroux and N. Sauvaitre, Denitrification by new strains of Thiobacillus denitrificans under non-standard physicochemical conditions. Effect of temperature, pH, and sulphur source, *Environmental technology*, 1998, **19**, 601–610.