# Electronic supplementary material (ESI)

# BIOCHAR AS SUBSTITUTE OF GRAPHITE ON MICROBIAL ELECTROCHEMICAL TECHNOLOGIES

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Table S1- Summary of biochar and graphite properties

| Material | Relative quinone content (FTIR) | ZP (mV) (pH 5.5) | Mass Yield (%) |
|---|---|---|---|
| 500 °C | 1 | -37.60±6.75 | 33.82±2.87 |
| 600 °C | 0.66 | -42.23±6.10 | 30.92±2.21 |
| 800 °C | 0.04 | -45.00±6.60 | 30.08±0.29 |
| 1000 °C | ND | NM | NM |
| Graphite | ND | NM | - |

*ND: not detected, NM: not measured*

**Oxygen and carbon ratio and biochar composition**

Oxygen and carbon were measured by EDS and XPS. Both techniques gave similar results of oxygen to carbon ratio for all the analyzed temperatures (Figure S1).

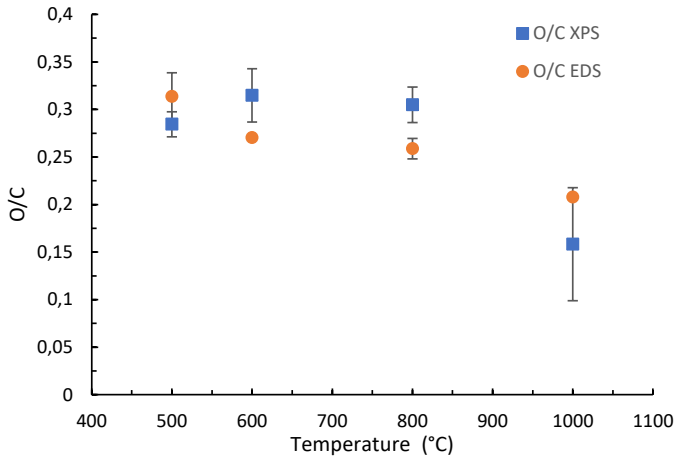


Figure S1 – Oxygen to carbon ratio (O/S) for biochar obtained at different pyrolysis temperatures, measured by XPS and EDS.

Besides oxygen and carbon other elements were detected and quantified through EDS. Two samples of each material were analyzed in randomly chosen spots. Mean values for each element are shown on Table S2.

Table S2 – Elemental composition of each material, determined through EDS

| Material | Weight fraction (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | O | K | Na | Ca | Cl | Si | Mg | S | P | Fe | Ti |
| E500 | 73,51 | 23,04 | 1,11 | 0,50 | 0,33 | 0,61 | 0,22 | 0,17 | 0,11 | 0,33 | N.D | N.D |
| E600 | 70,44 | 19,06 | 3,55 | 1,63 | 0,99 | 3,30 | 0,11 | 0,23 | 0,15 | 0,52 | N.D | N.D |
| E800 | 73,15 | 18,91 | 3,36 | 0,97 | 0,18 | 2,69 | 0,15 | 0,11 | 0,16 | 0,46 | N.D | N.D |
| E1000 | 80,37 | 16,71 | 0,25 | 0,11 | 0,93 | 0,17 | 1,29 | 0,04 | 0,03 | 0,09 | N.D | N.D |
| Graphite | 98,725 | N.D | 0,23 | 0,59 | 0,09 | 0,295 | N.D | N.D | N.D | N.D | 0,05 | 0,02 |

Spatial distribution of the Carbon and Oxygen on the surface of the E600 and E1000 obtained from EDS are shown in Figure S2.

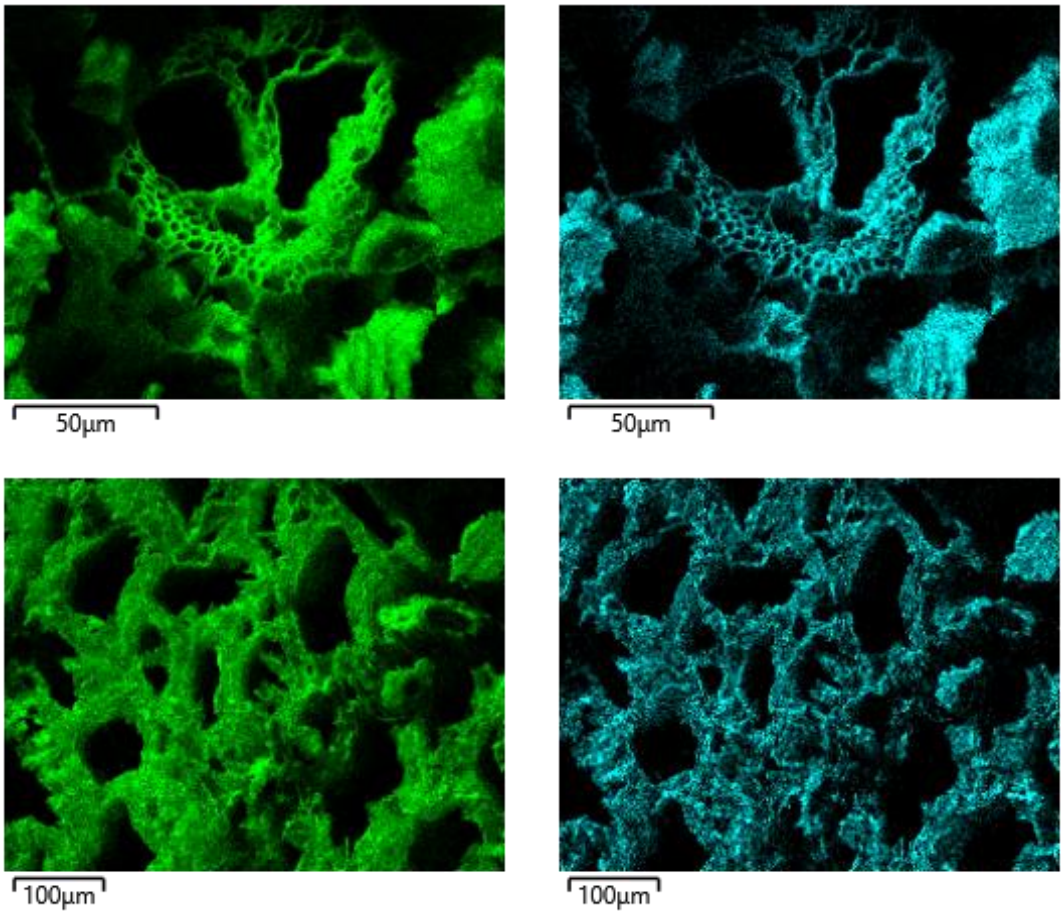


Figure S2 – Distribution of Carbon (left column) and Oxygen (right column) on E600 (top row) and E1000 (bottom row) determined by EDS.

## XRD Results

Table S3 - Crystalline compounds detected by XRD analysis.

| Biochar | Crystalline faces detected by XRD |
|---|---|
| 400, 500 and 600 | Sodium chloride (NaCl) (Ref Patt: 77-2064)<br>Potassium chloride (KCl) (Ref Patt: 75-0296)<br>Calcium carbonate ($CaCO_3$) (Ref Patt: 85-1108) |
| 800 | Sodium chloride (NaCl) (Ref Patt: 77-2064)<br>Potassium chloride (KCl) (Ref Patt: 75-0296)<br>Dolomite ($CaMg(CO_3)_2$) (Ref Patt: 84-1208)<br>Calcium Carbonate ($CaCO_3$) (Ref Patt: 85-1108)<br>Chromium Oxide (CrO) (Ref Patt: 08-0252) |

## FTIR spectra

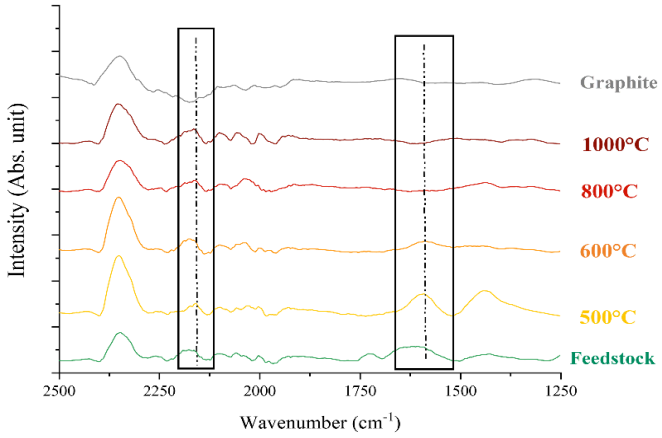


Figure S3 – FTIR spectra of dried feedstock, biochar obtained at different temperatures and graphite.

## Thermogravimetry assays (TGA)

TGA analysis performed on an oxygen atmosphere. Evolution of samples weight and the derivative of weight change are shown in figure S4. Heating rate was set on 10 ºC $min^{-1}$.

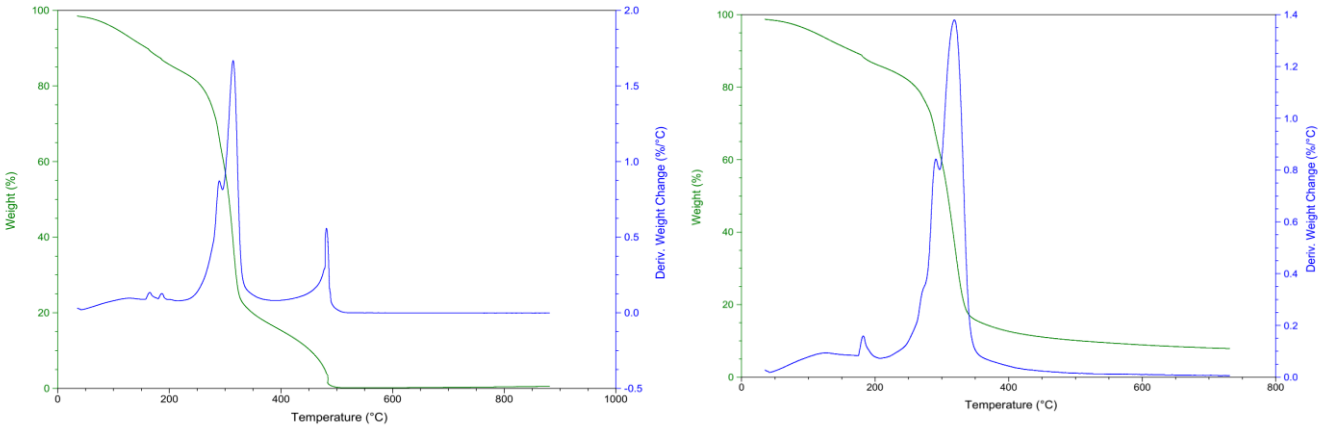


Figure S4 – TGA analysis of E1000 (left) and graphite (right)

## Zeta potential

The zeta potential (ZP) is a parameter that is used to characterize particles surface charge (at the interface between the solid surface and its liquid medium). In general, particles with a ZP in between ±30 mV are prone to form aggregates. The ZP depends on the particle characteristics (composition and size) and their environment (mainly pH and ionic strength). The ZP of the bacterial culture and the different biochar were determined. Under physiological conditions (PBS, pH 7.4), the ZP of *Geobacter sulfurreducens* was -25.20 mV which is in agreement with the negative charge of the cell generally observed for most bacteria.

The ZP of the different biochar materials was analyzed in several conditions, employing finely ground samples (≤ 10 µm), in a low and a high salt solution, at different pH. All the materials had a negative ZP at in every tested condition and the ZP values were very similar between the electrodes, showing a slight decrease with increasing pyrolysis temperature. Similarly, to other activated carbons, the ZP did not change significantly in the studied pH range (3-13), highlighting the stability of the material with pH.

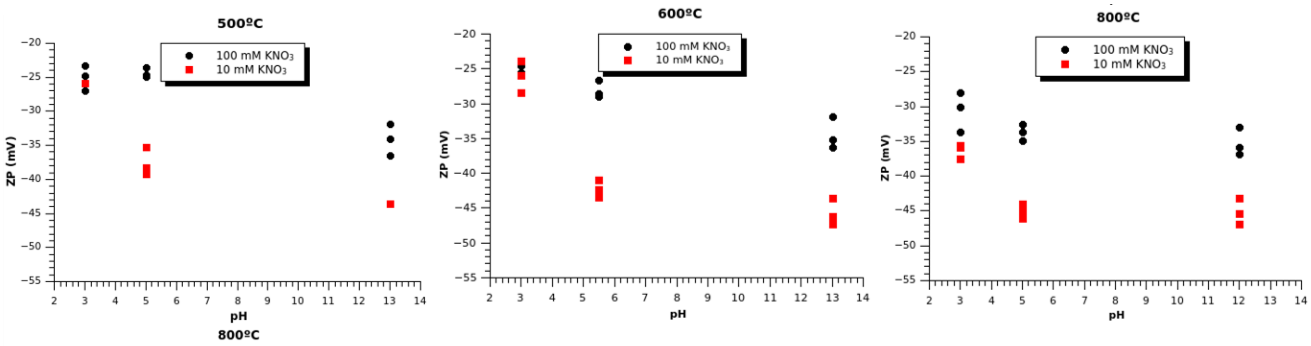


Figure S5 - Zeta potential of samples as function of pH, at different salt concentration (10 and 100 mM $KNO_3$) Results represent the mean of three independent measurements.

## Raman Spectra

Three major Raman peaks were observed at 1355, 1583, and 2710 $cm^{-1}$ (Figure S6), corresponding to D, G and 2D bands, which are typical features of pristine natural graphite. The D band arises from imperfections or hexagonal symmetry breaking of a graphite structure, whereas the G band arises from sp2 bonds of carbon atoms and corresponds to the high-frequency E2g phonon. The 2D band is the D-peak overtone and can be used to determine the number of graphene layers.

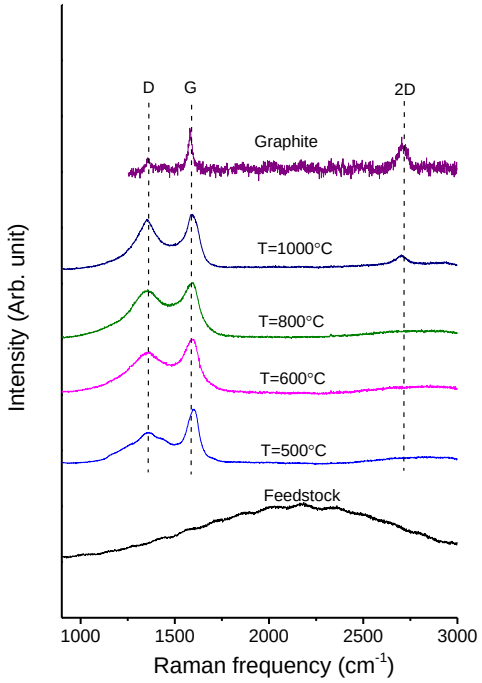


Figure S6 - Raman spectra of raw feedstock, samples pyrolyzed at different temperatures and graphite.

The evolution of the ratio of the intensities of D and G peak (ID/IG) with pyrolysis temperature can be used to follow the transition from amorphous carbon to a crystalline structured carbon. ID/IG for biochar obtained at different pyrolysis temperatures are shown in Table S4:

Table S4 - Ratio of peaks D and G intensity for different biochar

| Material | $I_D/I_G$ |
|---|---|
| E500 | 0.66 |
| E600 | 0.76 |
| E800 | 0.85 |
| E1000 | 0.95 |

Values shown in table S4 reveal a progressive increase in the order of the carbon atoms in biochar with pyrolysis temperature.

In order to perform a quantitative analysis of the peak area and following the work of Sheng et al. (2007), the band area ratios were obtained using five peaks corresponding to different chemical structures within the carbon structure, as shown in Fig. S7 for 1000 ºC.

Peaks were fitted using a free combination of Lorentzian functions. Initial values for peak positions were taken as 1585, 1350, 1620, 1500 and 1200 $cm^{-1}$ corresponding to peak G (ideal graphitic), D1 (disordered graphitic edges, in-plane imperfections), D2 (disordered graphitic surface), D3 (amorphous carbon, sp2 bonded) and D4 (disordered graphite lattice, ionic impurities). The relative value of the D peak and the total area (sum of all the peak areas) were obtained for all the temperatures to analyze the degree of graphitization. Also the band area ratios for each D peak and G peak were estimated to analyze the variation on the order of the graphitic structure (Figure S8).

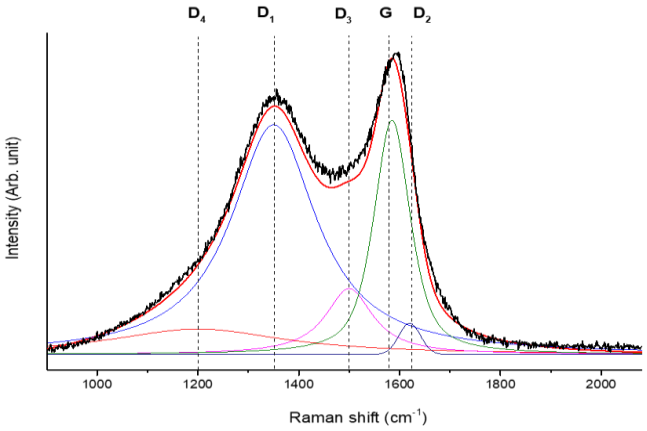


Figure S7 - RAMAN spectra fitting for the biochar obtained at 1000 °C.

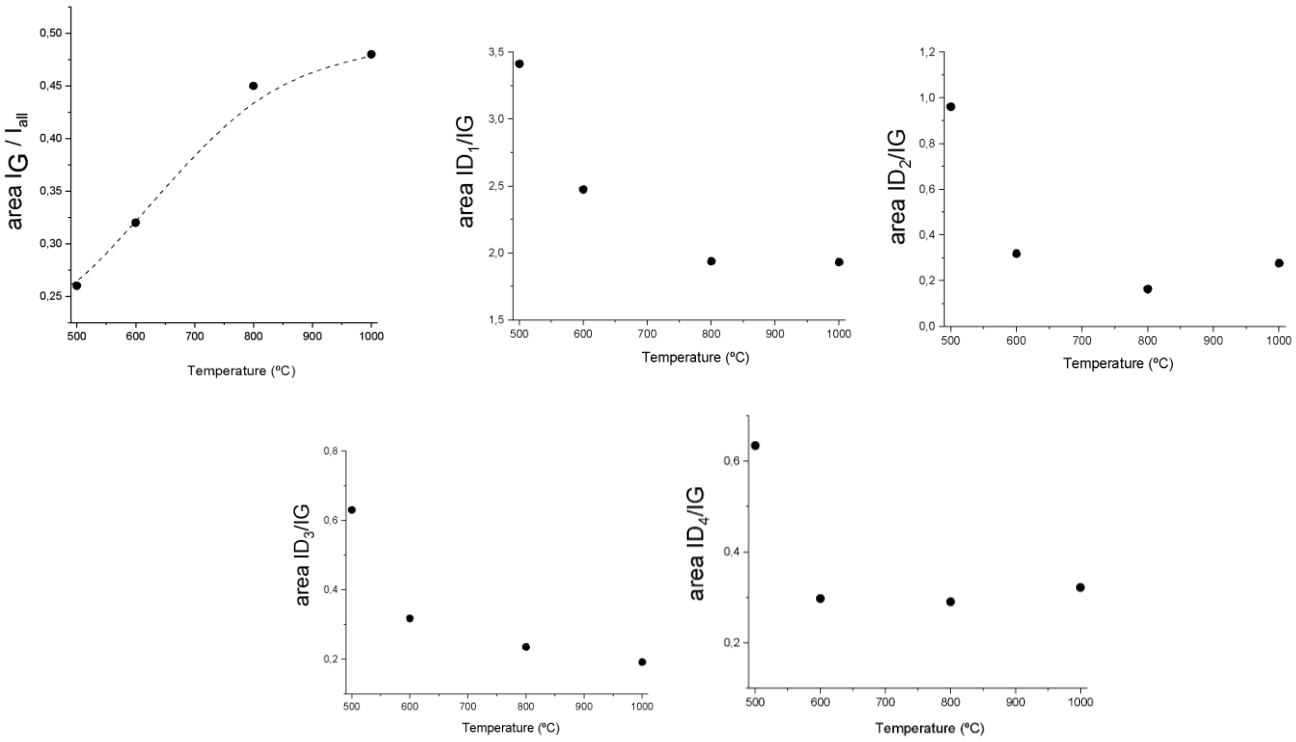


Figure S8 – Relative area of G peak ($I_G$) and the sum of all peaks ($I_{all}$) and relative area of each D peak ($ID_{\#}$) with G peak, for different pyrolysis temperatures.

An increase of biochar microstructural order under heat treatment can be observed through Raman parameters, in particular the band area ratios, indicated by the increase in $I_G/I_{all}$ and the decrease in $I_{D1}/I_G$, $I_{D2}/I_G$, $I_{D3}/I_G$ and $I_{D4}/I_G$ with increasing treatment temperature.

At temperatures below 500 °C the relative area of peaks representing disordered phases (ID1, ID2, ID3 and ID4) was much higher than at higher temperatures, indicating that biochar was formed mainly by amorphous carbon. With an increase in temperature, lower relative areas for those peaks and higher relative area for the peak of ideal graphitic structure (IG) were obtained, indicating a progressive decrease in the disorder and an increase in the degree of graphitization starting at 600 °C.

At temperatures of 1000 °C the 2D peak became prominent, further indicating an enhancement of graphitization degree with increasing temperatures and the formation of increasing number of graphene layers.

**Additional images**

Cross sections of 500, 600, 800 and 1000 °C electrodes were observed in SEM to analyze pore sizes and internal structure (Figure S9-A). The pore diameter measures (ImageJ, red lines) evidenced no differences between temperatures. Also, longitudinal sections of electrodes that were producing high current density (800 and 1000°C) were observed to check for colonization of the internal structure (Figure S9-B). No bacteria were observed on the internal pores on the analyzed samples.

Macrographs of cross sections of Cyperus *papyrus* were obtained (Figure S10) and compared with micrographs of the internal structure of biochar. The internal structure prevailed after pyrolysis.

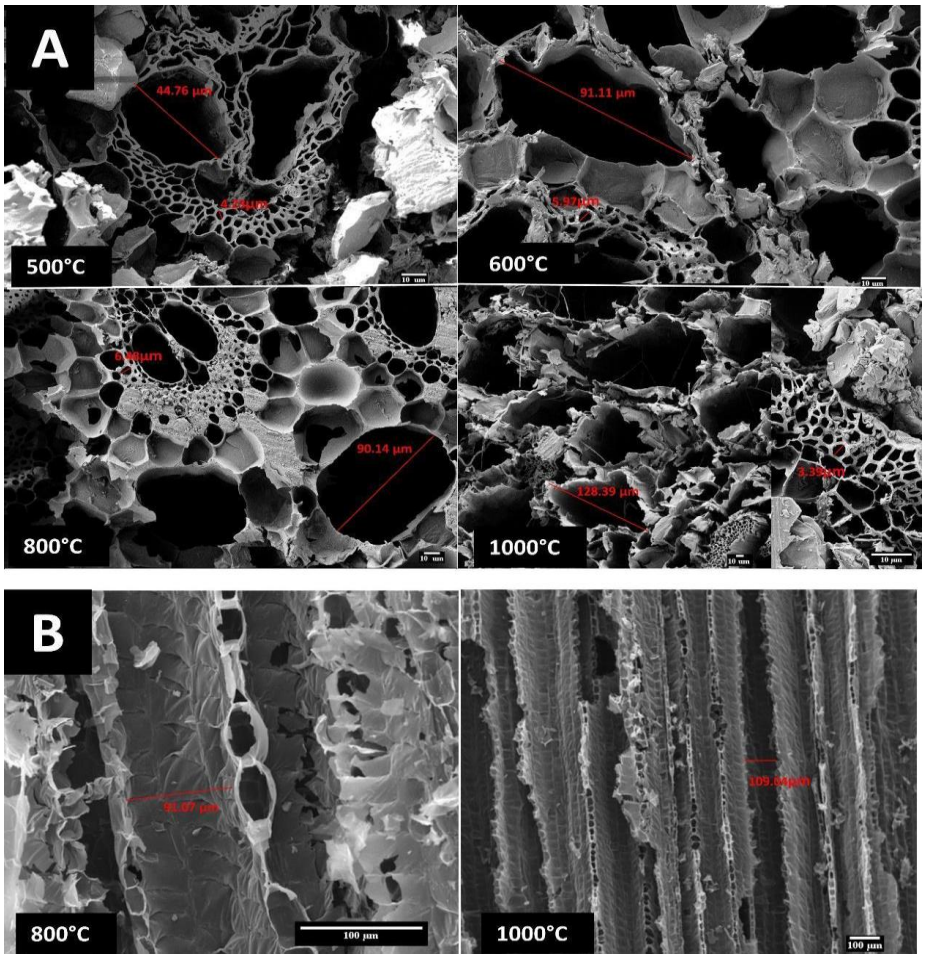


Figure S9 – Representative SEM images of the internal part of a biochar electrode obtained at different temperatures. In panel A cross sectional images of stems pyrolyzed at different temperatures are shown whereas in panel B longitudinal images are shown. Red lines show pore diameter.

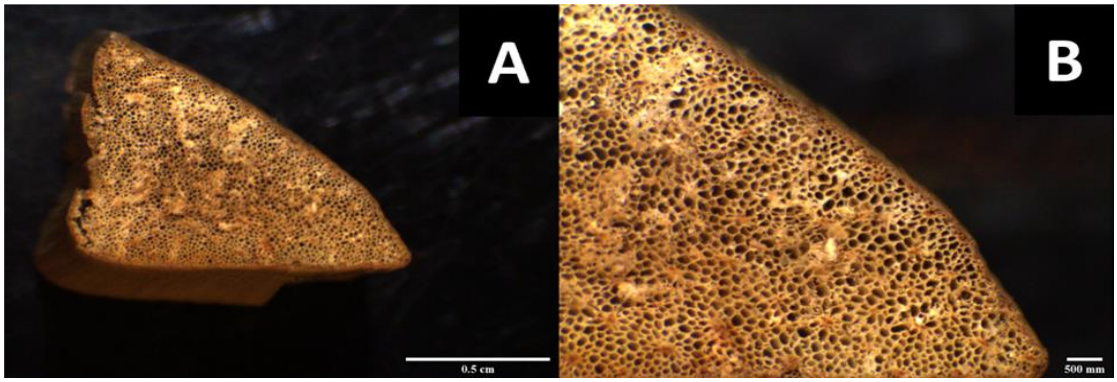


Figure S10 – Macrographs of cross section *Cyperus papyrus* showing internal structure. Image B is a magnification of image A where pore distribution is seen in more detailed.

**Growth of bacteria with biochar as electron acceptor**

The growth of *G. sulfurreducens* was evaluated using biochar as the only electron acceptor in vials with liquid medium. Vials with fumarate as electron acceptor were used as positive control, while in the experimental conditions fumarate was replaced by biochar at 400°C, 500°C, 600°C and 800°C. The negative control was not inoculated vials.

A biochar concentration of 3g/L of 400°C, 500°C, 600°C and 800°C materials was added to vials. After inoculating 3.07E5 bacteria/ml, vials were incubated at 30 °C and pH 7 for 30 days (N=2). Afterwards, samples of each condition were centrifuged 5 min at 2000 rpm and bacteria were counted using a Neubauer chamber (Marienfeld) under an inverted phase contrast microscope (Eclipse Ti, Nikon) with 40X magnification. To avoid bias during cell counting, vials were identified by numbers with a relation with the different biochar unknown by the operator. The negative control (blank) was subtracted from the bacterial counts. Results are shown in Figure S11.

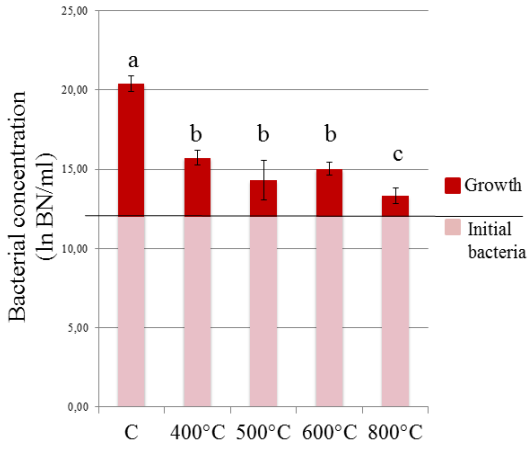


Figure S11 – Bacterial concentration (natural logarithm of bacterial number per ml - ln BN/ml-) obtained by Neubauer chamber for control (C) and biochar of 400, 500, 600 and 800°C as electron acceptor. Pink bars represent initial bacteria (3.07E5 bacteria/ml) and red bars growth of bacteria in different experimental conditions. Statistical analysis was performed with Fisher's F-Test and two-tailed Student T-Test. Prior to the analysis, a logarithmic transformation was applied to the data since they did not have a normal distribution. Those values that were negative after subtracting the blank were removed from the analysis. The significance level was 0.05 (p-value). Different letters indicate significant statistical differences.

FTIR of dry material from negative control and from experimental biochar of 500°C was performed and show a decrease on the peak assigned to quinones on the biochar used as electron acceptor (Figure S12).

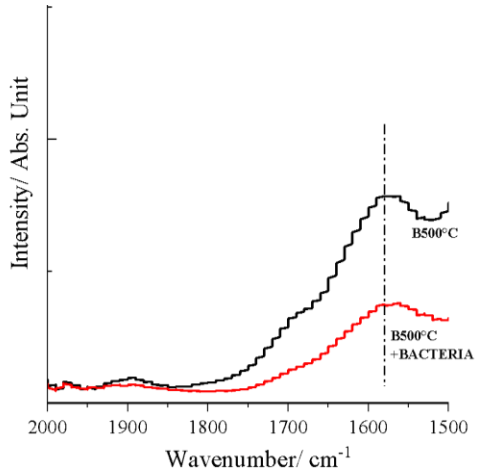


Figure S12 – FTIR spectra of 500°C biochar used (red curve, B500°C+BACTERIA) and not used (black curve, B500°C) as electron acceptor for *G. sulfurreducens* growth.