Supplementary Materials

2. Materials and Methods

2.1 Preparation, modification, and characterisation of biochar

2.1.1 Modification of biochar

To reduce the content of quinone groups, 1 g of biochar was placed in 100 mL of a 30% H_2O_2 solution. The biochar was subjected to shaking for 72 hours, followed by heating to 90°C for 2 hours. Following this, the biochar was cooled to room temperature and centrifuged at 14000 rpm for 15 minutes. The biochar was then rinsed three times with deionized water. The biochar collected by filtration was recorded as BC-1.

To introduce quinone groups to the biochar surface, 5 g of biochar was added to 100 mL of a methanol solution containing 13 mM p-benzoquinone (p-BQN) and 4 mM 9,10-anthraquinone (9,10-EQN). The mixture was then shaken for 72 h. Subsequently, the biochar was vacuumized through a 0.22-μm polysulfone membrane, followed by drying at ambient temperature. The biochar sample was labeled as BC-2.

2.1.2 Characterization of biochar properties

(1) Structural characterization of biochar

The biochar elemental composition was identified by means of an elemental analyzer (Vario EL Cube, Germany). The biochar surface morphology was observed with a scanning electron microscope (SEM, MIRA3, TESCAN, Czech Republic). samples were degassed at 105℃ for 12 h to remove moisture and impurities and the specific surface area and pore volume were calculated by adsorption and desorption of

N_2 at 77K with a pore structure analyzer (ASAP2460, Micromeritics, China). The surface functional groups of biochar were measured using a FTIR spectrometer (Nicolet 6700, Thermo Fisher, USA) in the 4000-400 cm^{-1} range. X-ray photoelectron spectroscopy (XPS, AXIS UltraDLD, Shimadzu, China) was used to test and analyze the C 1s peak, thereby facilitating the analysis of functional groups. X-ray diffraction analysis (XRD, Aeris, Malvern Panaco, China) was used to observe and analyze the degree of graphitization of biochar. The Raman spectra were analyzed by Raman micro-spectroscopy (LabRAM Solei, HORIBA, China). Following a five-day period of chloramphenicol biodegradation, the biochar in the reaction system was collected, fixed with 2.5% glutaraldehyde, and then treated with ethanol gradient dehydration, CO_2 critical point drying and gold spraying to observe the surface morphology of the biochar after the reaction.

(2) Cyclic voltammetry and electrochemical impedance spectroscopy

Biochar materials were utilized as the working electrode for cyclic voltammetry and electrochemical impedance spectroscopy tests, with platinum sheets and Ag/AgCl electrodes serving as counter electrode and reference electrode, respectively. Initially, a carbon paper electrode loaded with biochar was prepared. A biochar suspension was prepared by ultrasonicating a mixture of 20 mg of biochar, 0.2 mL of perfluorosulfonic acid polymer (Nafion) and 2 mL of ethanol solution for 1 hour. Thereafter, 50 μL of the biochar suspension was deposited onto the surface of the carbon paper electrode and desiccated at 60°C for a period of 8 hours, thus establishing the working electrode. The carbon paper electrode was then subjected to a cyclic voltammetry (CV) test within the

potential range of +1.0 V to -0.2 V. Electrochemical impedance spectroscopy (EIS) was subsequently conducted within the frequency range of 0.1 Hz to 10^6 Hz. The data obtained from the EIS were then fitted with the ZView software.

(3) Chronoamperometric experiments

For chronoamperometric experiments, the glass carbon electrode, platinum wire electrode and Ag/AgCl electrode were designated as the working electrode, opposite electrode and counter electrode, respectively. The electrolyte solution was composed of a mixture of 0.1M phosphate buffer (PBS, pH =7.0) and 0.1M KCl solution, at a ratio of 1:2. Prior to measurement, N_2 was injected into the electrolyte solution for 1 h, to remove dissolved oxygen. During the experiment, N_2 was continuously injected into the electrolytic cell tosequester oxygen. The electron accepting capacity (EAC) and the electron donating capacity (EDC) were measured using mediated electrochemical reduction (MER) and oxidation (MEO), respectively. The operating voltage was set at -0.49V/+0.61V. Following stabilization of the baseline, 1.0 mL of 10 mM methyl viologen dichloride and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were added as electron transfer mediators, respectively. Following restoration of the baseline, a biochar suspension was introduced at concentrations from 0.2-1.0 mg. The electron accepting capacity (EAC) and the electron donating capacity (EDC) of the biochar were calculated according to the following formula:

$$EAC = \frac{1}{m_{BC}} \int \frac{I_{red}}{F} dt$$

$$EDC = \frac{1}{m_{BC}} \int \frac{I_{ox}}{F} dt$$

where I_{red} and I_{ox} (A) are the baseline-corrected reduction and oxidation current,

respectively, F represents Faraday's constant (=96485 [s·A/mol e^-]), and m_{BC} (g biochar) is the mass of biochar. The electron exchange capacity (EEC) is defined as the total capacity for accepting and donating electrons where EEC = EAC + EDC.

2.3 Antibiotics biodegradation by biochar

Antibiotics concentrations were measured by filtrating the samples through a 0.22 μm filter to eliminate residual solids and microorganisms. Antibiotic concentrations were then determined by high performance liquid chromatography (HPLC) with a chromatographic column (2.1×50 mm, 1.8 μm) and an ultraviolet (UV) detector. The mobile phase comprised a 0.1% formic acid solution in phase A and an acetonitrile solution in phase B, with a flow rate of 0.8 $mL \cdot min^{-1}$ and an injection volume of 10 μL. The column temperature was set at 35 °C, and the ultraviolet wavelength was set at 278 nm.

Table S1 Experimental conditions for biodegradation

Environmental factor	Antibiotics concentration (mg/L)	Carbon source	Carbon source concentration (g COD/L)	Temperature (°C)
Chloramphenicol concentration	5, 10, 20, 40	NaAc+yeast extract	5	30
Antibiotic types	chloramphenicol, sulfadiazine, ofloxacin	NaAc+yeast extract	5	30
Temperature	10	NaAc+yeast extract	5	25, 30, 37
Carbon source	10	yeast extract+NaAc, yeast extract+glucose	5	30
Carbon source concentration	10	yeast extract+NaAc	5, 3, 1, 0	30

2.4 Determination of biochar effects on bacterial electron transport capacity

(1) Electron transfer system activity (ETSA)

Following a three-day culture period, bacterial cells were collected by centrifugation at 12000 rpm for three minutes. Thereafter, the cell pellet was washed thrice with 0.5 M PBS buffer (pH=7.4) and resuspended in 1 mL of PBS buffer. Following this, 200 μL of 0.5% 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazole chloride (INT) was then added to the bacterial suspension, after which the mixture was incubated in a dark box at 30°C for 30 minutes. The reaction was halted by the addition of 100 μL of methanol. Then, the bacterial cells were harvested by centrifugation at 11000 rpm for 2 min. This process was repeated twice. The clear upper layer was collected and mixed thoroughly. The extinction coefficient at a wavelength of 490nm was then determined. ETSA was calculated with the following formula:

$$ETSA(\mu g \cdot min^{-1} g^{-1}) = \frac{ABS_{490nm}}{15.9} \times \frac{V_1}{V_0 \times t} \times \frac{32}{2} \times \frac{1}{m}$$

where V_0 and V_1 are the sample volume and methanol volume (mL), respectively, t is the reaction time (min), and m is the dry weight of cells per unit sample (g/mL).

(2) NADH/NAD+

NAD+ and NADH are indicators of intracellular electron transfer in electroactive microorganisms. The total amount of NAD(H/+) is indicative of the total amount of intracellular electron carrier in electroactive microorganisms, and the ratio of NAD+/NADH signifies the intracellular redox state of microorganisms. The electro-activated bacterial cells were centrifuged at 12000 rpm and 4°C for 3 minutes, followed by culturing for a period of 3 days. The WST-8 NAD+/NADH detection kit (Biyantian

Biotechnology, China) was used to detect the content and ratio of NAD+ (oxidized coenzyme I) and NADH (reduced coenzyme I) in bacteria by colorimetry.

(3) Determination of bacterial extracellular polymers (EPS)

The extraction of the EPS was conducted with the hot extraction method. On the fifth day of culturing, 40 mL of the sample was subjected to centrifugation at 4500 rpm for 15 minutes. This was followed by two washes with 0.1M PBS buffer (pH =7.4). The cells were then suspended in 15 mL of 0.05% NaCl. The 0.05% NaCl solution was preheated to 70 °C, added to the initial volume of 40 mL, and vortexed for 3 minutes. The mixture was then subjected to centrifugation at 12000 g for a duration of 10 minutes, after which the loosely bound extracellular polymers (LB-EPS) present in the upper layer were collected. The cells devoid of LB-EPS were then suspended in 40 mL of 0.05% NaCl and vortexed for a further 3 minutes. Following incubation in a water bath at 60°C for 30 minutes and centrifugation at 12000 g for 10 minutes, the tightly bound EPS (TB-EPS) in the remaining sample was collected. The protein and polysaccharide content of the TB-EPS and LB-EPS samples was subsequently determined by the Coomassie Blue and anthrone-sulphuric acid colorimetric methods, respectively.

The Coomassie Blue method is outlined as follows: Initially, 100 mg of Coomassie brilliant blue G-250 is dissolved in 50 mL of 95% ethanol. Subsequently, 100 mL of 85% phosphoric acid was added, and the solution made up to 1000 mL. The solution should then be transferred to a brown bottle and stored away from light. A 2 mL water sample should then be taken, to which 5 mL of Coomassie brilliant blue reagent should then be added. The resulting mixture should then be measured for its ability to absorb

light at a wavelength of 595 nm, with this measurement being taken within 5-20 minutes. The protein content of the extracellular polymers is then calculated by plotting a standard curve using bovine serum albumin (BSA, Standard Grade).

The anthrone-sulphuric acid colourimetric method: A quantity of 0.1 g of anthrone was dissolved in 100 mL of 80 % concentrated sulphuric acid solution, ready for use. A 2-mL sample volume was then mixed with 6 mL of anthrone-concentrated sulfuric acid reagent. The mixture was heated in a boiling water bath at 100°C for 15 minutes and then immersed in an ice water bath for a further 15 minutes. The absorbances were then measured at 625 nm using a UV-visible spectrophotometer rapidly. Concurrently, standard curves were plotted with glucose solutions of varying concentrations to calculate the polysaccharide content in the extracellular polymers.

(4) Determination of cytochrome c

Shewanella oneidensis MR-1 cells were ccultured over a period of three days. The culture was centrifuged at 8000 rpm for 5 minutes and the cells were washed with 50 mM PBS buffer and subsequently suspended in 2 mL of SL solution (1 mg/mL lysozyme, 20% (w/v) sucrose, 0.01 M Tris). The cells were then incubated shaking at 100 rpm and 30°C for 1 h. Following this, the cultured cells were centrifuged at 8000 rpm for 10 min and the cells were suspended in a Tris-Mg^{2+} solution (0.01 M $MgCl_2$, 0.01 M Tris). The cells were then cultured shaking at 100 rpm and 30°C for 30 min. The cell-free layer was then centrifuged at 8000 rpm for 5 minutes. The samples were then analyzed using a UV-VIS spectrophotometer, with the optical density of the samples measured at a wavelength of 520 nm.

(5) Determination of riboflavin

Following a period of bacterial fermentation spanning three days, 2-mL samples were collected from the bioreactor. These samples were centrifuged at 12000 rpm for five minutes. Subsequently, the resultant material was filtered through a 0.22-μm PVDF membrane. To determine the concentration of riboflavin at a wavelength of 254 nm, 10 μL of the filtrate was then injected into a high-performance liquid chromatography (HPLC) apparatus. The flow rate was set at 1.0 mL/min in the solvent system, comprising a mobile phase of water, methanol, and acetic acid (68:32:0.1 v/v).

2.5 Determination of *Shewanella oneidensis* MR-1 functional genes and proteomes

(1) PCR parameters

The PCR reaction mixture comprised cDNA, SYBR Green PCR Mix, forward and reverse primers, and sterile water. The thermal cycle conditions were set to: (1) Denaturation at 95°C for 5 minutes; (2) Amplification: amplification at 95°C for 15 seconds, annealing at 60°C for 60 seconds, and then extension at 72°C for 20 seconds, repeated for 40 cycles. (3) Melting curve: The PCR instrument automatically and continuously recorded the fluorescence signal as the temperature increased from 65℃ to 97℃ at a rate of 0.3℃/s.

Table S2 qPCR Primers

Primer Name	**Primer Sequence (5’-3’)**
MtrCQF	CTCAAGAGTTTGCGGATGGT
MtrCQR	CATGTCGGATTCAACGTGAC
MtrAQF	CGGCACTTACCATCACAATG
MtrAQR	ATCCCACTTCGACGCATAAG
OmcAQF	AACTGTGCATCTTGCCACAC
OmcAQR	TCGCCACCTTTATGGATAGC
CymA FP	GATATCGAATTCATGAACTGGCGTGCACTAT
CymA RP	ACTAGTGGATCCTTATCCTTTTGGATAGGGGT

(2) Protein extraction

To facilitate proteomics analysis, the bacterial community and the culture medium were separated by centrifugation to exclude the influence of proteins in the culture medium. The harvested bacteria were washed with PBS buffer three times, after which they were suspended in 1.5 mL PBS. The suspensions were then subjected to centrifugation, after which the aqueous layer was removed. The remaining bacteria were then frozen in liquid nitrogen and stored at -80°C.

3. Results and Discussion

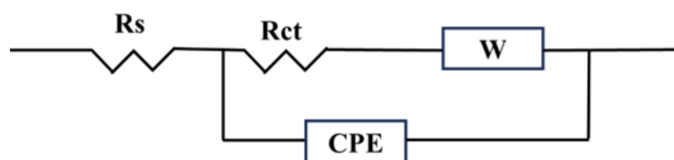

Figure S1. The equivalent circuit diagram obtained from the Nyquist plot.

Table S3 The resistance data fitted by ZView software

Biochar	R_s/Ω	R_{ct}/Ω
BC400	8.376	9.895
BC600	9.009	7.733
BC800	9.524	6.4
BC1000	9.332	6.287

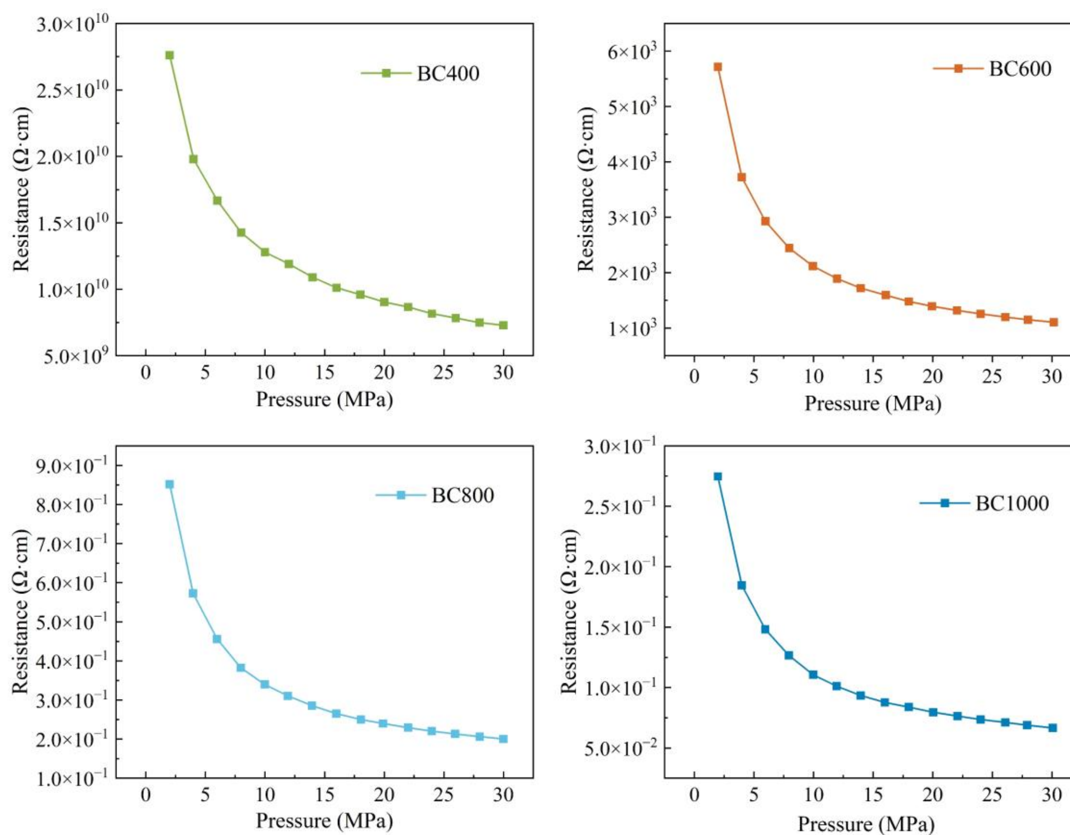

Figure S2. The electrical resistance-pressure (2-30 MPa) curve of biochar produced at 400℃, 600℃, 800℃, 1000℃. The electrical conductivity is calculated with the resistance at 20MPa.

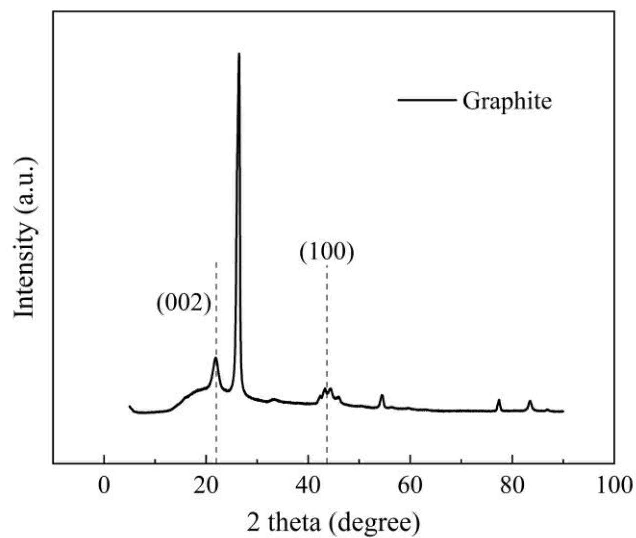

Figure S3. XRD of graphite

Table S4 XPS Results for the C 1s Binding State and Its Relative Atomic Percentage on the Biochar Surfaces.

Biochar	C-C/C=C	C-O	C-N	O-C=O
BC400	52.17	15.15	21.05	11.63
BC600	53.59	-	31.70	14.70
BC800	64.86	-	25.49	9.66
BC1000	65.25	-	30.25	4.49

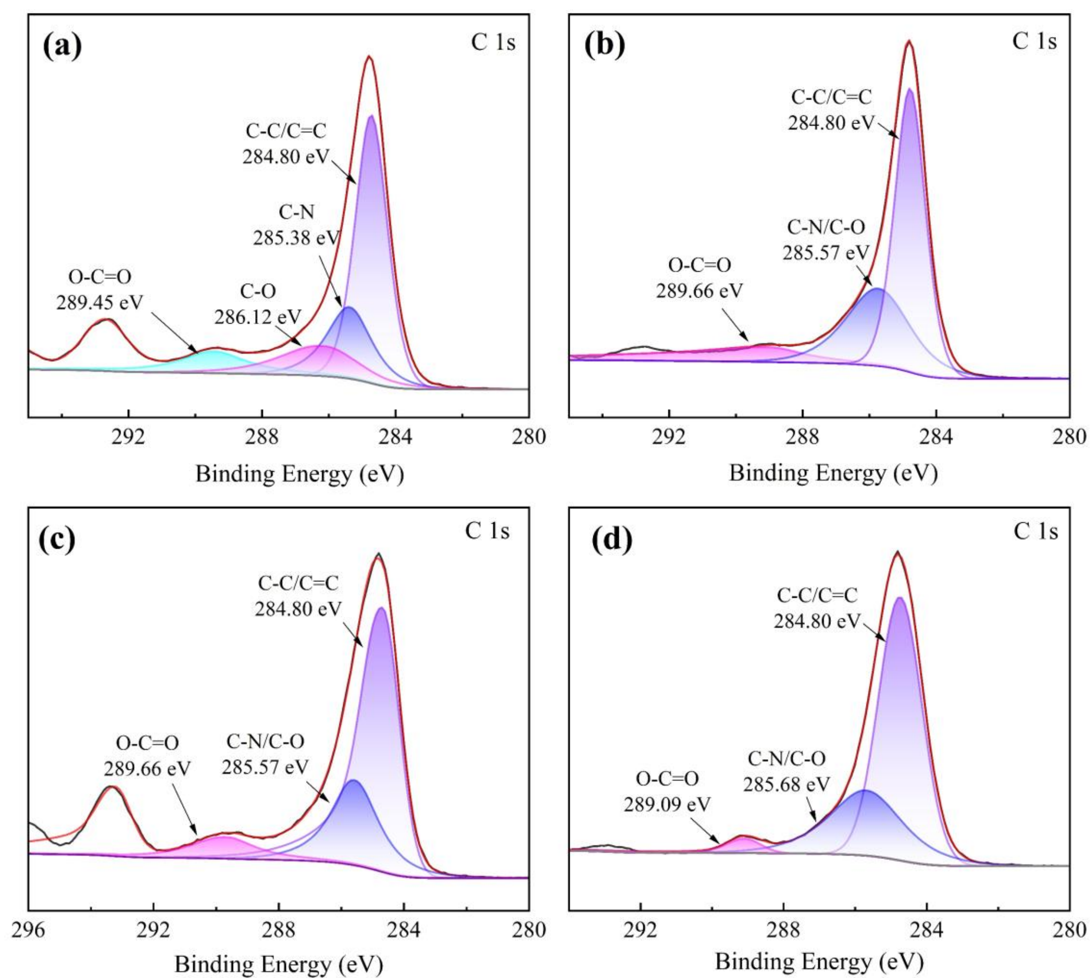

Figure S4. XPS of biochar produced at different temperatures: (a) BC400, (b) BC600, (c) BC800, (d) BC1000.

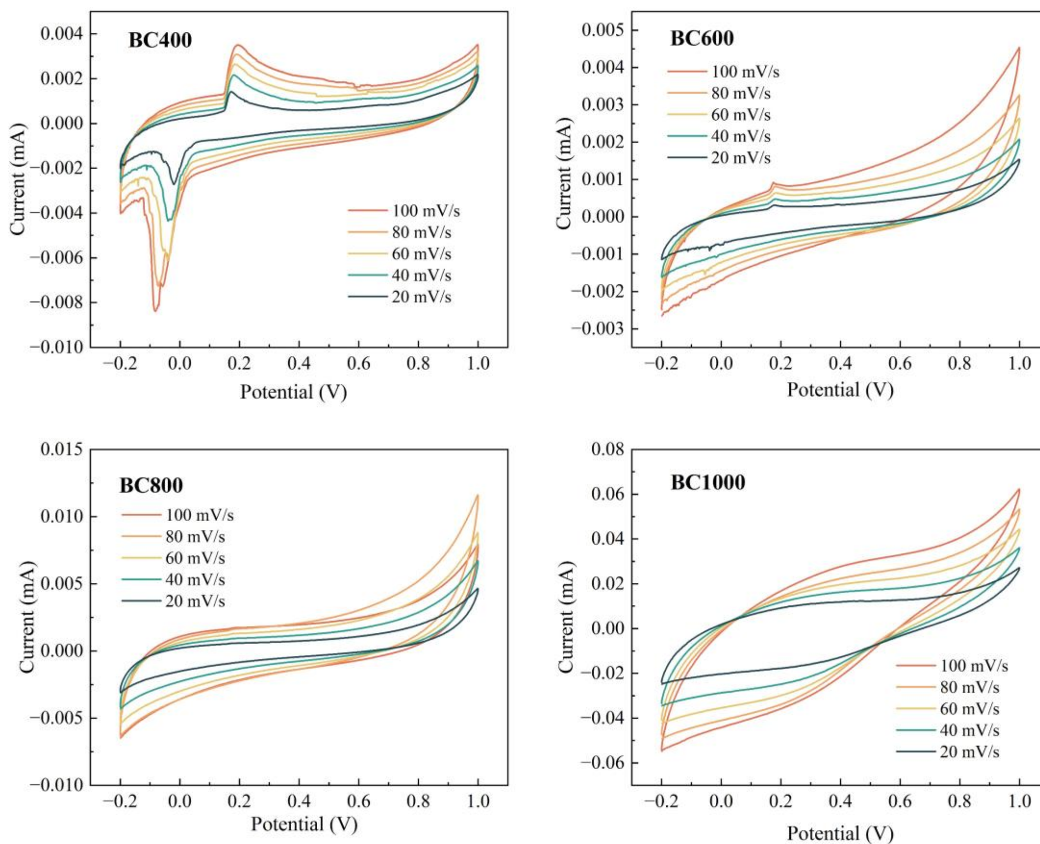

Figure S5. Cyclic voltammograms (CV) of biochar produced at different temperatures at different scan rates (20-100 mV/s).

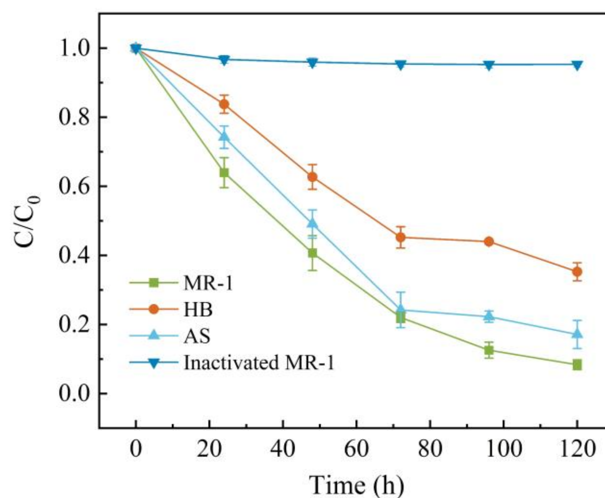

Figure S6. Biodegradation curve of chloramphenicol

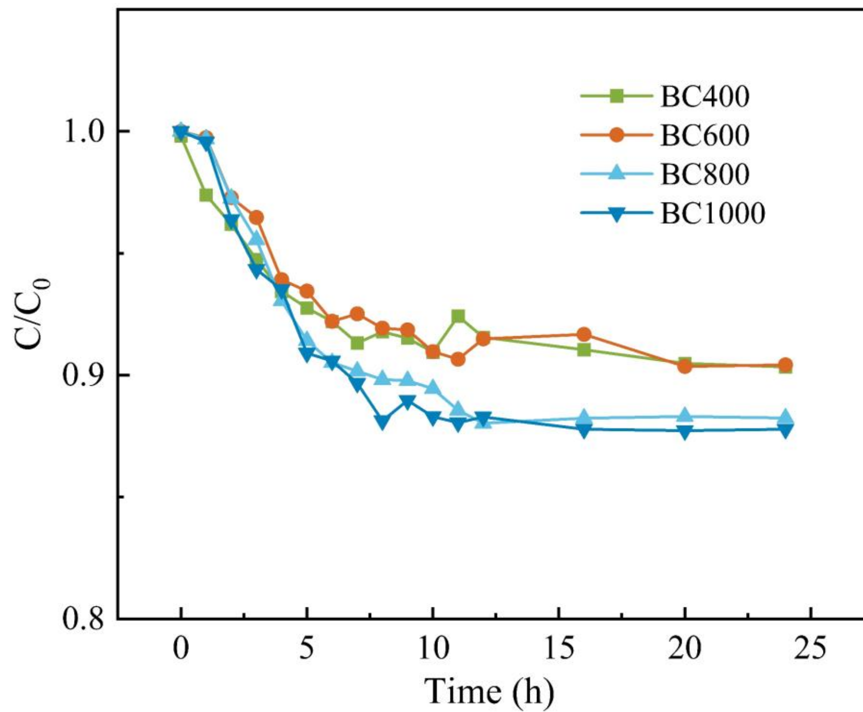

Figure S7. Adsorption of chloramphenicol by biochar produced at 400℃, 600℃, 800℃ and 1000℃. Chloramphenicol concentration in the presence of biochar was measured in the aqueous phase only.

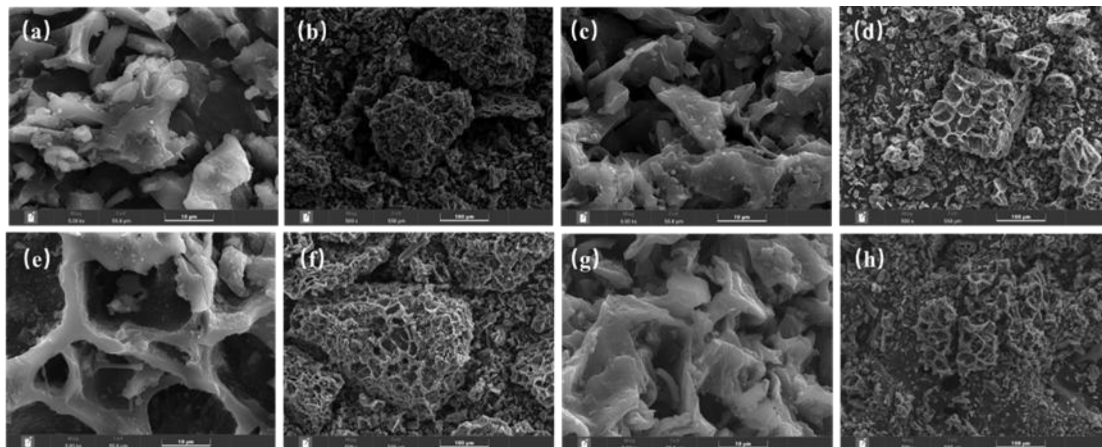

Figure S8. SEM analysis of biochar produced at different temperatures, (a) and (b) BC400, (c) and (d) BC600, (e) and (f) BC800, (g) and (h) BC1000.

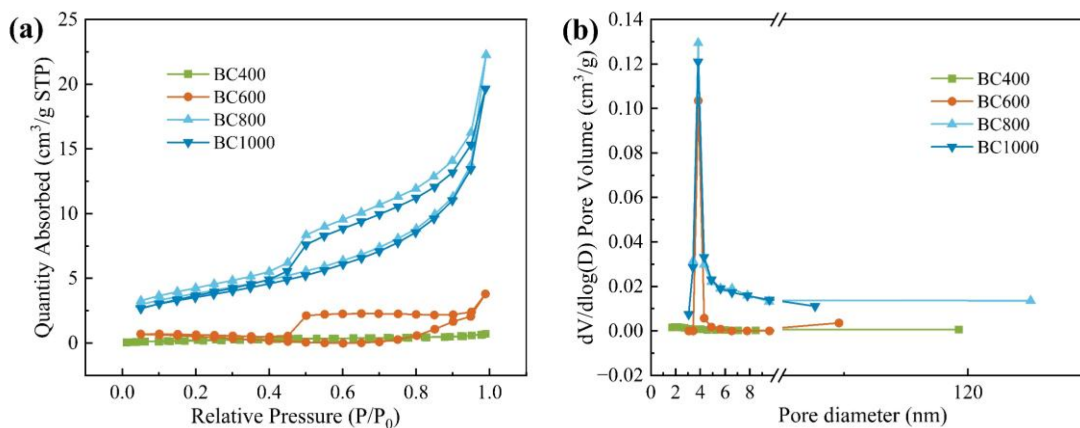

Figure S9. (a) Nitrogen adsorption and desorption isotherms (77K) and (b) pore size distribution of biochar produced at different temperatures.

Table S5 Specific surface area and pore structure of biochar

Biochar	Specific surface area ($m^2 \cdot g^{-1}$)	Pore diameter (nm)	Pore volume ($cm^3 \cdot g^{-1}$)
BC400	1.029	4.328	0.0011
BC600	1.038	3.832	0.0059
BC800	13.617	3.829	0.0344
BC1000	12.586	3.822	0.0304

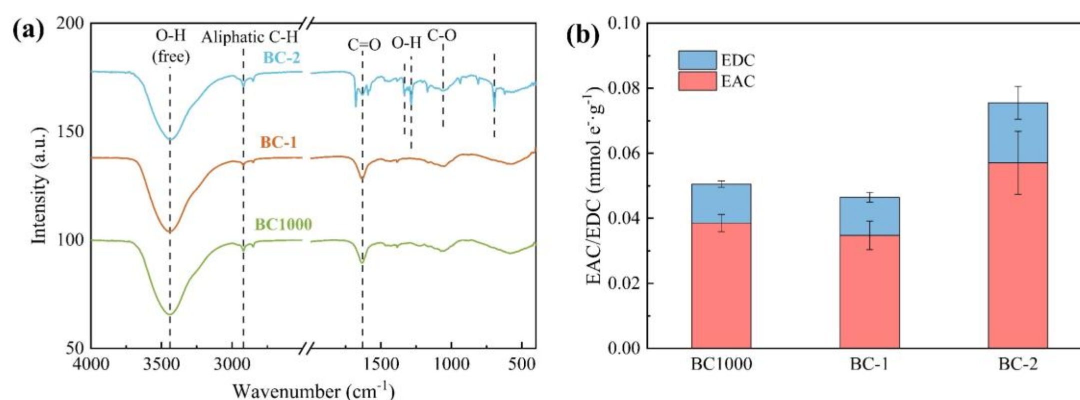

Figure S10. (a) Fourier Transform Infrared Spectrum of BC1000, BC-1 (H_2O_2-treated BC1000) and BC-2 (BC1000 modified by benzoquinone and anthraquinone). (b) Electron exchange capacity (EEC=EAC+EDC) of BC1000, BC-1 and BC-2.

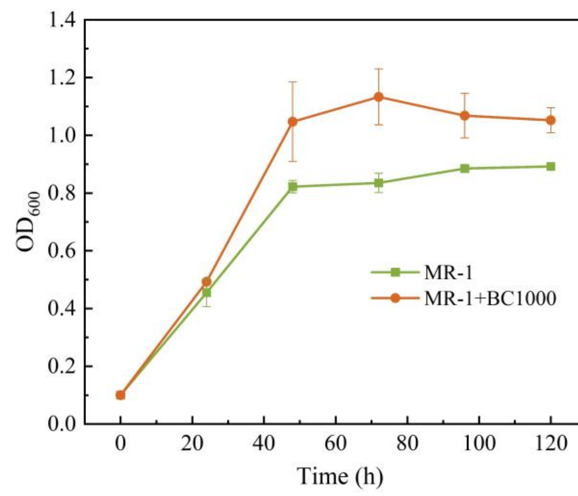

Figure S11. Growth curve of *S. oneidensis* MR-1 (OD_{600}) in the presence or absence of BC1000.

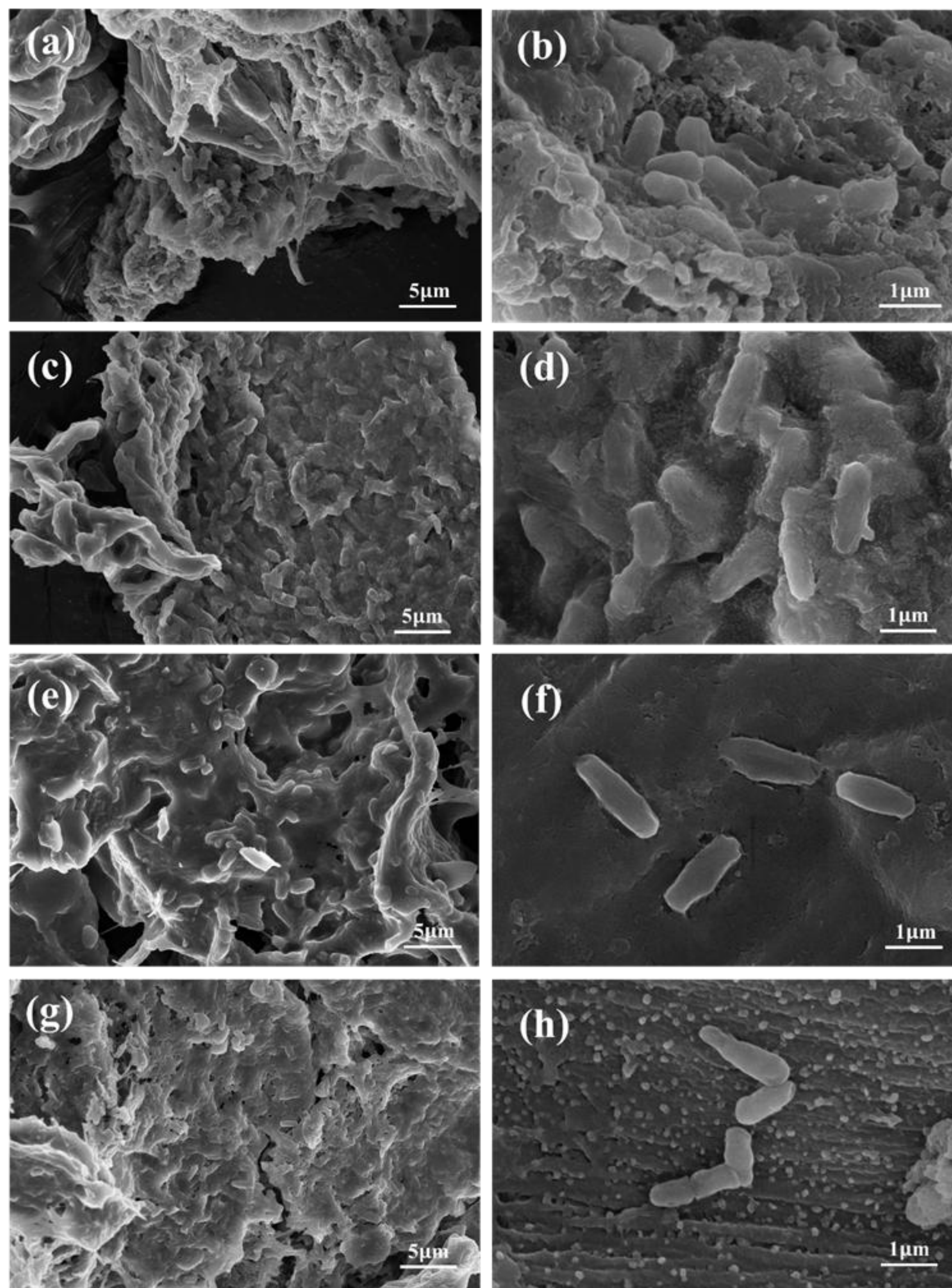

Figure S12. Surface morphology of bacteria attached to biochar probed by scanning electron microscopy, (a) and (b) BC400, (c) and (d) BC600, (d) and (f) BC800, (g) and (h) BC1000.

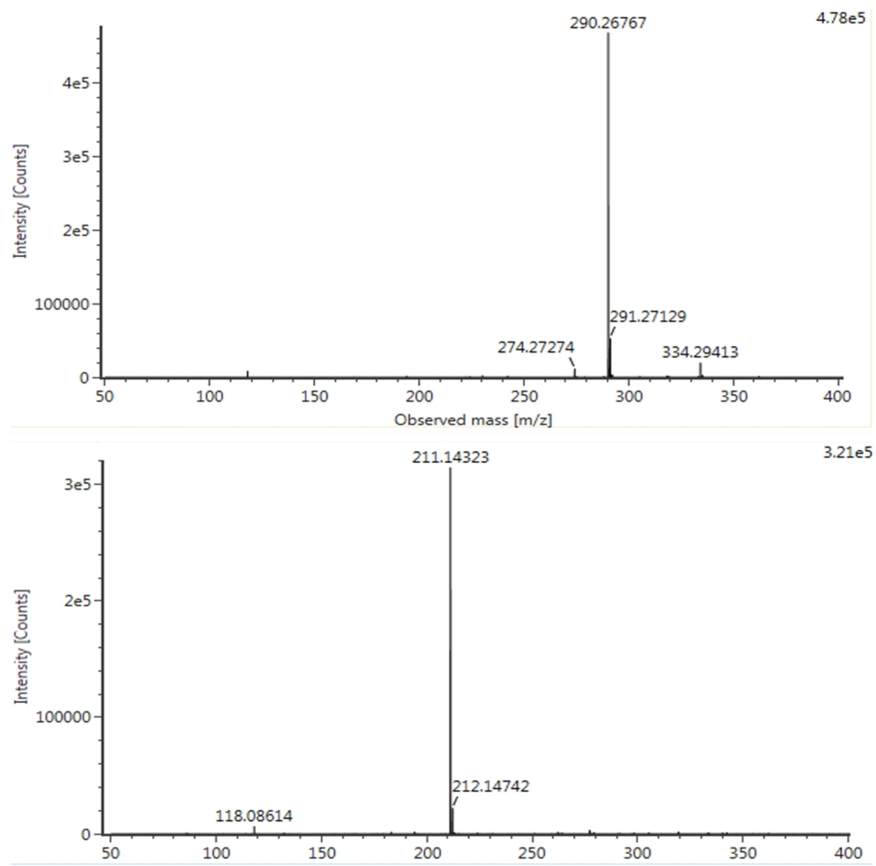

Figure S13. Analysis of degradation products of chloramphenicol