Electronic Supplementary Material (ESI) for Sustainable Energy & Fuels.

Supporting Information

Multifunctionsalisation of Woody Biochar Tuned for Sustainable Surface Microbiological Processes: A Case Study for Energy Application.

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3.1. Selection of woody biochar and fixation of their specifications

An appropriate selection of source of biomass and a pyrolysis temperature condition together determines the distinct physico-chemical characteristics of the biochar. Among different characteristics, surface functionalization plays an important role in bio-electrochemcial features too. The present study started with the challenge of choosing the right biomass for biochar synthesis. Based on the hypothesis of higher surface area, porosity and applications, lead to the selection of highly porous biomasses such as water plants namely *Salvinia molesta*, *Azolla pinnata* and *Pistia stratiotes*. Preliminary evaluation of porosity revelations were favorable. Later on, developing these anodes, none gave stable coatings in spite of using different ratios for casting. The wettability of these biochar is high that the concentration of binder used must be higher to make a coating which is another disadvantage of the earlier selected biomasses. Considering the functional-rich characteristics, woody biomass of *Acacia auriculiformis* selected for the present study is strategically carbonized to increase its redox-active functionalities specific to bacterial interaction.

3.2 Temperature controlled biochar synthesis

Pyrolysis is widely preferred for production of biochar applied to bioelectrochemical systems, which specifically guarantees higher surface area and porosity. Conventional methods of Biochar production, their specificity and application are illustrated in Table S1.

Table S1: Conventional methods of Biochar production, their specificity and applications

Source of biochar	Method of preparation	Specificity	Application	Reference
Palm oil empty fruit bunch	Microwave assisted synthesis	High yield, high surface area and porosity	Substitute for activated carbon	[1]
Pine cone	Slow pyrolysis	Low yield, high surface area and porosity, sufficient bulk density	Removal of organic and inorganic pollutants	[2]
Populus nigra wood	Steam assisted synthesis	Micropore volume increased	CO_2 adsorption and separation	[3]
Five seed shells namely pumpkin, walnut, coconut, pistachios, corncob	Gasification	The reactivity of each biochar is correlated with its ash level	Bioenergy with carbon capture and storage	[4]
Shrimp Shell	High temperature pyrolysis	At 750℃, the biochar showed a solid graphitic structure hiking electrical conductivity.	Supercapacitor electrode	[5]
Rice husk	Hydrothermal carbonization	Increased porosity and surface area, increased zeta potential, high yield	Wastewater treatment and biofuel production	[6]
Algal biomass	Hydrothermal liquefaction	Retains volatile organic materials and functional groups for adsorption, Higher energy denitrification ratio for high temperature biochar	Crucial for biofuel production Removal for dye ad pollutant adsorption	[7]
Bamboo	Combustion	Less moisture content, less hydroxyl/hydrophilic groups retained due to destruction, high energy density	Combustion systems	[8]
Oil palm waste	Torrefaction	Energy dense biochar, water resistivity, durability	Biofuels	[9]
Acacia wood	Torrefaction at 300°C	Partial degradation of cellulosic components, Specific functionalised biochar synthesised acts as a perfect substrate for bio-electrochemical reactions	Bio-electrochemical applications	**Present study**

Pyrolysis at high temperatures or low temperatures where oxygen is restricted increases the porosity and surface area, but lacks conducive redox-active functionalized biochar suitable for enhanced bio-electrochemical reaction (Data not shown). Thus, strategic methodology of torrefaction at 300°C is adopted for the present study. Torrefaction at particular temperature results in condensation of disordered carbon in the presence of oxygen results in conducive biochar with redox-active functionalization capable for faster electron transfer for long distance is developed and thereby increases the bio-electrochemical characteristics of the biochar.

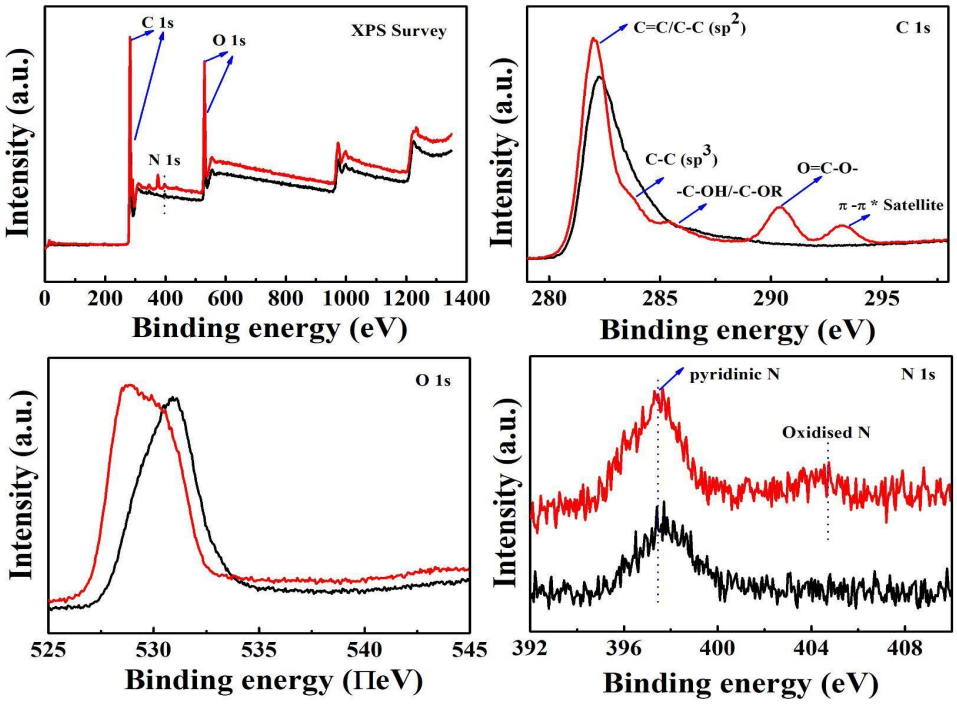

Figure S1: XPS spectra of (A) spectral survey, (B) C 1s, (C) O 1s, and (D) N 1s of BC (black) and CBC (red) showing its oxidation state and organic functionalization.

3.3. Methodology and strategies of anode preparation

3.3.1 Choice of perfect binder for biochar-based anode

Binders helps to keep coatings intact on any surface. Binders range from polysaccharides, polymers to chemicals. **Table S2** shows the different binders that used in the present study and their impact on nature of coating produced. Polyvinyl alcohol (PVA) is chosen as the coating agent which produces coatings that are conductive, hydrophilic nontoxic, biodegradable and

economical.[10,11] Moreover, PVA doesn't interfere with any of the reaction altering electrode bacterial interaction.

3.3.2 Addictive picked to enhance coating conductance

Activated charcoal is used to add on to conductivity of a coating. No significant enhancement in conductance is observed, moreover the coating exhibits lesser stability and irregularity on the surface.

Table S2: Choice of binder and nature of coating

Binder used	Nature of coating	Success/failure
PTFE	Hydrophobic, increased resistance, long term stability	X
PVDF	Super hydrophobic coating, increased resistance	X
Nafion resin	Not stable	X
Chitosan	Washed out	X
Agar	Washed out	X
Sodium Alginate	Washed out	X
Cellulose	Washed out	X
Starch	Washed out	X
PEI	Hydrophilic, bulged & peels off	X
PAN	White cast formed as a result of immediate solidification after melting, not feasible	X
PVA	Good, hydrophilic coating, long term stability, increases ionic conductance	✓

3.3.3. Standardization of casting approach

PVA has dense pores which makes biochar and bacterial interaction less accessible. To resolve this issue, calcium carbonate is added which in turn increases the porosity.[11] It is noted that, in spite of standardization of the casting protocol, the same procedure is not applicable to other biochar powders. The concentration of binder, amount of biochar and addictive vary depending on the wetting of different char with binder which attributes to differential surface area and porous nature of biochar particles.

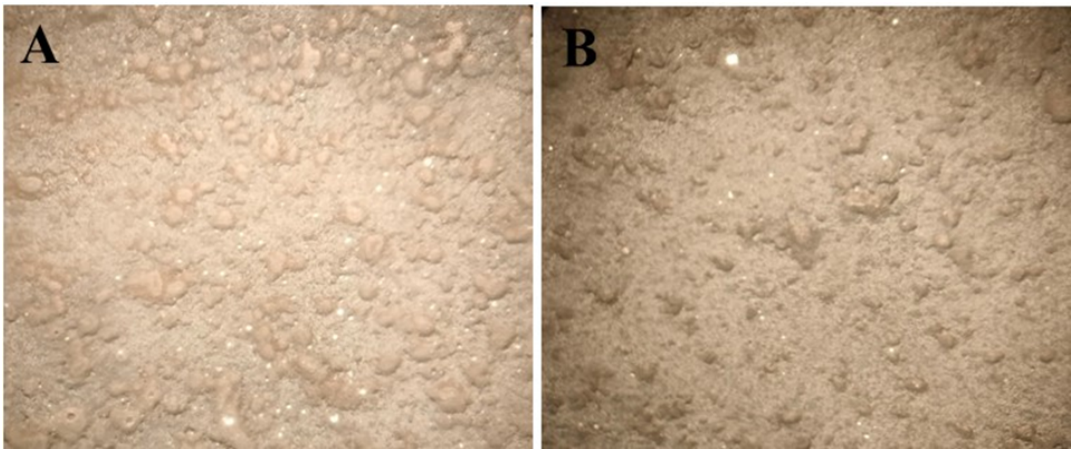

Figure S2: Optical images of (A) PVA/BC and (B) PVA/CBC coated electrodes

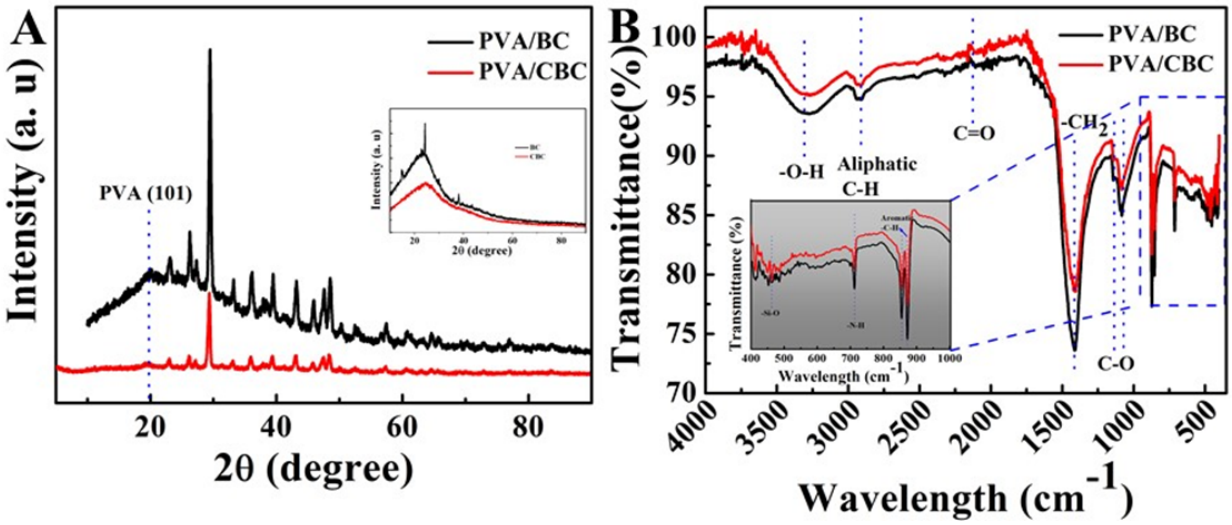

Figure S3: X-ray Diffraction analysis (A) and Fourier Transform Infrared spectra (B) of PVA/BC and PVA/CBC coated mild steel electrodes showing the retention of surface functional group with polymerization in PVA/BC, whereas amorphous carbon is transformed to crystalline with polymerization in PVA/CBC electrode.

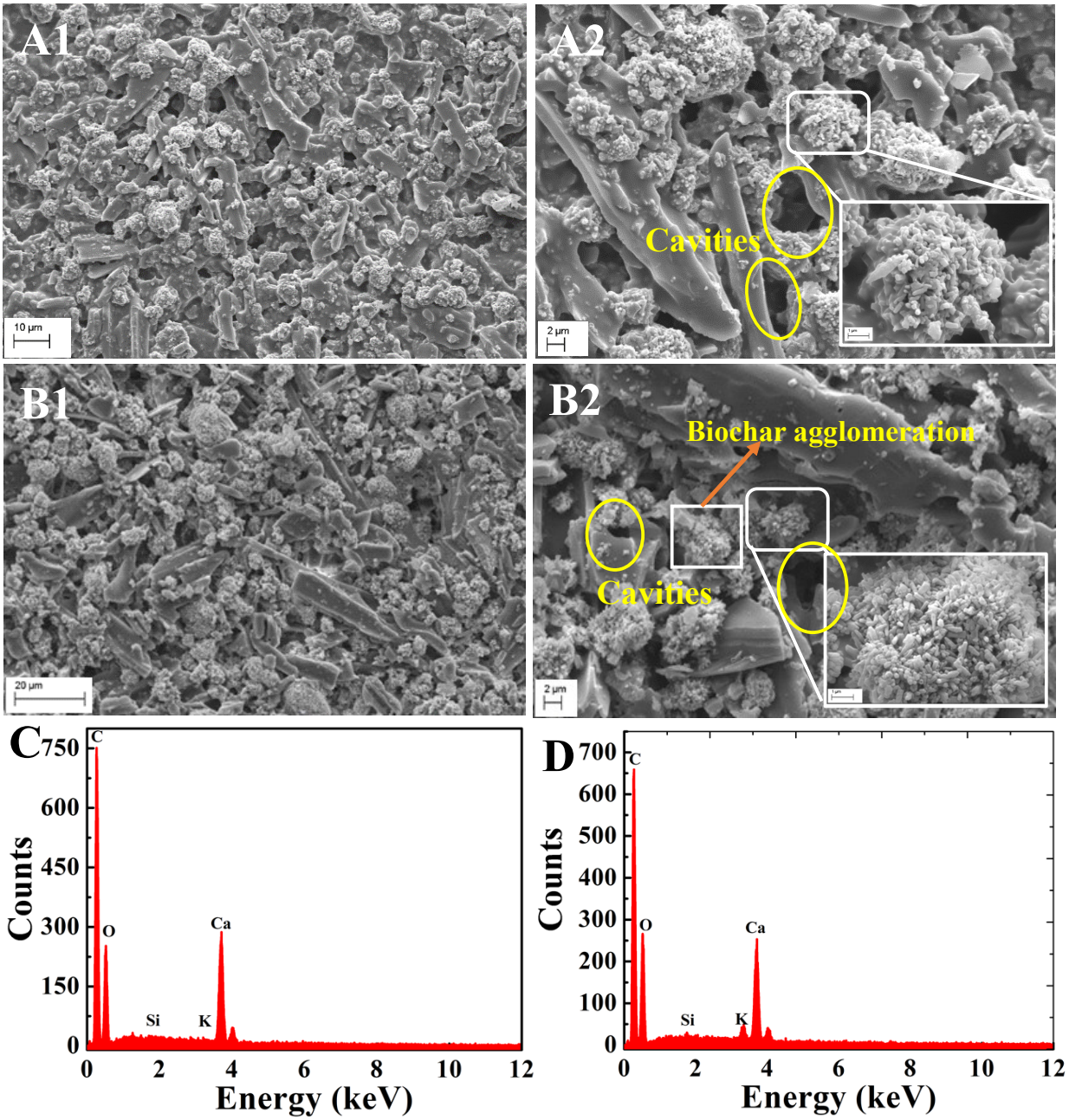

Figure S4: Morphological images of PVA/BC (A1 and A2) and PVA/CBC (B1 and B2) coated electrodes at different magnifications showing the compact arrangement of surface carbon and agglomeration of biochar with polymerization.

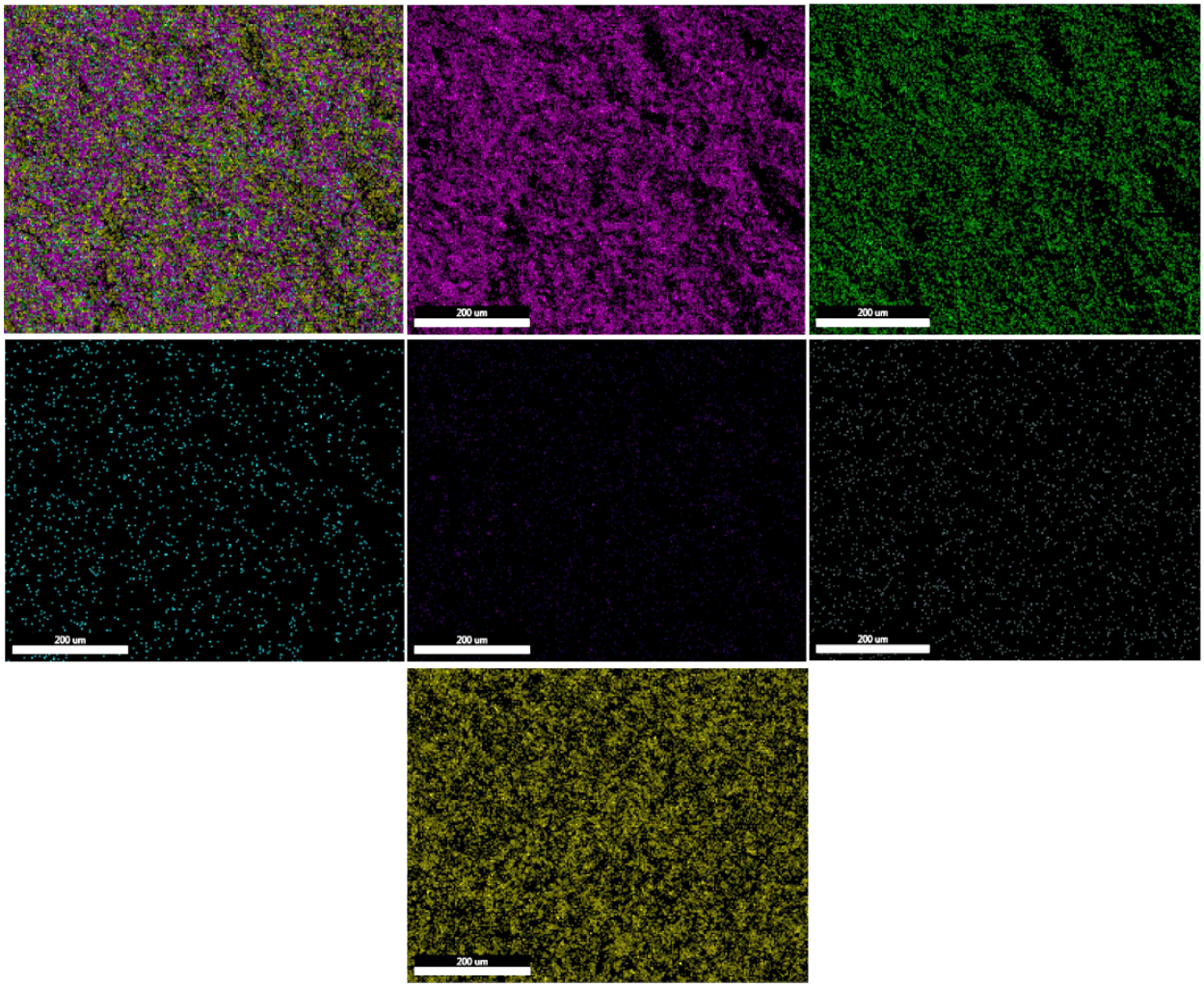

Figure S5: Elemental mapping revealing the distribution of (A) survey mapping, (B) C k, (C) O k, (D) N k, (E) Si k, (F) K k, and (G) Ca k on the surface of the PVA/BC electrode.

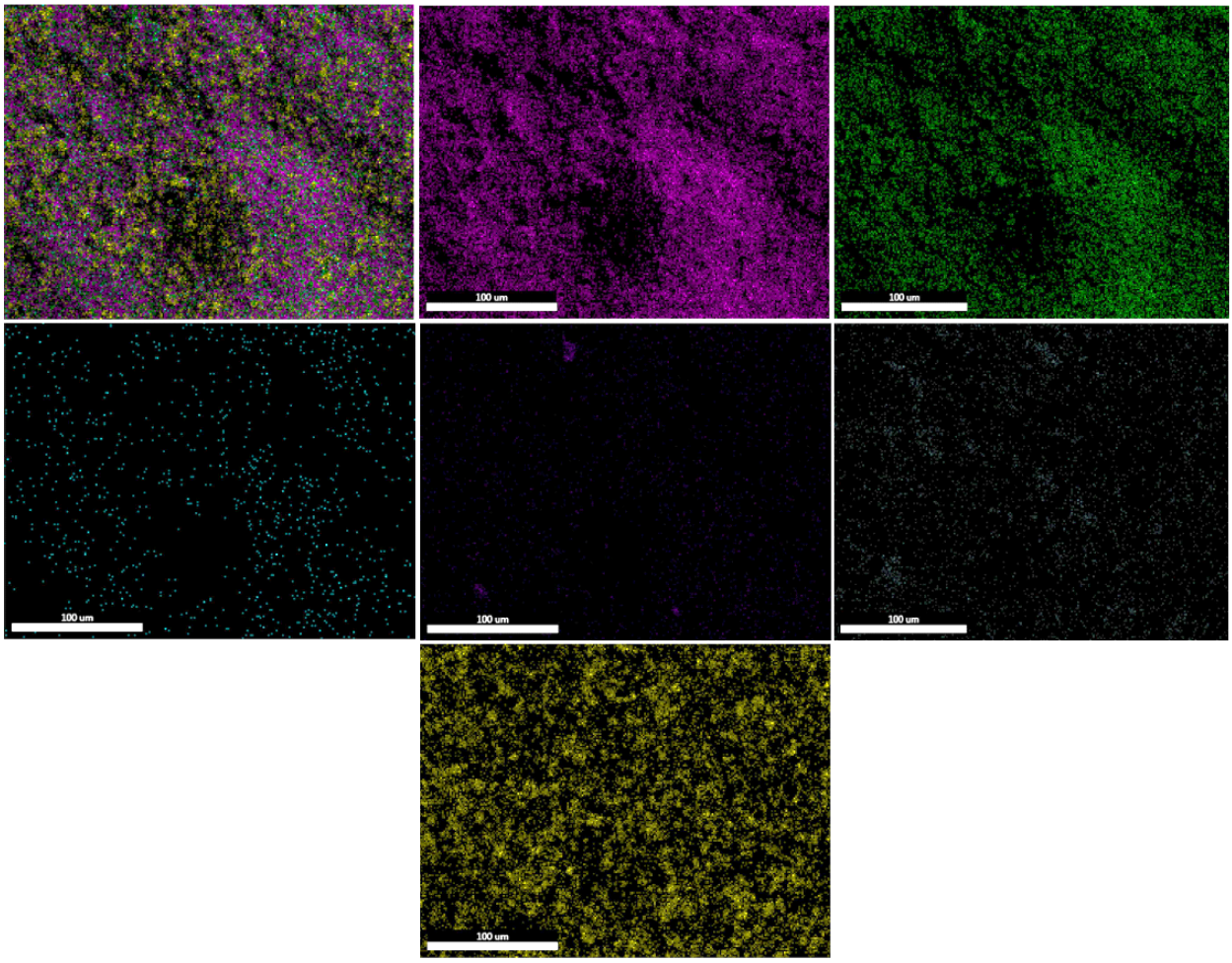

Figure S6: Elemental mapping revealing the distribution of (A) Survey mapping (B) C k, (C) O k, (D) N k, (E) Si k, (F) K k, and (G) Ca K on the surface of the PVA/CBC-1.0 electrode.

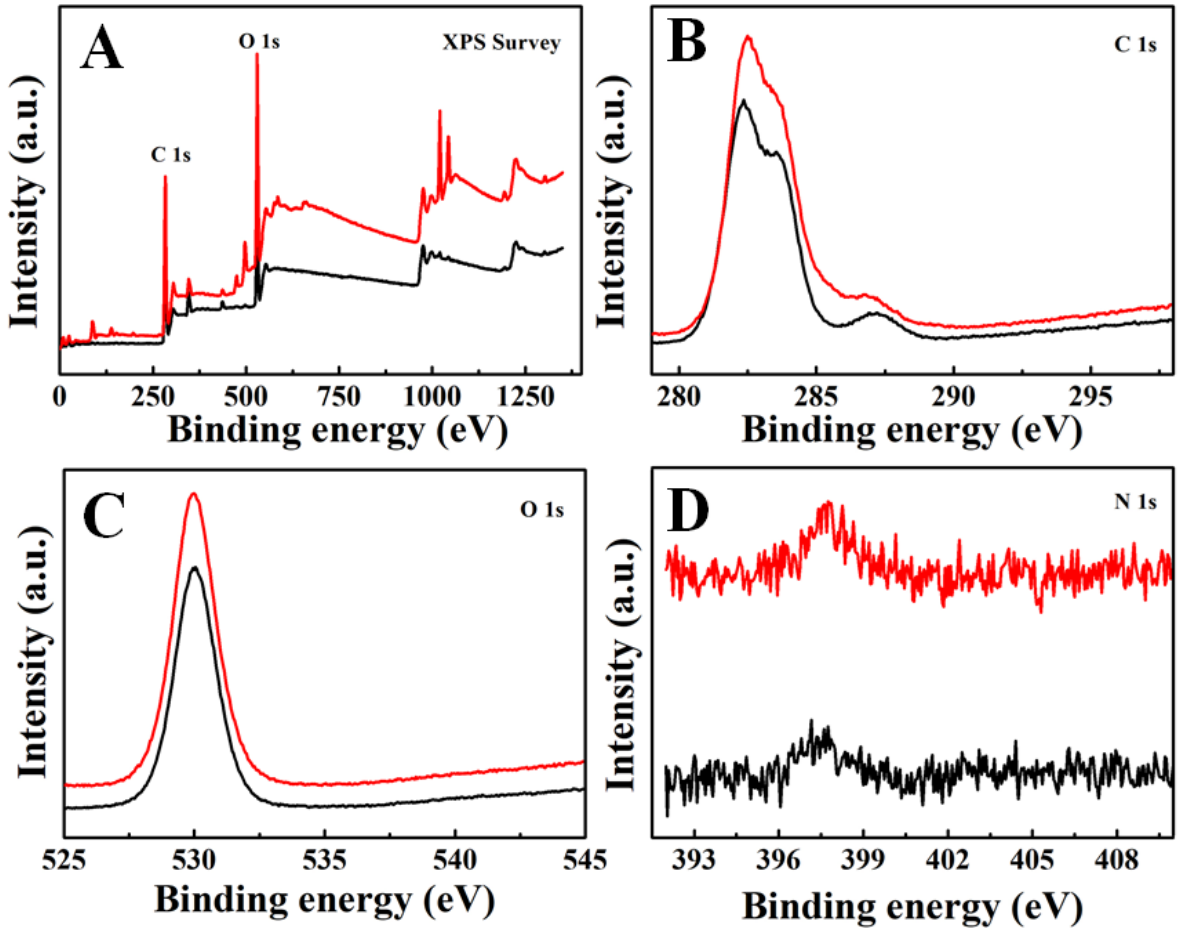

Figure S7: XPS spectra of (A) spectral survey, (B) C 1s, (C) O 1s, and (D) N 1s of PVA/BC (black) and PVA/CBC (red) electrodes showing the presence of multifunctional groups.

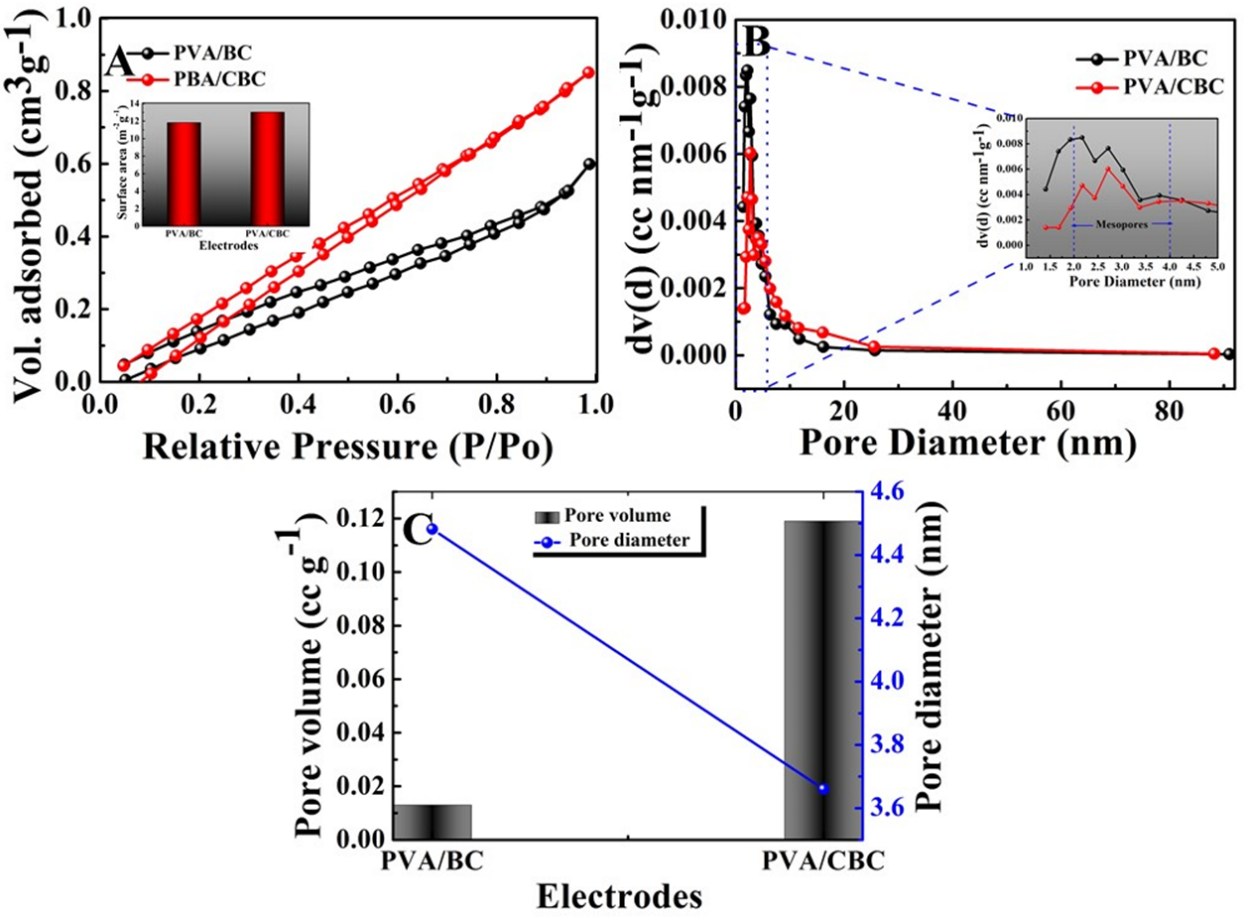

Figure S8: (A) N_2 adsorption/desorption isotherms (Inset: Comparison of surface area of PVA/BC and PVA/CBC electrodes), (B) pore size distribution and (C) comparison of pore volume and pore diameter of PVA/BC and PVA/CBC electrodes.

3.4. Standardization of reactivation methodology

Biofilm clogging is one of the major constrains that limits the performance of MFC. This could be overcome by reactivation of the anode surface at regular interval to sustain the performance. Researchers have adopted many methodologies including physical, chemical and biological methods for efficient reactivation. The feasibility of these methods for reactivation in current system is also evaluated as per the reported literatures. In the present study, the advantages of shear forces in biofilm have been explored with ultrasonic conjoined bubbling for reactivation. Physical reactivation is done by washing electrode thoroughly by aspirating distilled water to and fro through a micropipette. Sodium hypochlorite being a good surface disinfectant is employed as chemical method. Antimicrobial agent namely ciprofloxacin is used as biological treatment used

for the comparison study and the results are expressed as biofilm removal efficiency in percentage and illustrated in **Table S3**.

Table S3: Comparison of efficiency of different reactivation strategies

Method	Specifications	Biofilm removal efficacy (%)	Limitation/Advantage
Physical	Washing	31.70	● Not applicable to in-vivo system
Chemical	2.5% NaOCl	20.38	● Hinder the growth of existing bacteria in the anolyte raising overall cost
	5.0% NaOCl	26.36	
Biological	500µg/ml	16.65	
	1mg/ml	19.64	
Mechanical	US 5min	46.60	● Applicable to in-vivo system
	US 10min	68.23	
	B 5 min	60.45	● High removal efficiency
	B 10 min	62.01	
	USCB 5min	79.70	● No hindrance to bacterial metabolism
	USCB 10min	83.90	

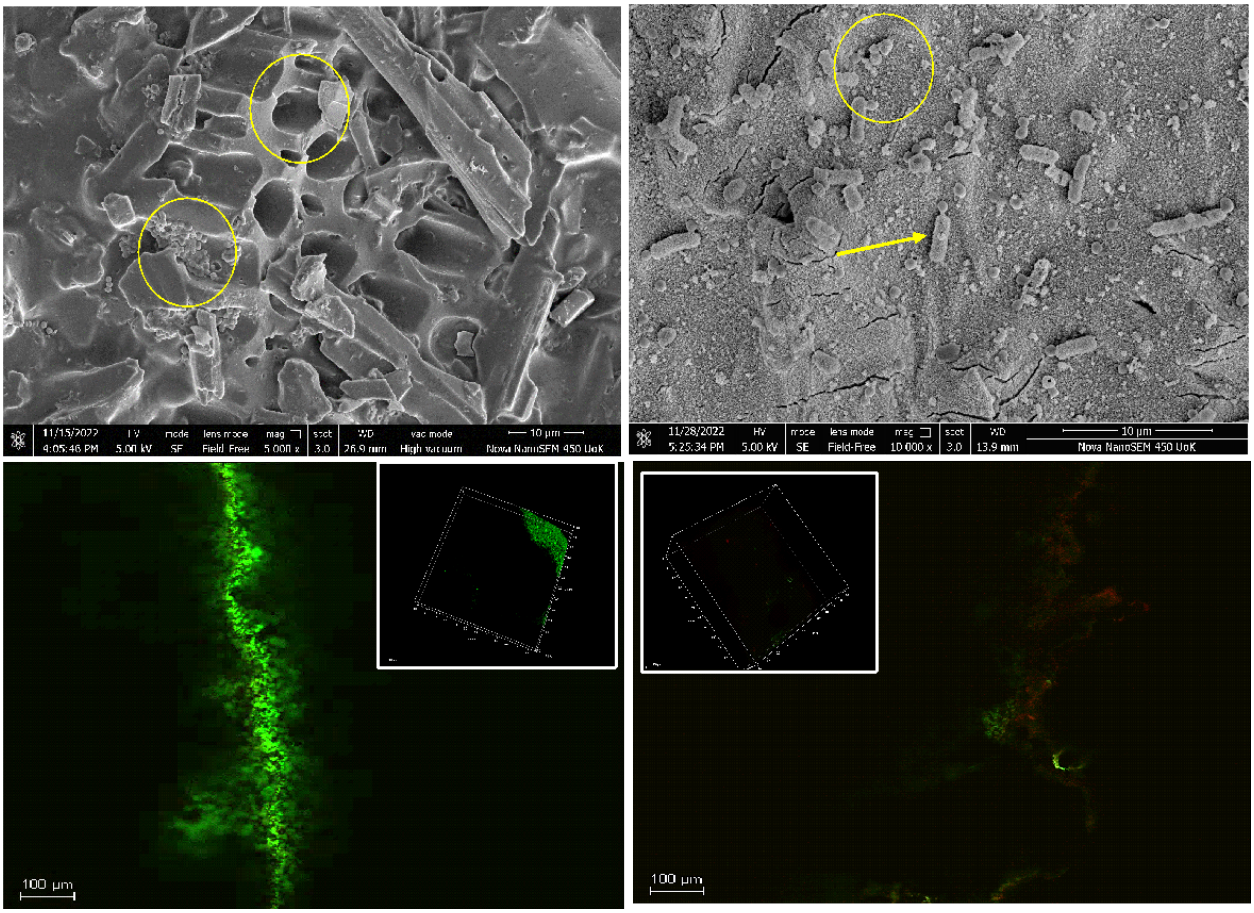

Figure S9: (A and B) FESEM images and (C and D) CLSM images (Inset: 3D images) of PVA/BC and PVA/CBC electrodes after reactivation of biochar-based electrodes by ultrasonication coupled bubbling revealing the retention of surface texture with high bacterial removal efficiency.

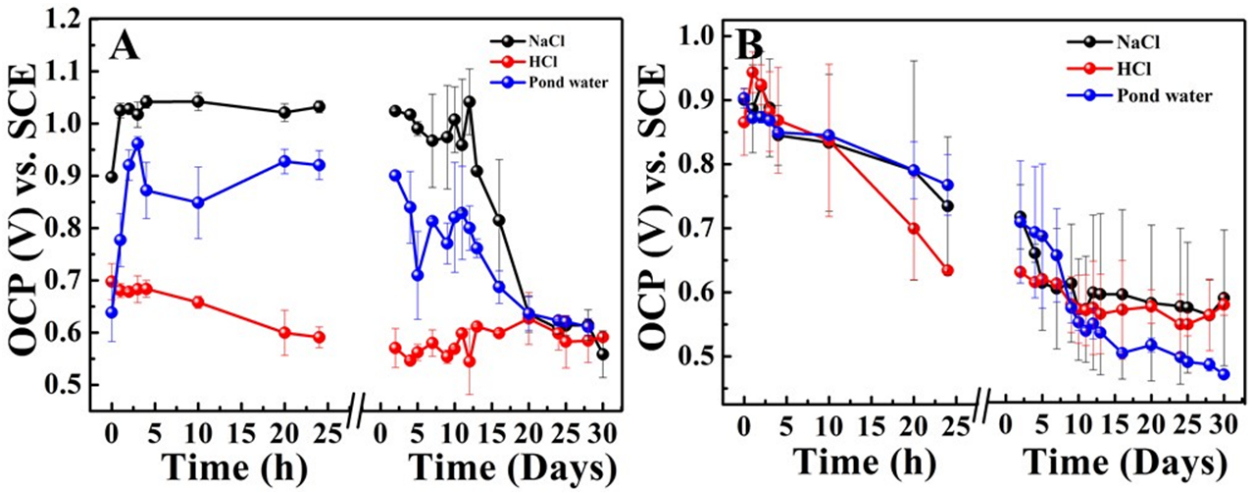

Figure S10: OCP curve of (A) PVA/BC and (B) PVA/CBC electrodes immersed in different aggressive environment namely 3.0M NaCl, 1.0 M HCl and pond water for a period of 30 days showing the stability of the coatings.

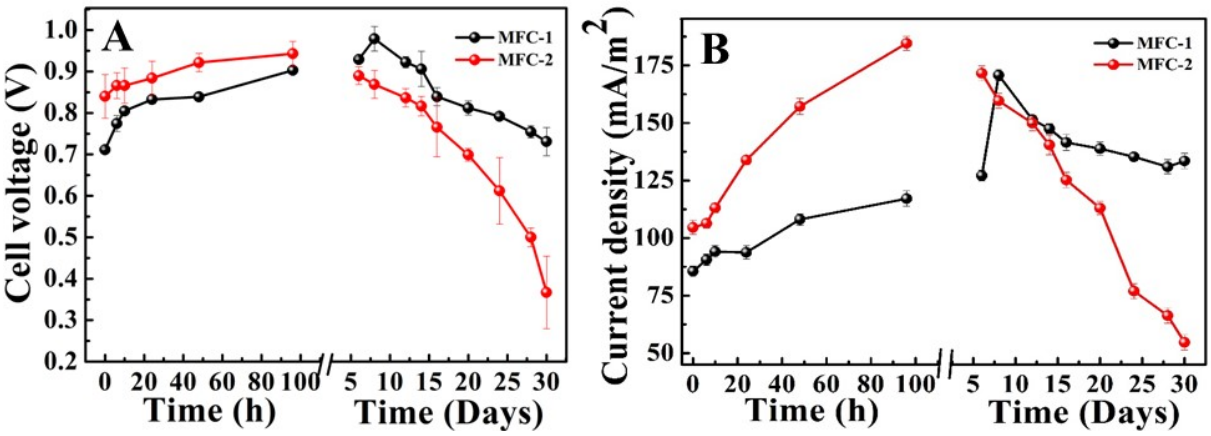

Figure S11: (A) Cell voltage and (B) Current density curve of reactivated biochar-based electrodes in MFC systems inoculated with pure culture of *S. putrefaciens* showing the reusability of the developed electrodes. MFC-1 and MFC-2 fabricated with PVA/BC and PVA/CBC-1.0 as bioanode respectively.

Table S4: Comparison of different literatures and reactivation strategies

Type	Anode	Performance		Reactivation strategies	Sustainability	Ref
		Current density	Power density			
SC-MFC	Graphite felt	-	105 mW/m^2	Replaced by fresh media	31 days	[12]
SC-MFC	Graphite brush	7.45 A/m^2	1330 mW m^2	Replaced by fresh media	15 days	[13]
DC-MFC	Carbon felt	-	6.1 W/m^3	Moderate shear stress (9.34 mPa)	40 days	[14]
DC-MFC	Carbon Brush	-	3920 mW/m^3	Ultrasonics driven biofilm removal	6 days	[15]
DC-MFC	Carbon felt	233 mA/m^2	3.1 ± 0.6 W/m^3	Replaced by fresh media	7 days	[16]
DC-MFC	Carbon fiber mesh	4.96 mA/cm^2	399.3 ± 7.4 mW/m^2	Replaced by fresh media	-	[17]
DC-MFC	Carbon cloth	150mA/m^2	250mW/m^2	Replaced by fresh media	4-5 days	[18]
DC-MFC	Biochar	253.5mA/m^2	349.9 mW/m^2	USCB	15 days	Present study

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