Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Materials

Natural tea polyphenol functionalized graphene anode for simultaneous power production and degradation of methyl orange dye in microbial fuel cells

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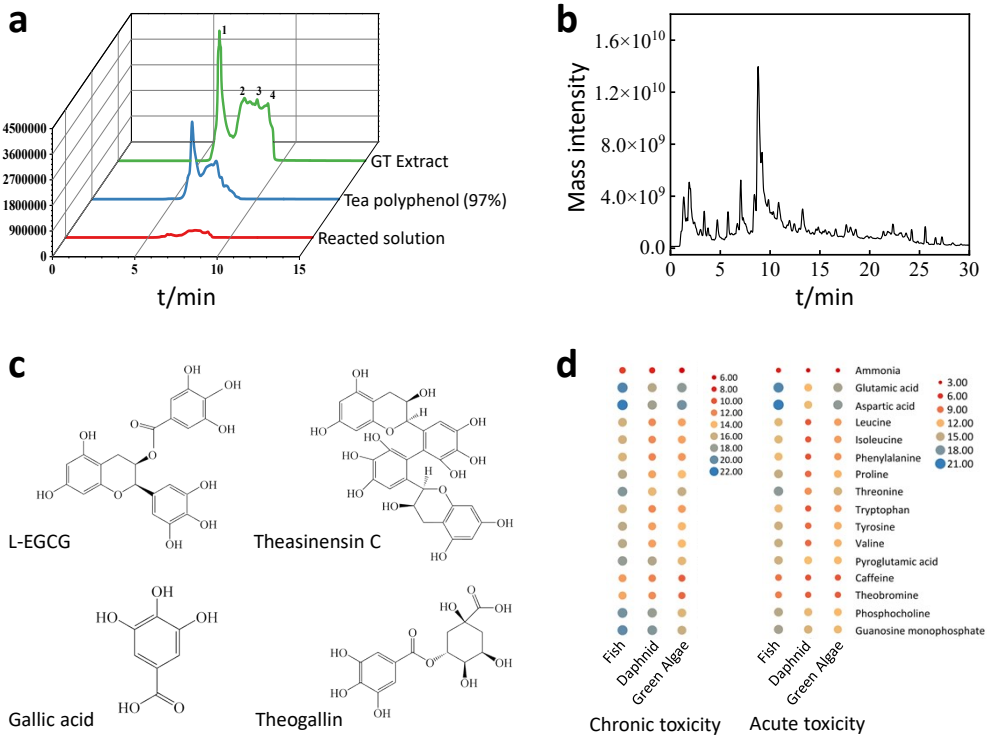

Fig. S1 (a) HPLC analysis diagram (1. L-EGCG; 2. Theasinensin C; 3. Gallic acid; 4. Theogallin); (b) Total ion current (TIC) chromatogram of the green tea extracts by UHPLC-Q-Exactive/MS; (c) Molecular structural formulae of the four main components of green tea extracts; (d) Toxicological data pertains to the impurities found in green tea extracts.

Table S1. Information on compounds identified in green tea extracts.

Compound	Retention time	Mass-to-charge ratio		Compound name	Formula
		Theoretical	Measured		
Tea polyphenols	10.823	290.0785	291.0858	Epicatechin	$C_{15}H_{14}O_6$
	13.608	306.0735	289.0702	(-)-Epigallocatechin	$C_{15}H_{14}O_7$
	9.625	458.0844	459.0917	Epigallocatechin gallate	$C_{22}H_{18}O_{11}$
	6.268	306.0734	307.0807	Gallocatechin	$C_{15}H_{14}O_7$
	8.487	290.0782	291.08548	Catechin	$C_{15}H_{14}O_6$
	3.783	610.1321	611.1393	Theasinensin C	$C_{30}H_{26}O_{14}$
	7.935	730.1533	731.1606	Procyanidin B2 3'-	$C_{37}H_{30}O_{16}$

				O-gallate	
	2.456	170.0213	171.0286	Gallic acid	$C_7H_6O_5$
	1.366	192.0631	193.0704	Quinic acid	$C_7H_{12}O_6$
	4.036	344.0739	345.0811	Theogallin	$C_{14}H_{16}O_{10}$
	10.515	336.0818	337.0891	Chlorogenic acid	$C_{16}H_{18}O_9$
Amino acids	1.268	147.0529	180.0863	Glutamic acid	$C_5H_9NO_4$
	1.233	133.0374	134.0447	Aspartic acid	$C_4H_7NO_4$
	23.934	131.0946	132.1019	Leucine	$C_6H_{13}NO_2$
	2.99	131.0945	132.1018	Isoleucine	$C_6H_{13}NO_2$
	7.842	165.0789	166.0862	Phenylalanine	$C_9H_{11}NO_2$
	3.522	115.0634	116.0707	Proline	$C_5H_9NO_2$
	2.107	119.0583	120.0656	Threonine	$C_4H_9NO_3$
	6.673	204.0896	188.0703	Tryptophan	$C_{11}H_{12}N_2O_2$
	7.666	181.0737	182.0811	Tyrosine	$C_9H_{11}NO_3$
	1.762	117.0791	118.0863	Valine	$C_5H_{11}NO_2$
	5.609	129.0426	130.0499	Pyroglutamic acid	$C_5H_7NO_3$
Other	5.386	194.08024	195.08752	Caffeine	$C_8H_{10}N_4O_2$
	7.28	180.06447	181.07175	Theobromine	$C_7H_8N_4O_2$
	4.061	183.0656	184.07321	Phosphocholine	$C_5H_{14}NO_4P$
	1.754	363.0573	364.06458	Guanosine monophosphate	$C_{10}H_{14}N_5O_8P$

Text1: The components of the green tea extracts were determined using a high performance liquid chromatography system (HPLC，LC-2030 Plus, Shimadzu). A Shimadzu, shim-pack packed column with dimensions of 4.6*250 mm was utilized for the chromatographic separation. The mobile phase consisted of two components: mobile phase A, which consisted of a methanol- 0.01 % phosphoric acid solution, and mobile phase B, which was pure methanol. The separation was carried out using a

gradient elution program at a flow rate of 1 mL·min^{-1}. The column temperature was maintained at 30℃, and an injection volume of 20 μL was used. The detection of the components was performed at an ultraviolet wavelength of 278 nm. The standard solution used for calibration purposes was prepared using Tea Polyphenol Reagent (97%; Shanghai Macklin Biochemical Co., Ltd.).

The HPLC analysis in **Fig. S1** demonstrates that the green tea extracts is abundant in tea polyphenols, particularly catechins, as compared to the tea polyphenol standard (97%). Based on previous research, it has been deduced that green tea extracts contains various constituents, including L-EGCG, theasinensin C, gallic acid, theogallin [1,2], with L-EGCG being among the primary compounds identified. Following the reaction of reducing graphene oxide, the green tea extracts experienced a significant decrease in tea polyphenol content. Particularly, the L-EGCG content exhibited a more pronounced decrease, indicating that not only did green tea extracts contribute to the reduction process of graphene oxide, but the L-EGCG played a crucial role in this reduction process. By considering the changes observed in functional groups during infrared analysis, it can be inferred that the decrease in tea polyphenol content may be associated with the binding of biomolecules with RGO during the reduction of graphene oxide. The extraction process used water as the extraction solvent. The remaining water insoluble components are dehydrated to obtain green tea residue. As reported in the literature, the components in green tea residue are also rich in polysaccharides, proteins and fat-soluble vitamins, etc. Assuming the tea residue can be further processed into reusable values, it would not only protect the environment but also beneficially contribute to the high-value utilisation of green tea residue [3,4].

Text2: The determination was conducted using ultra performance liquid chromatography and quadrupole-electrostatic orbitrap mass spectrometry (UHPLC-Q-Exactive/MS, Thermo Scientific UltiMate 3000 HPLC). Chromatographic conditions included a mobile phase A consisting of 0.1% formic acid in water, and phase B

comprising methanol. The injection volume was 3 μL, the flow rate was 0.4 $mL \cdot min^{-1}$, and the column temperature was maintained at 40℃. The mobile phase was eluted using a linear gradient. For mass spectrometry, electrospray ionization (ESI) was employed with scanning in positive ion mode. The capillary voltage was set at 3,500 V, and the drying gas temperature and flow rate were 300°C and 8 $L \cdot min^{-1}$, respectively. The nebulizing gas pressure was maintained at 35 psi, while the sheath gas temperature and flow rate were 300°C and 11 $L \cdot min^{-1}$, respectively. Mass spectrometry scanning was conducted over a mass-to-charge ratio (m/z) range of 100~1,000. In the green tea extracts, the identified compounds are presented in **Table S1**. Among these, amino acid compounds were identified as the main components of impurities in green tea extracts.

The purity of the tea polyphenols was determined to be 27.64% using the ferrous tartrate method [5]. The toxicological data of impurities in green tea extracts are depicted in **Fig. S1 (d)**. In the toxicological data for the reducing agent ammonia, high toxicity to fish, daphnid, and green algae was observed even at lower concentrations, indicating significant toxicity. Compared to ammonia, amino acids exhibited better organism survival at higher concentrations, indicating lower toxicity. This suggests that the green tea extracts used as a reducing agent can effectively reduce the use of toxic reagents in the preparation process and minimize environmental side effects. Additionally, it is less toxic to microorganisms and promotes excellent biocompatibility of the anode.

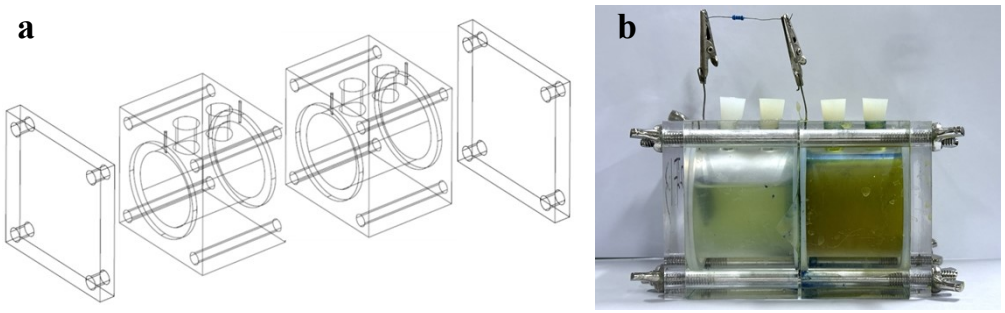

Fig. S2 (a) MFC structure design sketch; (b) Physical drawing of MFC device.

Table S2. Concentrations of vitamins in solution added to inoculum.

Composition	$mg \cdot L^{-1}$
Biotin	2
Folic acid	2
Pyridoxine HCl	10
Riboflavin	5
Thiamin	5
Nicotinic acid	5
Pantothenic acid	5
B-12	0.1
P-aminobenzoic acid	5
Thioctic acid	5

Table S3. Concentrations of trace element solution in solution added to inoculum.

Composition	$g \cdot L^{-1}$
NTA	1.5
$MgSO_4$	3
$MnSO_4 \cdot H_2O$	0.5
NaCl	1
$FeSO_4 \cdot 7H_2O$	0.1
$CaCl_2 \cdot 2H_2O$	0.1
$CoCl_2 \cdot 6H_2O$	0.1
$ZnCl_2$	0.13
$CuSO_4 \cdot 5H_2O$	0.01
$AlK(SO_4)_2 \cdot 12H_2O$	0.01
H_3BO_3	0.01
Na_2MoO_4	0.025
$NiCl_2 \cdot 6H_2O$	0.024
$Na_2WO_4 \cdot 2H_2O$	0.025

Table S4. The gradient elution procedure

Samples	$R_s(\Omega)$	$R_{ct}(\Omega)$
CC	43.3	235.4
RGO@CC	50.2	49.5
TP-RGO@CC	52.0	14.6

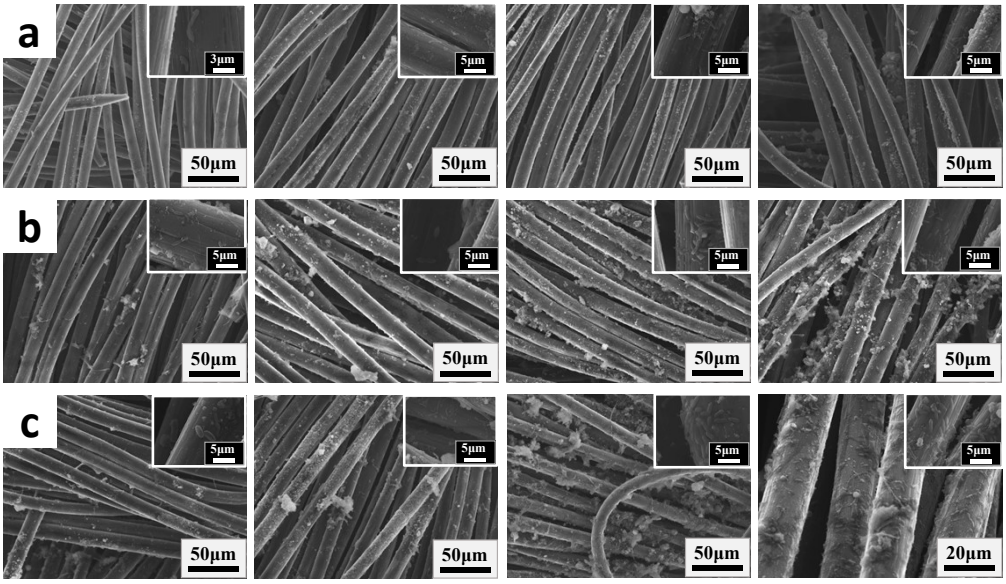

Fig. S3 SEM images of biofilm growth on (a) CC, (b) RGO@CC and (c) TP-RGO@CC anodes (Each set of graphs from left to right is 1, 3, 7, 14 days).

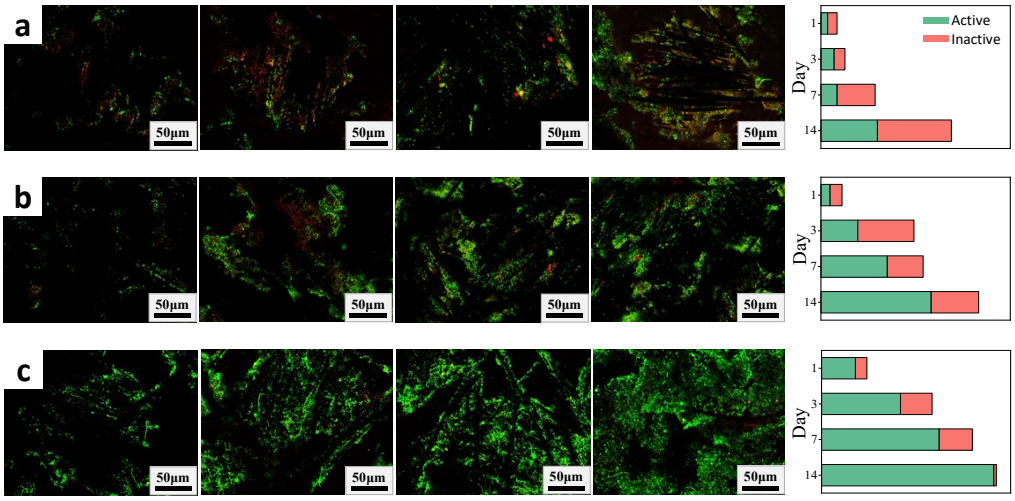

Fig. S4 CLSM images and fluorescent intensity statistics of biofilm growth on (a) CC, (b) RGO@CC and (c) TP-RGO@CC anodes (Each set of graphs from left to right is 1, 3, 7, 14 days).

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