SUPPORTING INFORMATION

Pd Nanoparticles Supported On Reduced Graphene-*E. Coli* Hybrid With Enhanced Crystallinity In Bacterial Biomass

Rachel E. Priestley[†], Alexander Mansfield[†], Joshua Bye[†], Kevin Deplanche[‡], Ana B. Jorge[§], Dan Brett[§], Lynne E. Macaskie[‡], Surbhi Sharma*[†]

[†]Centre for Hydrogen and Fuel Cell Research, School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

[‡]Unit of Functional Bionanomaterials, School of Bioscience, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

[§]UCL Chemical Engineering Department, Electrochemical Innovation Lab, Torrington Place, WC1E 7JE, London, UK

*Address correspondence to: Dr S. Sharma; *Email address:* s.sharma.1@bham.ac.uk;

Preparation of bacterial culture

E. coli strain MC4100 from a stock maintained at -80 °C in 75% glycerol was used to inoculate nutrient broth no. 2 (Oxoid, 5 ml) and cultured aerobically (37 °C, 16 hours). 2 ml was then used to inoculate (under oxygen-free nitrogen (OFN)) five sealed 200ml bottles of minimal media (MM; per litre: 12 g Tris buffer; 0.62 g KCl; 0.96 g $(NH_4)_2HPO_4$; 0.063 g $MgSO_4.7H_2O$; 0.00033 g $FeSO_4.7H_2O$; 4 g sodium fumarate; 5ml glycerol; adjusted to pH 7.2 ± 0.2 using HCl. Sterile glucose (1 M; 20 ml) was added when inoculating. Growth of *E. coli* was anaerobic (37 °C, 48 hours). During the exponential growth phase (OD_{600} of between 0.4 and 0.6), complete 200ml inocula were transferred under oxygen-free nitrogen into 2 litres of the same media in sealed bottles and cultured for 4-6 hours (37 °C), harvesting during the exponential growth phase. The harvested culture was washed 3 times using 20 mM MOPS-NaOH buffer (pH 7.0) and then re-suspended in the same buffer to a concentration of approximately 30 mg dry weight/ml. Cell concentration was determined (OD_{600}) using a UV/Vis spectrometer and the biomass concentration was estimated by conversion to dry cell weight via a previously determined calibration (OD600=1 for 0.482 mg/ml).

Preparation of graphene oxide (GO)

The GO was prepared by the modified Hummers' method, described in detail by Ganguly et al. (2011) and was diluted from its initial aqueous solution of 0.0047 g/ml for further use.

Preparation of palladium (II) salt solution

A 2 mM solution of palladium (II) salt was made by dissolving an appropriate amount of sodium tetrachloropalladate (Na_2PdCl_4) in 0.01 M HNO_3. The pH was adjusted to pH = 2 ± 0.2 using NaOH and HNO_3 solution; this pH had been previously reported to be optimal for production of active catalyst using whole cells (Yong et al., 2002).

Table S1
List of all control samples and their acronyms used in this study

Control Sample	**Acronym**
Without *E.Coli* cells	
GO, H_2	GO(H)
GO, formate	GO(F)
Pd salt, H_2	Pd(H)
Pd salt, formate	Pd(F)
GO, Pd salt, H_2	GO/Pd(H)
GO, Pd salt, formate	GO/Pd(F)
GO only	GO
Without any electron donor	
E.coli, GO	B-rGO
E.coli, Pd salt	B-Pd
E.coli, GO, Pd salt	B-rGO/Pd
E. coli only	*E.coli*

Preparation of samples for analysis
A small amount of material (a few mg) was prepared for TEM analysis by centrifuging and resuspending in 2.5% (w/v) glutaraldehyde buffer at 4 °C. Cells were then sectioned and placed on copper grids before staining with uranyl acetate and lead citrate by standard methods. Material was also prepared for TEM by diluting the resulting solution and dropping a thin layer onto a copper grid. The two methods of preparing for TEM gave different types of images (figure 1). The biomass supported reduced palladium and/or graphene oxide was collected by centrifugation and washed three times in distilled water and once in acetone before being left to dry overnight, and ground to a fine powder. This was then used for analysis by powder x-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA). Some was suspended in distilled water and sonicated intermittently in an Ultrawave U300 ultrasonic bath at 10kW in cold water (replaced every 10 minutes to prevent heat damage) for ~1 h, and then drop-dried onto silicon wafers to produce thin films for x-ray photoelectron spectroscopy (XPS) and Raman spectroscopy. No effect is expected from sonication other than to evenly distribute the samples in suspension. The same processes were used on the control samples. For a further control study, a sample of *E. coli* was collected by centrifugation and left to dry as a powder, and another sample was drop-dried from solution onto a silicon wafer to produce a thin film. The same procedure was used for GO-only samples.

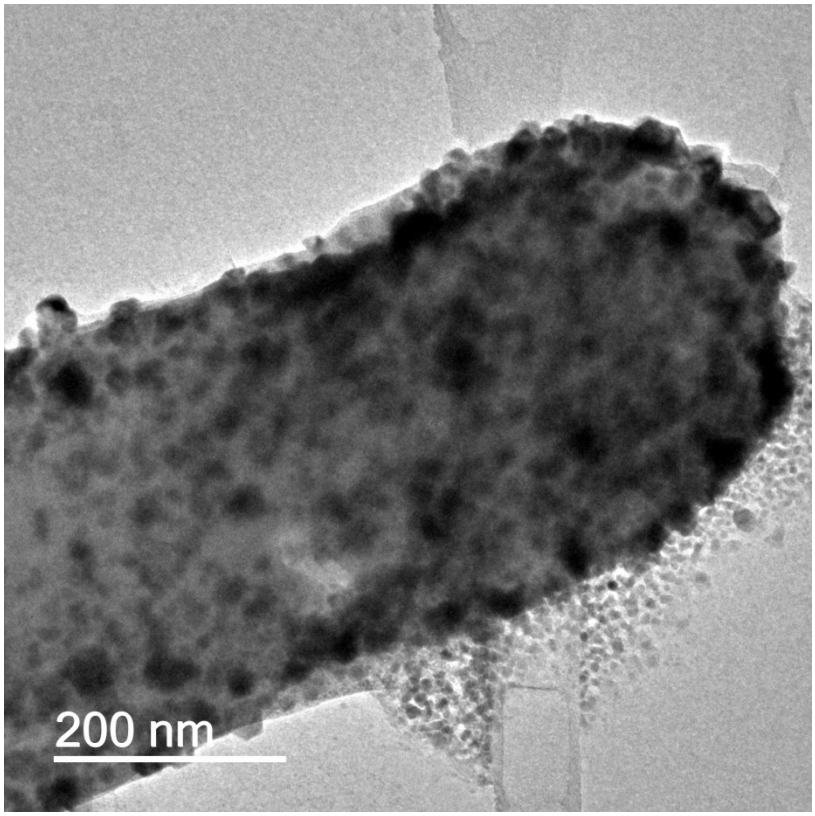


Figure S1a: High resolution image of Figure 2(c)

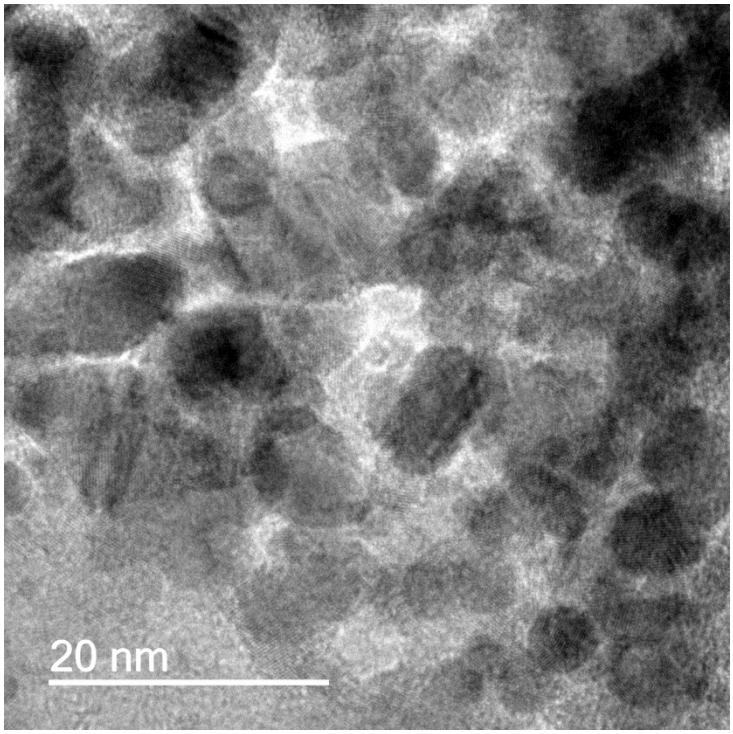


Figure S1b: High resolution image of B-rGO/Pd(F) showing PDNPs on rGO sheet

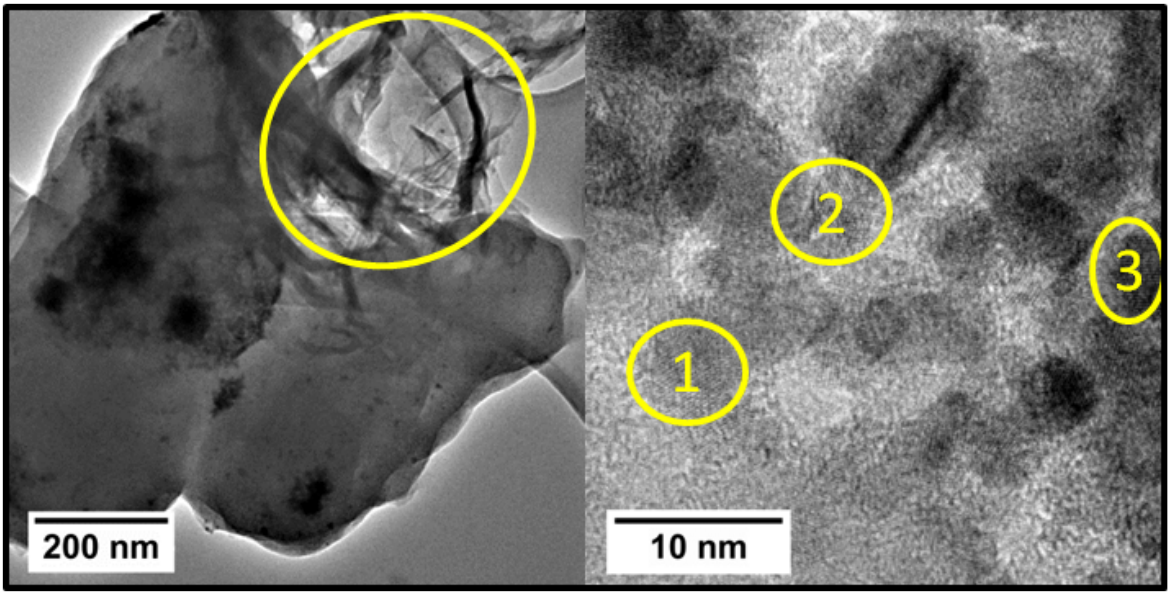


Figure S1c: TEM images of samples produced by drop drying samples of B-rGO/Pd(H): (left) rGO sheets crumpled and associating with cell surfaces; (right) High resolution image showing lattice fringes in PdNPs measuring 5-10 nm.

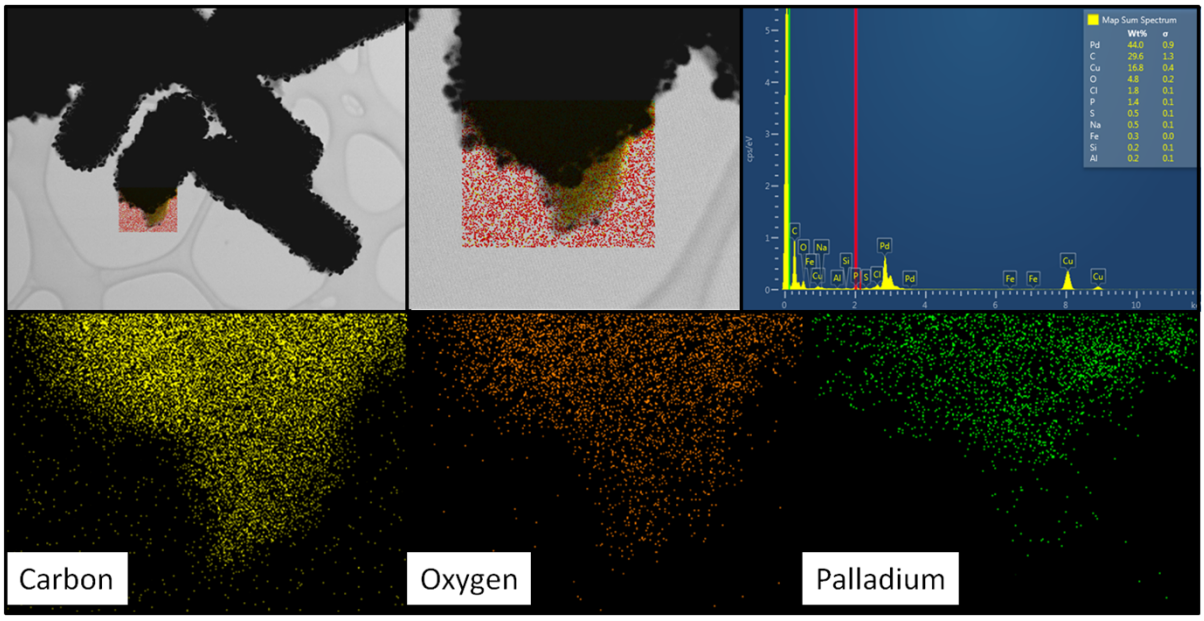
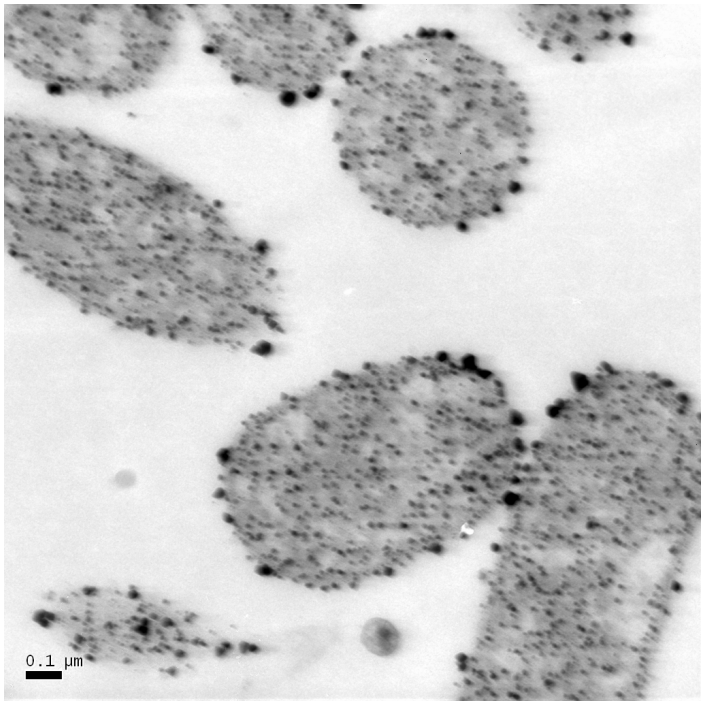


Figure S2: EDX on a section including a bacterial cell and rGO sheet.



TEM image of bacterial sample prepared without U/Pb staining.

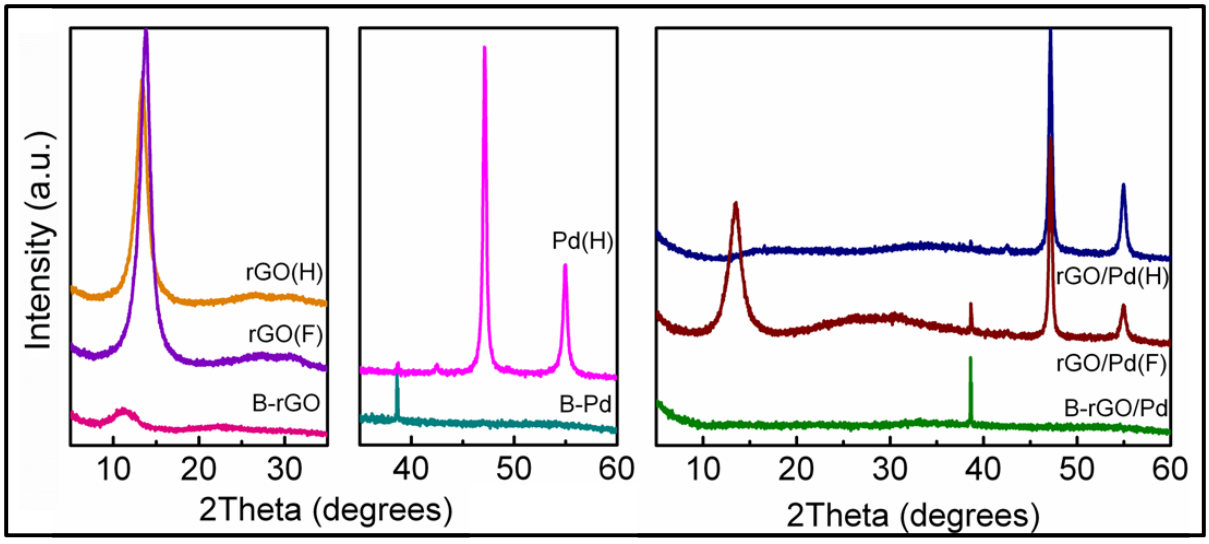


Figure S3: XRD analysis of control samples

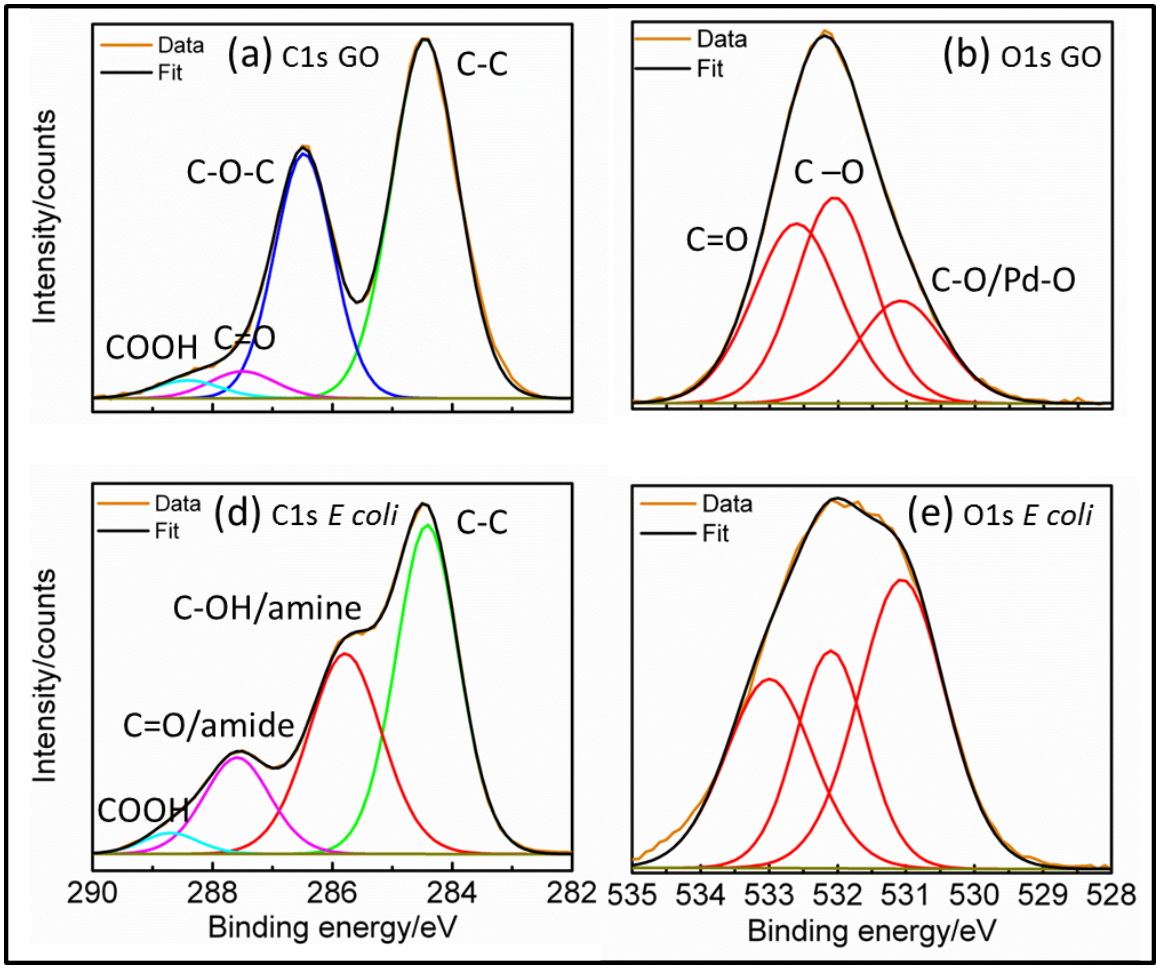


Figure S4 a,b: High resolution C1s and O1s XPS spectra for GO alone; c,d: High resolution C1s and O1s XPS spectra for E coli alone.

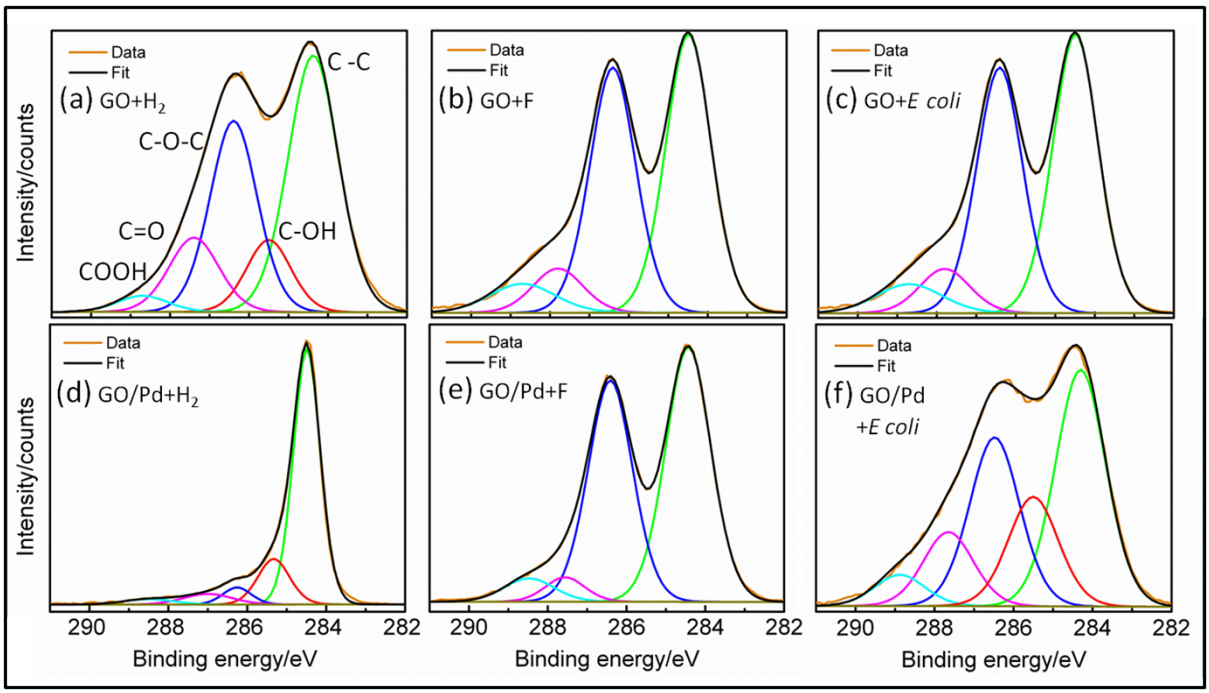


Figure S5: High resolution C1s XPS spectra for control samples

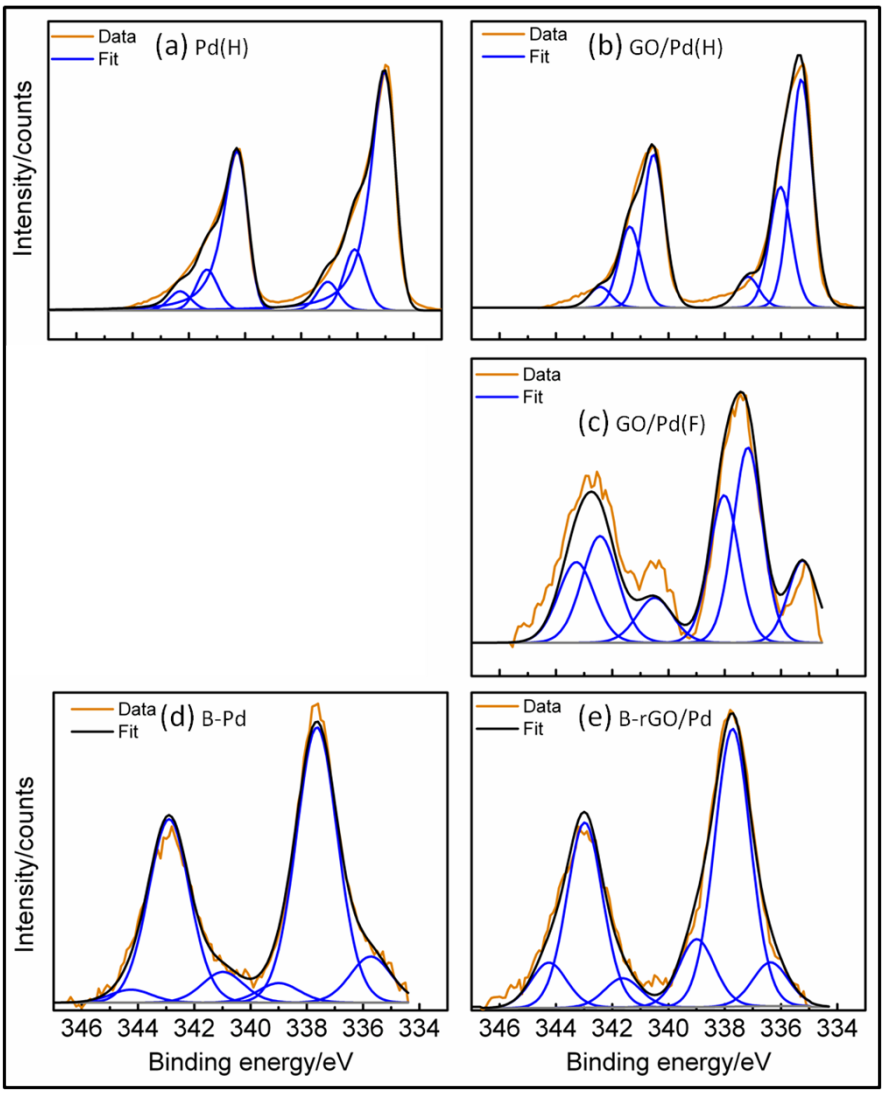


Figure S6: High resolution $Pd3d_{5/2}$ XPS spectra for control samples.

Component , % \ Sample	B-rGO(H)	B-rGO(F)	B-Pd(H)	B-Pd(F)	B-rGO/Pd(H)	B-rGO/Pd(F)	GO	*E. coli*
C1s								
C-C	57.9	54.4	60.1	62.2	57.2	53.1	58.5	48.4
C-OH hydroxyl	16.5	15.7	16.9	19.2	18.1	19.5	-	32.4
C-O-C epoxide	12.3	14.0	11.3	7.7	11.8	13.2	35.4	-
C=O carbonyl	9.3	13.9	8.2	8.0	8.5	10.2	4.6	15.4
COOH carboxyl	4.0	2.1	3.5	2.9	4.4	4.0	2.4	3.8
$Pd3d^{5/2}$								
Pd(0)	-	-	86.9	93.9	88.2	88.5	-	-
Pd(II)	-	-	9.1	6.1	9.9	8.0	-	-
Satellite peak	-	-	4.0	0.0	1.9	3.5	-	-
O1s								
C=O	40.6	51.3	34.8	37.6	36.0	40.0	20.5	46.6
C-O/Pd3p3/2	29.1	25.1	40.2	30.3	33.8	30.4	43.8	25.3
C-O	30.3	30.2	25.0	32.1	28.9	29.6	35.6	28.2

Table S1: Average percentages of various carbon bonds, palladium oxidation states and oxygen bonds observed in XPS analysis

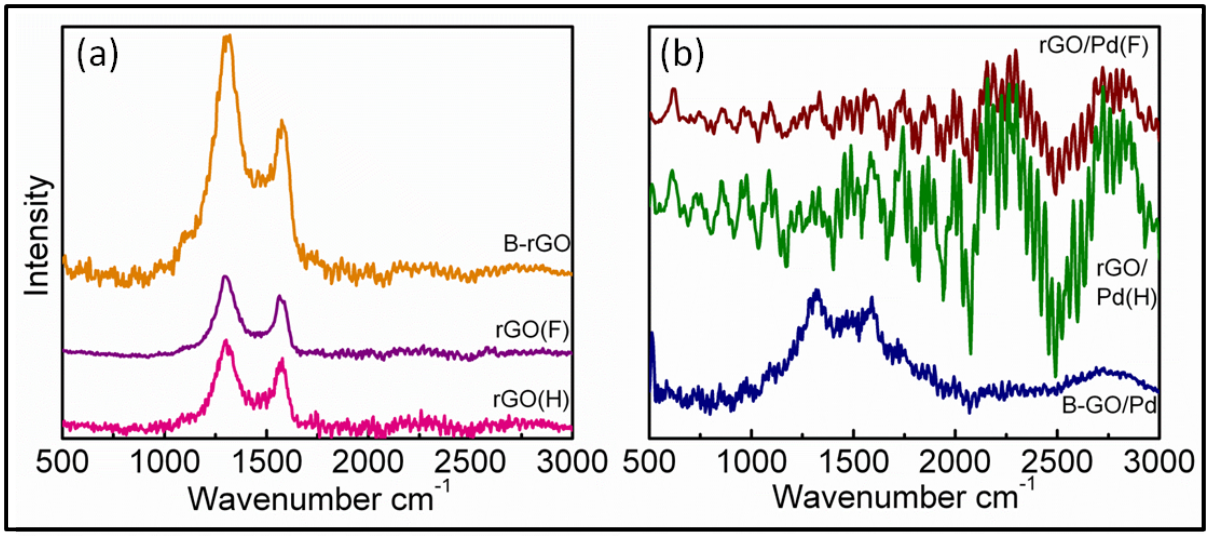


Figure S7: Raman spectra for control samples