Supporting information

Natural Eumelanin-Based Porous N-Doped Carbon as Active Bio-catalyst for Base- and Initiator-Free Aerobic Oxidation of Olefins and Alkyl Aromatic Hydrocarbons

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1. Physicochemical methods

The structures and crystal phases of the adsorbents were investigated using a Powder X-ray diffractometer (XRD) Philips X'pert MPD system for powder X-ray Diffractometer with Cu-Kα radiation of 1.54056 Å wavelength and a Ni filter. Fourier transform infrared (FT-IR) spectra were recorded on a Perkin-Elmer GX-FTIR spectrophotometer with KBr pellets in the wavelength range of 400-4000 cm^{-1}. UV/VIS spectra of melanin were acquired by the dissolution of melanin in distilled water and scanned in the wavelength range of 200 to 800 nm in a Thermo Scientific Evolution 201 UV-Vis spectrophotometer. The presence of free radicals in purified melanin was detected by the MS-5000 benchtop ESR/EPR spectrometer (Magnettech). For this, 2 mg melanin powder was added into a thin-walled glass tube and EPR signals were recorded. The parameters used to acquire the spectra were as follows; modulation amplitude: 0.16 mT; modulation frequency: 100 kHz; center field: 325 mT; sweep width: 25 mT; sweep time: 2 min; microwave frequency: 9.1 GHz; microwave power: 0.1 mW; at room temperature. Raman spectroscopic analysis of the catalyst was done with 532 nm argon source laser excitation with 10 mW power in the range of 1000-2000 cm^{-1}. The surface microscopic features of the sample were investigated using Field Emission Scanning Electron Microscopy (FE-SEM, JSM-7100F) equipped with INCA Oxford Energy dispersive X-ray (EDX), having silicon detector, and High-Resolution Transmission Electron Microscope (HRTEM, JEOL, JEM 2100). The detailed elemental composition and chemical state at the surface of synthesized materials were studied by an X-ray photoelectron spectrometer (XPS) instrument Thermo Fisher Nexsa spectrometer equipped with Al-Kα as a monochromatic X-ray radiation source and energy of 1486.6 eV. For the survey and narrow scan, the pass energy was set at 400 eV and 50 eV, respectively. Regarding the low-energy electrons and ions, dual-beam charge neutralization has been used. The surface of the sample was kept at the right angle (90°)

with the input lens axis. The standard spectra used were one of the individual core-level spectra by charge correction C1s at 284.8 Ev. An Advantage software with a smart function for background correction was used to fit the individual core-levels. A gas chromatograph equipment GC-MS (Shimadzu, QP-2010) with an HP-5 column (30 m × 250 μm × 0.25 μm) using tetradecane as an internal standard was used to identify the obtained products after the reaction.

2. Chemicals and Materials

Acetone was purchased from Qualigens (India). Microbial media components including L-tyrosine, L-asparagine, and glycerol were purchased from HiMedia (India). γ-Valerolactone (99.0%) was purchased from Sigma-Aldrich. Ethylbenzene (99.0%), styrene ((>99%), 4-methyl styrene (>99%), 1-choloro 4-ethylbenzene (98%), 4-bromostyrene (>95%), diphenylmethane (99.0%), and cyclohexene (99.0%) were purchased from TCI Chemicals, India. All these chemicals were of AR grade and were used as received without further purification.

3. Isolation, identification and extraction of melanin

Isolation of endophytic microorganisms

The plant *Salicornia brachiata* Roxb. was collected from Victor port, Amreli district, Gujarat on Feb. 26, 2018 (N 20° 58′ 53.2″, E 071° 33′ 21.2″). These plant samples were brought to the laboratory under cold conditions in a sterile Ziploc bag. Further processing of plant samples was done according to a previously reported method.[1,2] Plant samples were surface sterilized for isolation of endophytic microorganisms and subjected to pre-treatment. The surface sterilized plant samples (extract) were treated with 1.5 % v/v phenol and incubated at 28 °C for 15 min. Immediately after incubation dilutions of treated samples were made up to 10^{-4} and 100 μL of these dilutions were spread on ISP-4 agar supplemented with nystatin and cycloheximide (60 μg/mL each). The inoculated plates were incubated at 28 °C for 2 to 4 weeks, following which colonies appearing on ISP^{-4} agar plates were picked up.

Screening for melanin production

The isolated colonies were screened for melanin production according to the reported method.[1,3] The isolates were inoculated into ISP-6 broth (Peptone Yeast Extract Iron media) and ISP-7 (Tyrosine broth) incubated at 28 °C for 7-10 days. Production of colored pigment in the culture medium was selected as positive for melanin production. Melanin pigment production was quantified by measuring the O.D. of the supernatant of culture broth spectrophotometrically at 400 nm.

Identification and phylogenetic analysis of microbial strain

The melanin-producing microorganism isolated from halophyte *S. brachiata* was identified by 16S rRNA analysis. The genomic DNA was isolated manually by the phenol: chloroform: isoamyl alcohol method. Further genomic DNA sample was sent to Macrogen, Inc. is a South Korean public for DNA purification and 16S rRNA sequencing. 16S rRNA gene was amplified using universal primers (785F 5′- GGATTAGATACCCTGGTA- 3′, 907R 5′ CCGTCAATTCMTTTRAGTTT 3′). The 16S rRNA gene sequence was identified by a database search using the BLAST program (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov).

Medium for melanin production

For seed culture preparation, 8 mm diameter of well-grown culture was inoculated into 30 mL tyrosine broth contained in a 250 mL conical flask and incubated at 28 °C for 8 days. After incubation, the 3% seed culture was inoculated into 250 mL tyrosine broth contained in a 1L conical flask, and incubated at at 28 °C for 8 days.

Melanin extraction and purification

After 8 days of incubation, the fermented broth was harvested and centrifuged at 8000 rpm for 10 min at 25 °C to separate cell biomass. The supernatant was collected and an equal amount of acetone was added and allowed to stand for 12 h. After 12 h, the mixture was centrifuged at 8000 rpm for 10 min at 25 °C to collect precipitate. The precipitated melanin was washed with acetone and water to remove impurities and then dried by freeze drying for further use.

4. Synthesis of *N*-doped porous eumelanin-based biomaterial

In a typical synthesis, a 100 mL two-necked round-bottom flask was charged with 1 g of eumelanin, cetyltrimethylammonium bromide (CTAB) (3 mmol), and 30 mL of cyclohexane. The flask was stirred at 50 °C for 30 min or until the uniform dispersion of eumelanin and CTAB with the dropwise addition of hydrogen peroxide (H_2O_2). Subsequently, 2 mL distilled water and 4.8 mL ammonium hydroxide were slowly added and stirred for 15 min. To this solution, 1 g tetraethyl orthosilicate (TEOS) was slowly added, and it was stirred for an additional 2 h. The reaction was quenched by the addition of 40 mL isopropanol followed by ultra-sonication for 5 min. The final SiO_2-incorporated melanin was collected by simple filtration using Whatman filter paper. Then, the collected solid material was dried at room temperature and then pyrolyzed at 600 °C for 3 h at a heating rate of 5 °C/min under N_2 atmosphere to get SiO_2@*N*-doped carbon. Further, the resulted SiO_2@*N*-doped carbon was treated with 1 M hydrofluoric acid (HF) aqueous solution in a polypropylene bottle with a screw cap for 24 h at room temperature to remove SiO_2. Finally, the sample was washed with deionized water and dried overnight at 70 °C. The obtained sample was labelled as porous *N*-doped carbon (PNDC).

5. Synthesis of *N*-doped porous material from chitosan

In a typical synthesis, a 100 mL two-necked round-bottom flask was charged with 1 g of Chitosan, CTAB (3 mmol), and 30 mL of cyclohexane. The flask was stirred at 50 °C for 30 min or until the dissolution of chitosan and CTAB with the dropwise addition of H_2O_2. Subsequently, 2 mL distilled water and 4.8 mL ammonium hydroxide were slowly added and stirred for 15 min. To this solution, 1 g TEOS was slowly added and stirred for an additional 2 h. The reaction was quenched by adding 40 mL isopropanol followed by ultra-sonication for 5 min. The final SiO_2-incorporated melanin was collected by simple filtration using Whatman filter paper. Then, the collected solid material was dried at room temperature and then pyrolyzed at 600 °C for 3 h at a heating rate of 5 °C/min under N_2 atmosphere to get SiO_2@N-doped carbon. Further, the resulting SiO_2@N-doped carbon was treated with 1 M HF aqueous solution in a polypropylene bottle with a screw cap for 24 h at room temperature to remove SiO_2. Finally, the sample was washed with deionized water and dried overnight at 70 °C. The obtained sample was labeled as PNDCC.

6. General procedure for the oxidation of alkenes derivatives in an oxygen atmosphere

In a 30 mL reaction tube fitted with a rubber septum, PNDC (10 wt% to substrate catalyst), styrene derivatives (1 mmol), and 1 mL of 1,4-dioxane 0.1 mL tetradecane (internal standard) were added. The mixture was heated at the desired temperature for 12 h with continuous stirring. Progress of the reaction was monitored by withdrawing aliquots from the reaction mixture at a definite time interval and analyzing through gas chromatography (GC). After completion, the catalyst was recovered by filtration of the reaction mixture, washed twice with methanol, dried in an oven at 80 °C overnight, and reused for the next cycle. The resulting mixture was stirred at 80 °C under 1 atm of O_2.

7. General procedure for the oxidation of aromatic alkanes in oxygen atmosphere

In a 30 mL reaction tube fitted with a rubber septum, PNDC (10 wt% to substrate catalyst), aromatic alkanes (1 mmol), and 1 mL of GVL 0.1 mL tetradecane (internal standard) were added. The mixture was heated at the desired temperature for 24 h with continuous stirring. Progress of the reaction was monitored by withdrawing aliquots from the reaction mixture at a definite time interval and analyzing through gas chromatography (GC). After completion, the catalyst was recovered by filtration of the reaction mixture, washed twice with methanol, dried in an oven at 80 °C overnight, and reused for the next cycle. The resulting mixture was stirred at 80 °C under 1 atm of O_2.

Table S1. XPS elemental composition of PNDC and PNDCC

Sample Code	C1s (at.%)	N1s (atomic%)				O1s (at.%)
		Graphitic-*N*	Pyridinic-*N*	Pyrrolic-*N*	Quaternary-*N*	
PNDC	88.9	1.7 (20.56)	1.7 (20.67)	1.7 (45.06)	1.7 (13.71)	7.44
PNDCC	80.33	4.31 (0)	4.31 (22.53)	4.31 (41.72)	4.31 (35.74)	15.36
Other trace element present on PNDC: Cl (0.67%) and Si (1.3%)						

Table S2. Metal-free selective oxidation of olefins and hydrocarbons

S.No	**Catalyst**	**Substrate**	**Reaction conditions**	**Oxidant and Additive**	**Conv.(%)**	**Sel. of C=O (%)**	**Ref.**
1.	g-C_3N_4	Styrene	Subs.;10 mmol, C.; 100 mg, NHPI: 1 mmol, S.; ACN, 1 atm	O_2, NHPI	98	97	4
2.	(N)G	Styrene	Subs.;1 mL, C.; 10 mg, P.; balloon O_2 pressure at 100 °C for 10 h	O_2	58	49	5
3.	(N)G	Ethylbenzene	Subs.;2 mL, C.; 5 mg, P.; balloon O_2 pressure at 120 °C for 24 h	O_2	9	94	5
4.	g-C_3N_4@G	Styrene	Subs.; 0.8 mmol, C.; 20 mg, S.; 1 mL ACN, at 80 °C for 4 h	TBHP	NR	NR	6
5.	PDNSC-800	Ethylbenzene	Subs.; 1 mmol, Oxidant: 500 µL, C.; 20 mg, S.; 3 mL H_2O, at 80 °C for 10 h	TBHP	>99	98.7	7
6.	NGG-4-900	Ethylbenzene	Subs.; 0.5 mmol, Oxidant: 500 µL, C.; 10 mg, S.; 6.5 mL H_2O, at 80 °C for 12 h	TBHP	99	99	8
7.	*N*-HCSs	Ethylbenzene	Subs.; 0.5 mmol, Oxidant: 500 µL, C.; 10 mg, S.; 6.5 mL H_2O, at 50 °C for 12 h	TBHP	98.4	99.5	9
8.	g-C_3N_4-NS	Styrene	Subs.; 4.37 mmol, Oxidant: 8.7 mmol, C.; 50 mg, S.; 5 mL ACN, at 80 °C for 12 h	TBHP	81.2	12.9	10
9.	NPS-HCS	Ethylbenzene	Subs.; 1 mmol, Oxidant: 1 mL, C.; 10 mg, S.; 2 mL H_2O, at 80 °C for 12 h	TBHP	99	99.5	11
10.	GS1000	Ethylbenzene	Subs.; 1 mmol, Oxidant: 1 mL, C.; 10 mg, S.; 1 mL H_2O, at 80 °C for 4 h	TBHP	85	98	12

NHPI: *N*-hydroxyphthalimide, ACN: Acetonitrile, g-C_3N_4: Graphitic carbon nitride, (B,N)G: boron and nitrogen-doped carbon, PDNSC-800: Polymer-derived N,S co-doped carbon catalysts, *N*-HCSs: *N*-doped hollow carbon spheres, S.: Solvent, Subs.: Substrate, C.: Catalyst, Conv.: Conversion, Sel. of C=O: Benzaldehyde for styrene and acetophenone for ethylbenzene, NR: Not reported

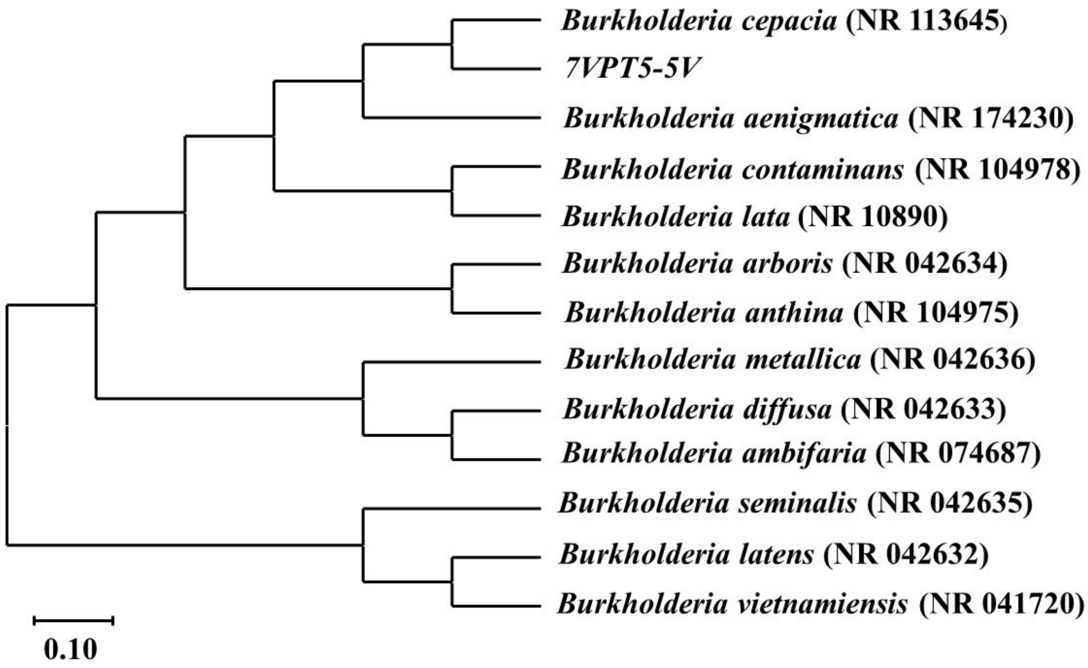


Figure S1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences. Bar, 0.10 substitution per nucleotide position.

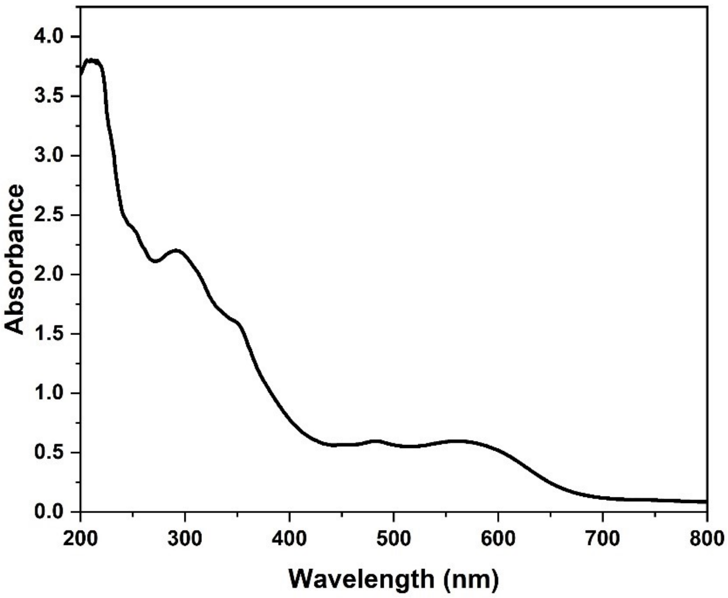


Figure S2. UV/VIS spectrum of eumelanin.

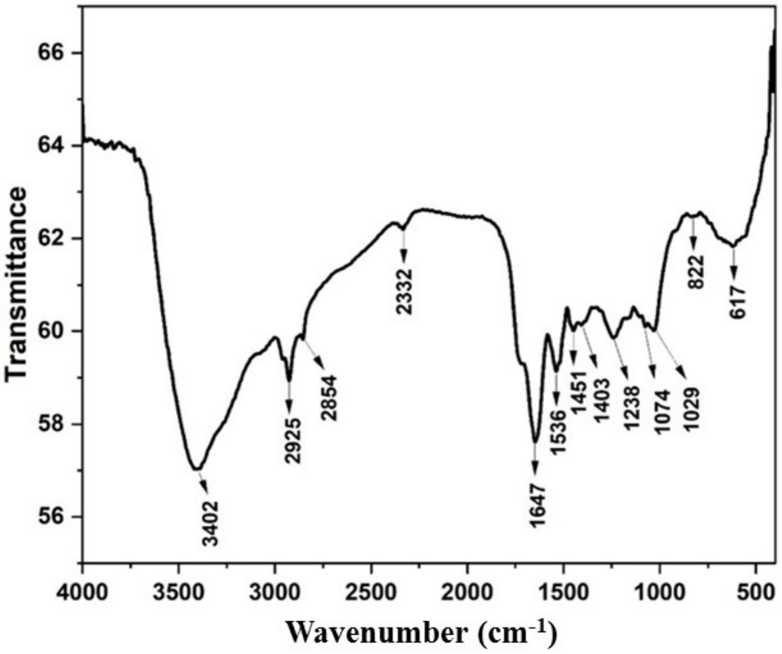


Figure S3. FT-IR spectrum of eumelanin.

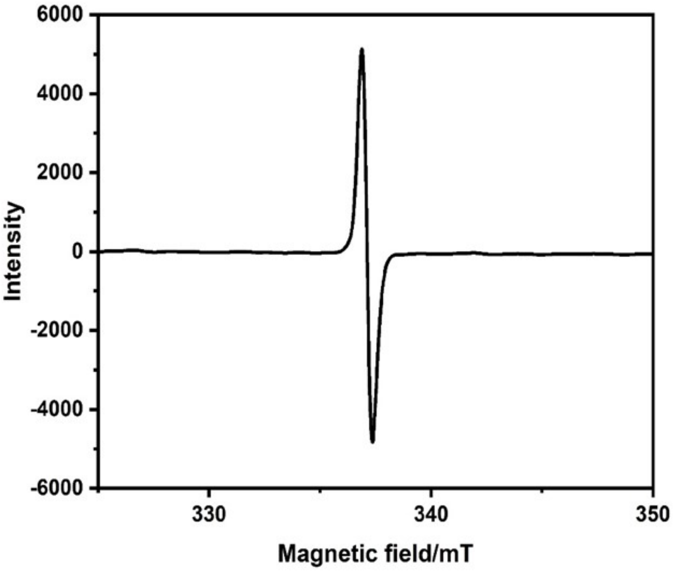


Figure S4. EPR spectrum of eumelanin.

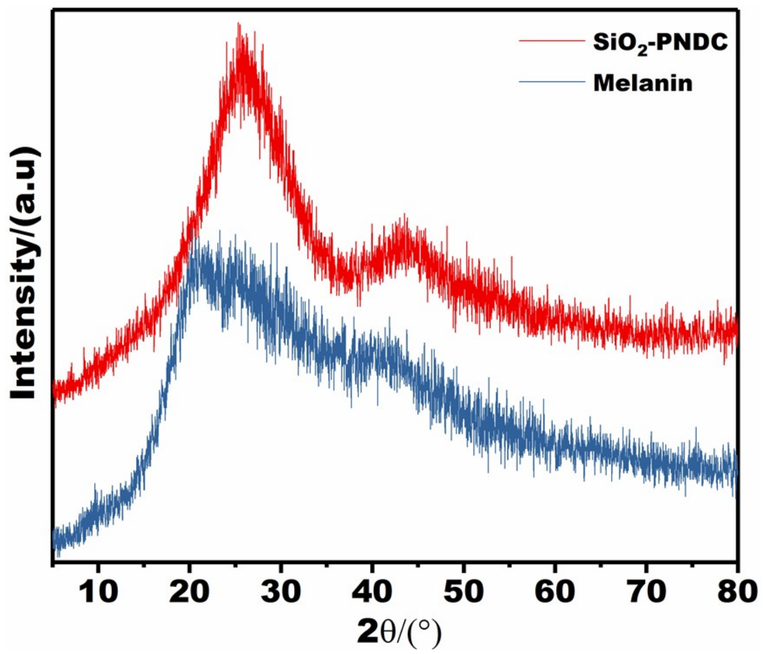


Figure S5. PXRD data of eumelanin and PNDC-SiO$_2$.

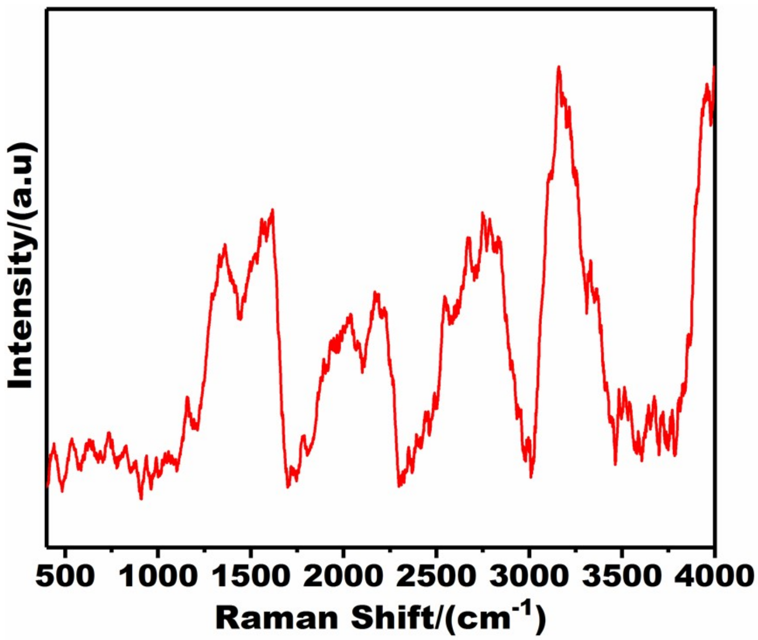


Figure S6. Raman spectrum of eumelanin.

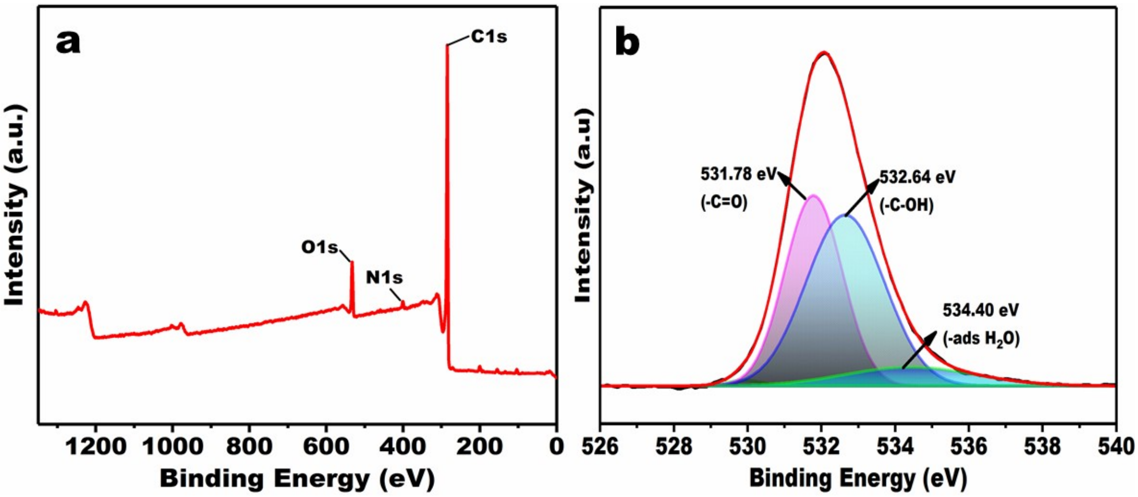


Figure S7. XPS analysis of PNDC (a) survey spectrum and (b) O 1s spectra.

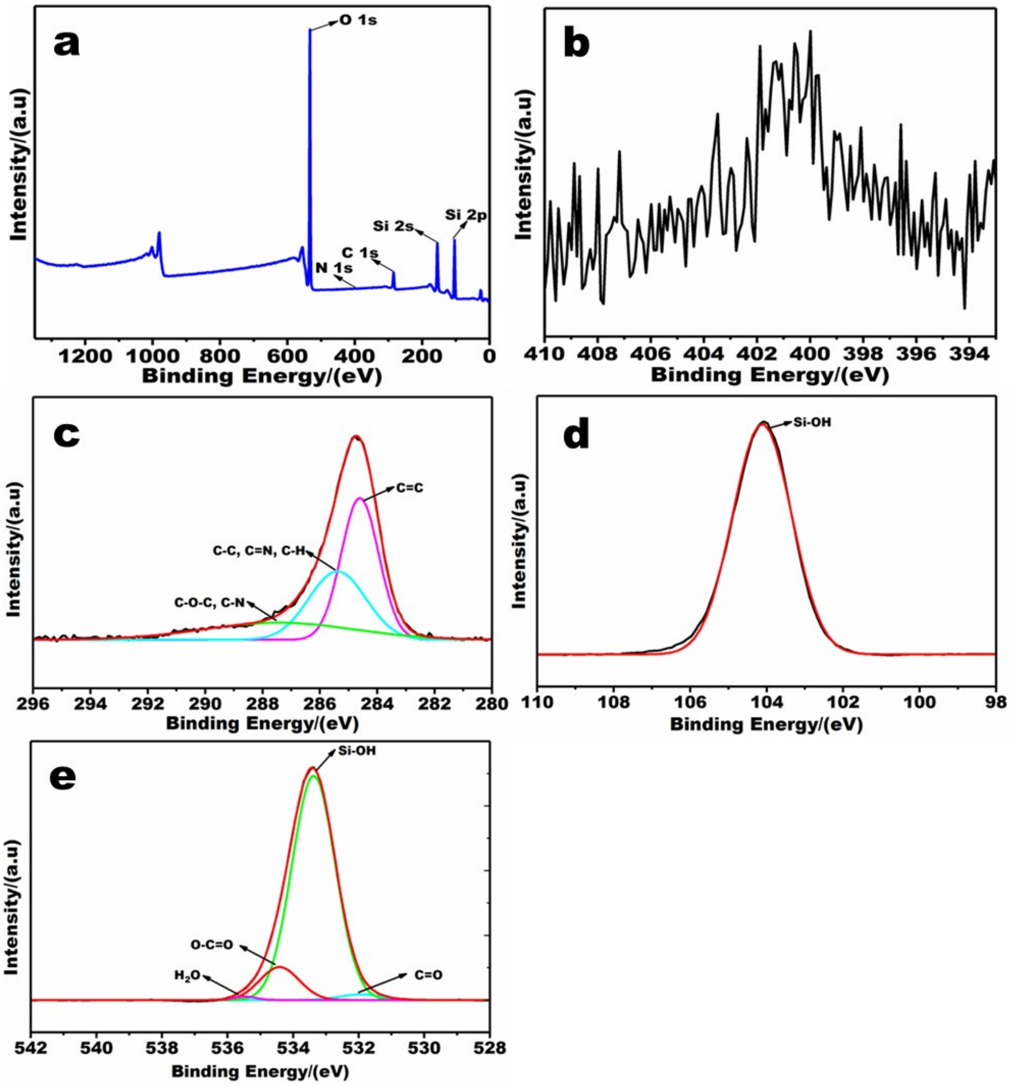
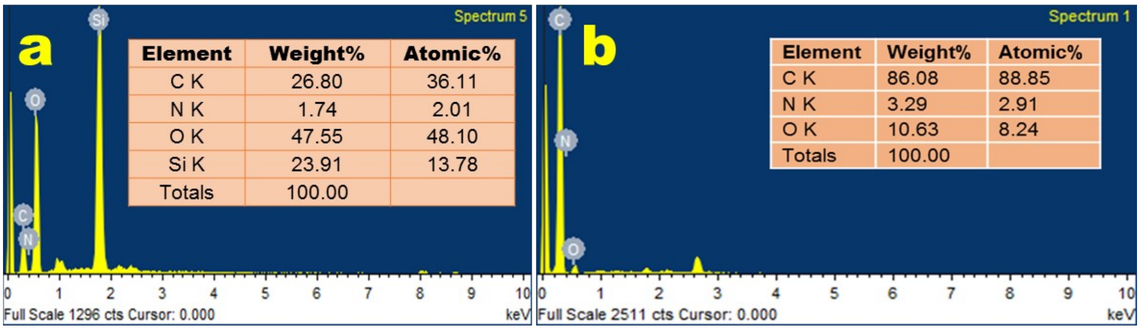


Figure S8. XPS analysis of PNDC-SiO_2, (a) survey spectrum, (b) N 1s spectra, (c) C 1s spectra, (d) Si 2p spectra, and (e) O 1s spectra.



Element	Weight%	Atomic%
C K	26.80	36.11
N K	1.74	2.01
O K	47.55	48.10
Si K	23.91	13.78
Totals	100.00	

Element	Weight%	Atomic%
C K	86.08	88.85
N K	3.29	2.91
O K	10.63	8.24
Totals	100.00	

Figure S9. FESEM elemental composition of PNDC-SiO_2 (a) and PNDC (b).

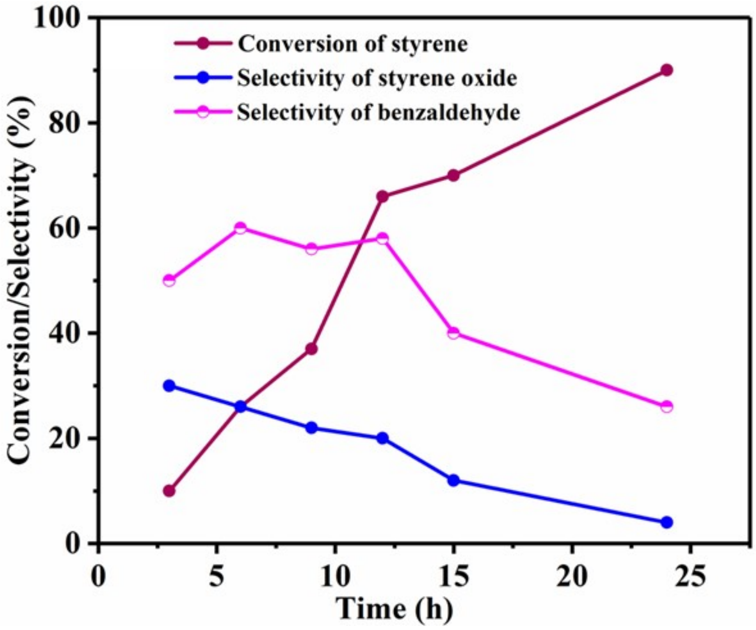


Figure S10. Time variation studies for oxidation of styrene to benzaldehyde.

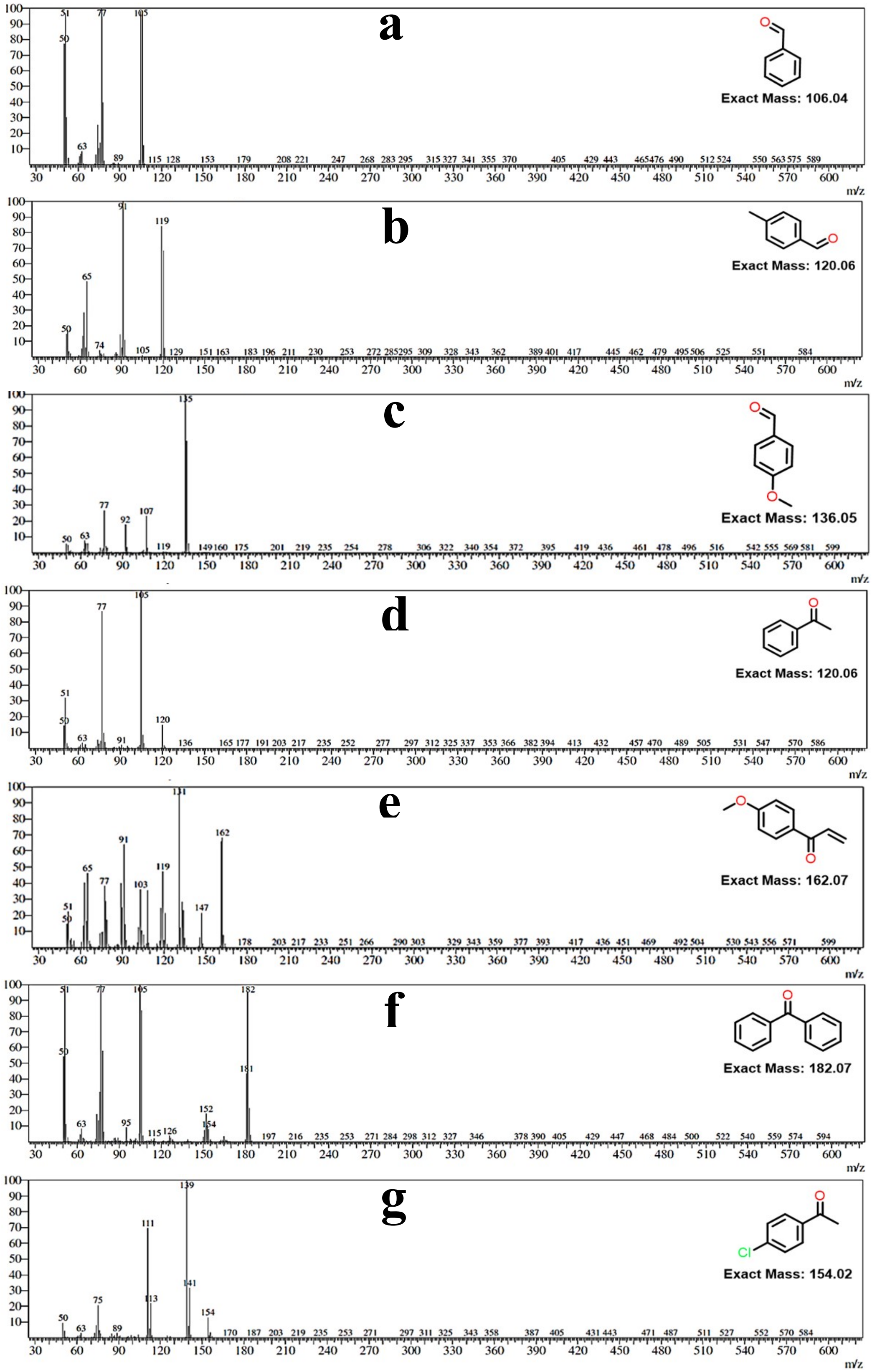


Figure S11. GC-MS spectra of (a) benzaldehyde, (b) 4-methyl benzaldehyde, (c) 4-methoxy benzaldehyde, (d) acetophenone**,** (e) 1-(4-methoxyphenyl)prop-2-en-1-one, (f) benzophenone, (g)1-(4-chlorophenyl)ethan-1-one.

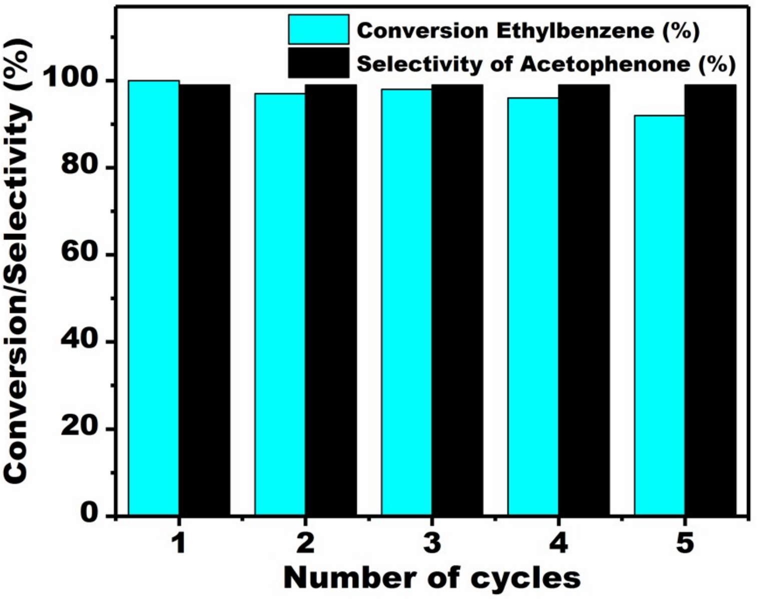


Figure S12. Recyclability studies: 1 mmol of EB and 10 wt% to substrate catalyst was dissolved in 1 mL of GVL at 120 °C for 24 h in the presence 1 atm O_2.

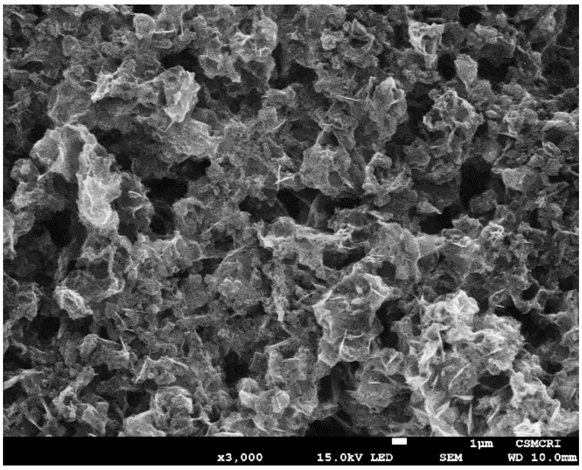


Figure S13. FESEM image of PNDC after 5th cycle.

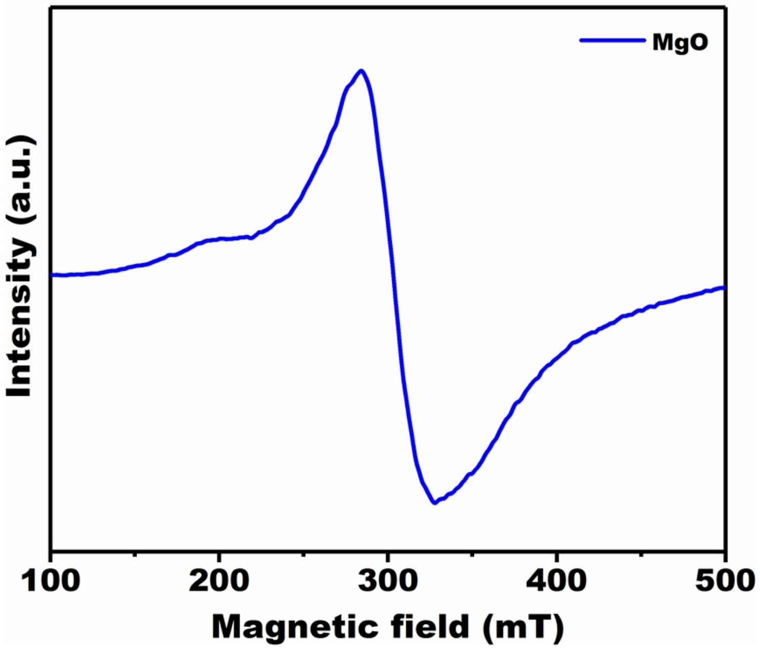


Figure S14. EPR spectrum of MgO for the oxidation of styrene under the optimized conditions.

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