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-Supporting information-

Hydroxyl radical generation by cactus like copper oxide nanoporous carbon catalysts for microcystin-LR environmental remediation

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2. Materials and methods

2.1. Preparation of copper oxide incorporated on nanoporous activated carbon

The heterogeneous catalyst CuO-NPAC was prepared from rice husk agricultural solid waste containing silicon as the major inorganic element in the matrix. The preparation procedure of nanoporous activated carbon was presented by following the procedure detailed elsewhere by the authors [1]. The carbon sample soaked in hydrofluoric acid (HF) for one week to remove the silicon present in rice husk. It was washed several times until obtaining neutral pH in the washed water. The washed samples were dried at 110 °C. The obtained activated carbon material was labelled as NPAC. Copper oxide incorporated on nanoporous activated carbon (CuO-NPAC) was prepared using hydrothermal method in two steps: NPAC was oxidised using 5 M nitric acid (HNO_3) at its boiling point for 3 h and the sample was dried at 110 °C. 5 g of oxidized NPAC were introduced into 150 mL of 0.05 M $Cu(NO_3)_2.3H_2O$ solution in acidic media nitric acid for 2 h. Sodium borohydride ($NaBH_4$, 0.132 g) was added to the reaction mixture as a reducing agent under continuous rapid stirring at 600 rpm. This mixture was heated at 100 °C for 8 h under inert atmosphere. The samples were subsequently annealed at three different temperatures 300, 400, and 500 °C under nitrogen atmosphere in a muffle furnace for 8 h. After cooling, the copper oxide incorporated on nanoporous activated carbon was washed several times using deionized water until pH became neutral. The washed samples were dried at 110 °C to get the final products and labelled as CuO-$NPAC_{300}$, CuO-$NPAC_{400}$, and CuO-$NPAC_{500}$ corresponding to annealing temperatures of 300, 400, and 500 °C, respectively. Cell lines and cultures, 3T3 cell line was purchased from National Centre for Cell Science, Pune, India. Cell lines were routinely grown in DMEM supplemented with 10% serum with antibiotics and incubated in a humidified incubator with 5% carbon dioxide at 37°C

2.2. Characterization of NPAC and CuO-NPAC

The physical and chemical characteristics of the synthesized copper oxide incorporated on nanoporous activated carbon were examined by Thermogravimetric Analysis (TGA), Electron paramagnetic resonance (EPR), X-Ray Photoelectron Spectroscopy (XPS), Transmission Electron Microscope (TEM), X-ray diffraction (XRD), Scanning Electron Microscope (SEM), Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) analysis, Fourier Transform Infrared spectroscopy (FT-IR), cyclic voltammetry (CV), Fluorescence spectroscopy and UV-Visible Spectrophotometry. The TEM images were recorded using TECNAI (PHILIPS, and Netherlands). The crystalline properties were examined using XRD Rich Siefert 3000 diffractometer under Cu $K\alpha 1$ radiation (λ=1.5406 Å). The surface morphology of CuO-NPAC was determined using a Quanta 200 FEG scanning electron microscope (SEM). The TGA data were collected at heating rate of 10 °C per minute in nitrogen atmosphere. CuO-NPAC samples were characterized for surface area, pore volume, and pore size distribution using an automatic adsorption instrument (Micromeritics ASAP 2020). The free electron density of CuO-NPAC was determined using Electron paramagnetic resonance (EPR) spectroscopy at room temperature using wilmad EPR tube in a Bruker ESP 300E spectrometer. The spectrometer was operated at a microwave frequency of 9.399 GHz, with microwave power of 3.188 mW, modulation frequency of 100 kHz and modulation amplitude of 3.0 G, center field of 3342 G, sweep width of 3000 G and sweep time of 20.972 s. The elemental composition (carbon, hydrogen and nitrogen content) of the CuO-NPAC was determined using Vario MICRO CHNSO 15091002 (model Carlo–Erba analyser). The elemental composition of the CuO-NPAC catalysts before and after MC-LR oxidation was analysed using ICP-OES Perkin Elmer Optima 5300 DV elemental analysis instrument. X-ray photoelectron spectroscopy (XPS) was carried out in a SPECS XPS system

using 150 W Al-*Ka* radiation. The 2p core level spectrum of Cu and 1s core level spectra of C, N and O were obtained at 25 eV pass energy. FT-IR Spectroscopy (Perkin-Elmer) was used for the investigation of the surface functional groups in NPAC and CuO-NPAC. Spectra analysis was recorded in the range of 4000 to 400 cm^{-1} for 20 scans. NPAC and CuO-NPAC samples (0.1 g) were mixed with spectroscopy grade KBr (1 g) (Merk, Darmstadt, Germany) in a mortar. The NPAC and CuO-NPAC samples mixed with KBr were then were pelletized as disks with dimensions of 1 mm in thickness; and 13 mm in diameter. UV visible absorption spectra were collected in a CARY 100 conc UV–Visible Spectrophotometer. Fluorescence spectra were collected from a Varian Cary Eclipse Fluorescence spectrophotometer. The photoluminescence spectra were measured by using a Varian Cary eclipse (ELO7023695) fluorescence spectrophotometer with an excitation source from a Xe lamp in the wavelength range of 350 – 700 nm. The ESI-MS (Electron Spray Ionization-Mass Spectrum) was recorded on Micromass QuattroII triple quadrupole mass spectrometer that was set in the positive ionization mode. The MC-LR was dissolved in methanol and applied into the ESI source through a syringe pump at flow rate of 5µL/min. The ESI capillary was set at 3.5 kV and the cone voltage was 40 V. The source temperature was 120 °C and the dessolvation temperature was 300 °C. The spectrum was collected in 6 s scans.

2.3. Destruction of Microcystin-LR by heterogeneous catalyst (CuO-NPAC)

A fluidized bed device was developed by polyacrylic sheet of thickness 5 mm. The design of the fluidized device was patented by the authors; Indian patent application number (2728/DEL/2012)). The volume of reactor was 550 mL with the working volume of 500 mL. Two (2) g of the $CuO\text{-}NPAC_{400}$ catalyst was added in the reactor. The reactor was provided with coarse bubble air spargers to distribute air in the fluidized bed to facilitate oxygen transfer for degradation of MC-LR and to fluidize the $CuO\text{-}NPAC_{400}$ catalyst. The MC-LR solution was applied to the reactor in the batch experiment through contaminated water

distribution system provided at the bottom of the reactor. The batch reactor was maintained in such a way that the reaction time was provided at 80 min. The aliquot of samples were collected and degradation of MC-LR was determined. The selection of optimal hydrogen peroxide concentration was measured by varying the concentration of hydrogen peroxide. The collected samples were analysed for the degradation of MC-LR. The effects of time, pH, catalyst loading, and hydrogen peroxide concentration were studied. The effect of time was investigated performing the experiment at different time intervals from 5 to 90 min. The samples were collected and analysed to determine the concentration of MC-LR. The effect of pH was studied by adjusting the pH of the aqueous MC-LR solution at 3, 7 or 9 using H_2SO_4 or NaOH while keeping aeration in the system.

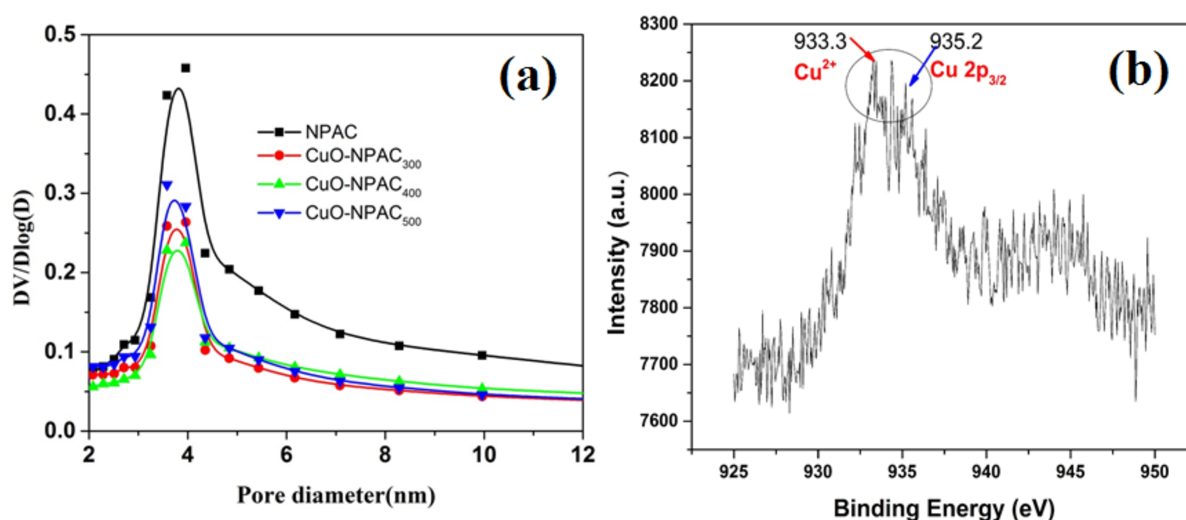

Fig. S1 (a) Pore size distribution vs. pore diameter of NPAC and CuO-NPAC, (b) XPS spectrum of CuO-NPAC annealed at 500 °C

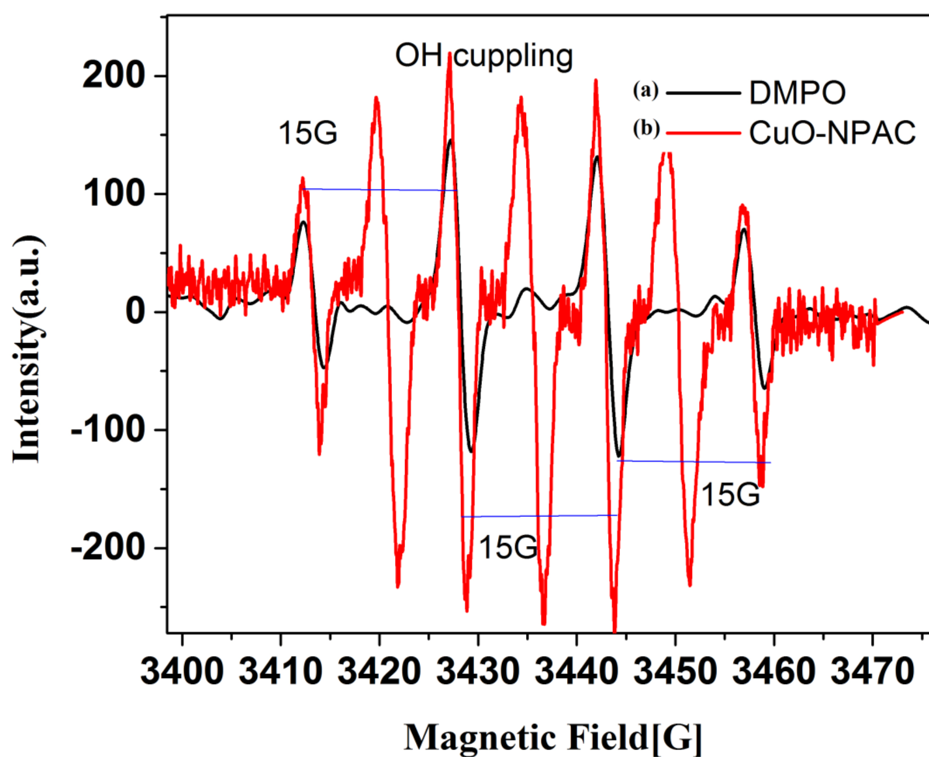

Fig. S2 (a) The generation of hydroxyl radicals was verified by spin trap technique using Fenton oxidation process, (b) shows generation of hydroxyl radical using CuO-NPAC with hydrogen peroxide

The EPR spectrum was obtained at room temperature using a quartz liquid flat cell (Wilmad WG-812-Q) in a TE102 cavity of a Bruker ESP 300E spectrometer. The spectrometer was operated at a microwave frequency of 9.399 GHz, with microwave power of 3.188 mW, modulation frequency of 100 kHz and modulation amplitude of 3.0 G, centre

field of 3200 G, sweep width of 500 G and sweep time of 20.972 s. The Fenton oxidation system yielded an EPR signal with four lines with intensity ratio of 1:2:2:1 (Fig. S2 (a)), corresponding to the DMPO-•OH adduct. When the CuO-$NPAC_{400}$ with hydrogen peroxide system showed strong EPR signal to confirm the generation of both •OH and •O_2^- radicals; DMPO-•OH and DMPO-•O_2^- were observed using heterogeneous oxidation system [2, 3].

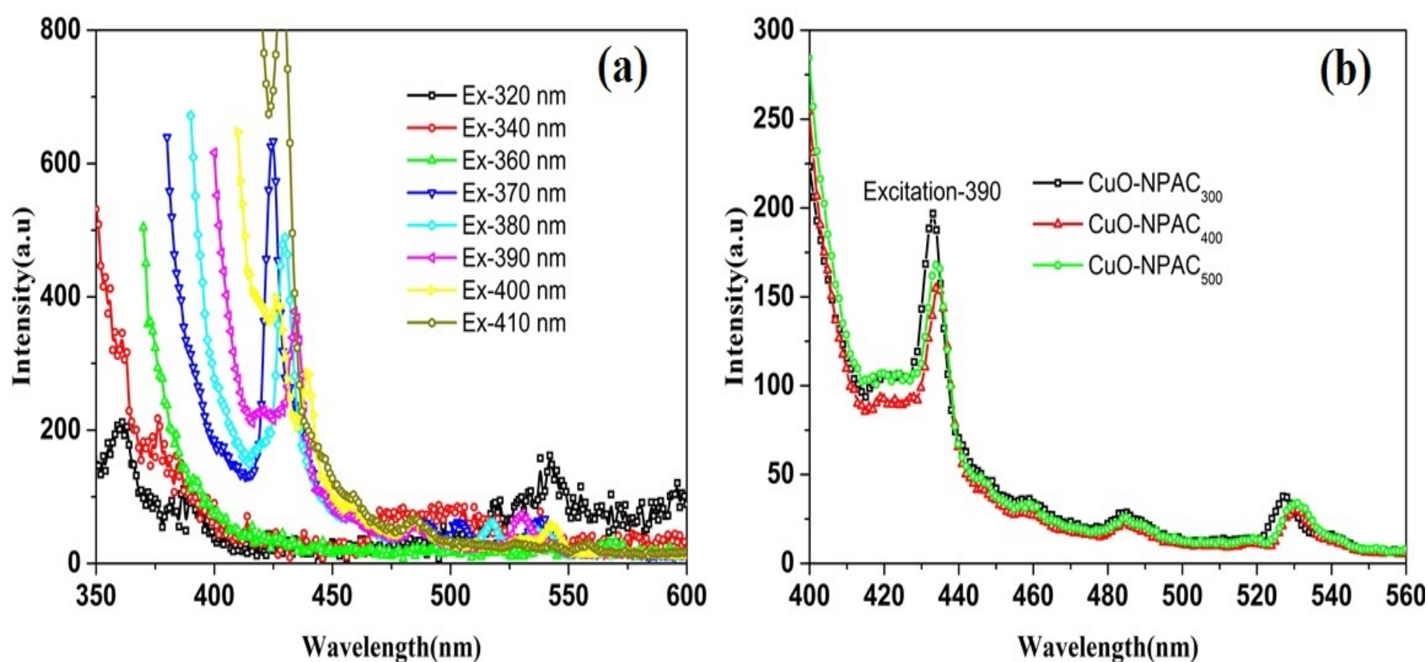

Fig. S3 (a) Photoluminescence spectrum of NPAC, (b) CuO-NPAC.

Table S1 Effect of CuO weight percentage on NPAC and leaching of copper after MC-LR oxidation

Sample	CuO wt.% after loading	Cu leaching wt. % after MC-LR oxidation	Rate constant (k) $\times 10^{-2}$ min^{-1}
CuO-NPAC, 0.1 wt. %	0.086	0.00042	2.93
CuO-NPAC, 0.5 wt.%	0.42	0.00065	3.48
CuO-NPAC, 1.0 wt.%	0.94	0.0012	3.81
CuO-NPAC, 1.5 wt.%	1.10	0.0026	4.17
CuO-NPAC, 2.0 wt.%	1.56	0.0038	4.26

2.3. Photoluminance property

Figs S3 (a) and Fig S3 (b) represent the photoluminescence spectra of NPAC and CuO-NPAC. Hydrothermal and phosphoric acid activation produced the oxygen containing functional groups on the surface of the starting material. One factor leading to tunable performance was the presence of different particle sizes, as observed in semiconductor nanocrystals. Another factor was due to the different oxygen-containing groups. PL is considered to be one of the most fascinating features of C-dots and thus, the PL spectrum recorded at longer wavelength, 320-410 nm, suggests the presence of C-dots in NPAC. Only two emission peaks were observed when copper oxide was present on NPAC matrix at excitation wavelength of 390 nm. This may indicate the copper was equally dispersed in the NPAC network structure.

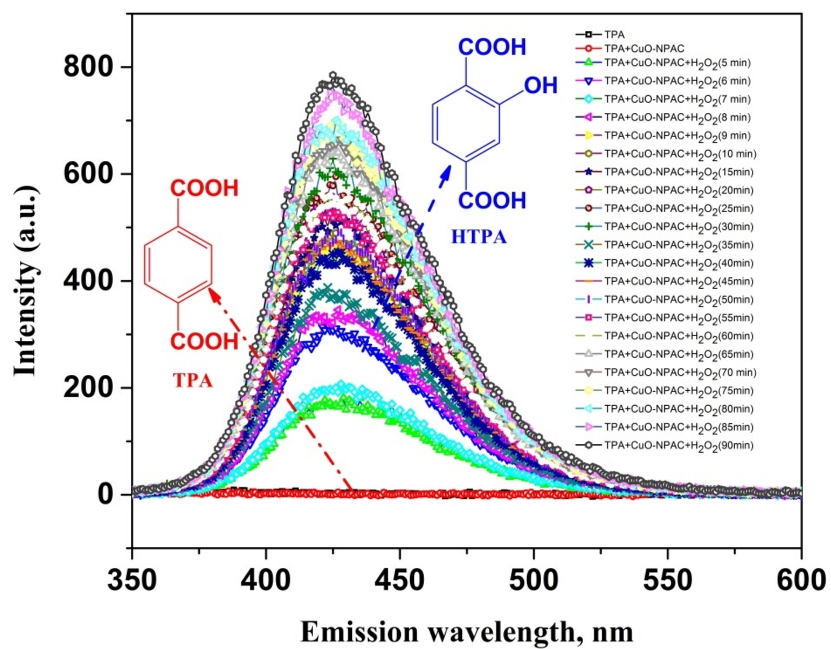

Fig. S4 TPA and HTPA fluorescence spectroscopy at different time interval.

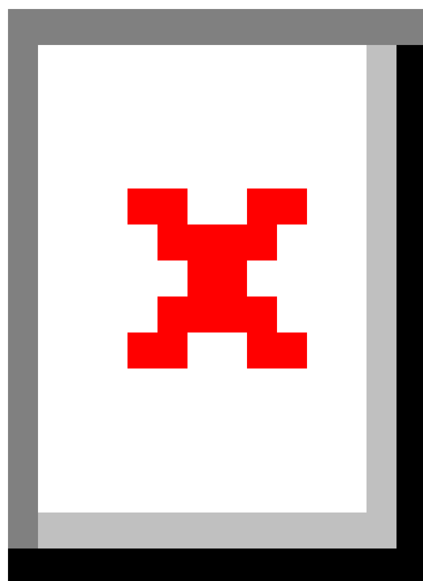

Fig. S5 (a) LC-MS spectrum of MC-LR.

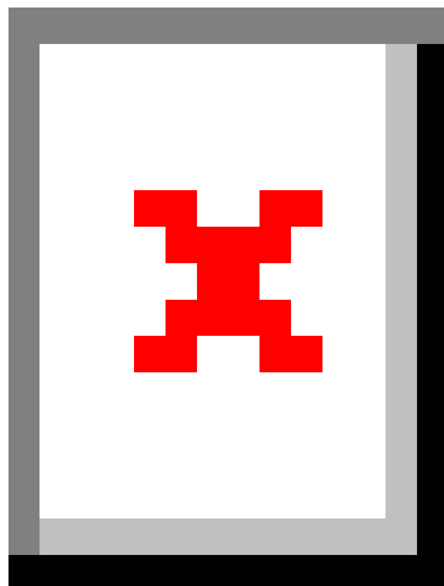

Fig. S5 (b) LC-MS spectrum of intermediates of MC-LR (m/z at 1029)

2.4. Toxicity studies of cells with waste water (treated and untreated)

3T3 10^4 cells per well were incubated in 48 well plate until reach the semi confluence and then treated with waste water and incubated for 24 hours. Morphological and Cell viability (MTT reduction assay) were assessed after 24 hours. The Morphological assessments after incubation, cells were viewed under inverted microscope to analyse the morphological pattern of the cells. MTT reduction assay was conducted after incubation time, the medium was removed and 100 µl of MTT solution (50 mg/ml) was added to each well.

After incubation for 2-4 hours at 37 °C, the solution was removed. Formazan crystals were solubilized using 100 µl of DMSO. The plate was shaken for 5 min on a plate shaker and the absorbance was measured at 540 nm in micro plate reader (Biotek instruments). Finally the morphological assessment of 3T3 cells reveals, cells were slightly damaged and cell viability was reduced after 500 µl of waste water.

Reference

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