Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Adsorption, photodegradation and antibacterial study of Graphene-Fe_3O_4 nanocomposite for multipurpose water purification application

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Supplementary data.

Cell Preparation: 5 ml of overnight grown culture of *E.coli* were added to flat bottom conical flask containing 100 ml sterile nutrient broth. To plot the growth curve OD was measured at 540 nm and mid-log growth phase was calculated. *E. coli* were grown in Nutrient Broth (NB) medium at 37 ºC, and harvested in the mid exponential growth phase. It was then centrifuged at 6000 rpm for 10 min to pellet cells, and cells were washed three times with isotonic saline solution to remove residual macromolecules and other growth medium constituents. The pellets were then re-suspended in isotonic saline solution. Bacterial cell suspensions were diluted to obtain cell samples containing 10^6 to 10^7 CFU/mL. 100 µg of graphene and G-Fe_3O_4 nanocomposite was individually dispersed in 1 ml isotonic saline solution (0.9 w/v% NaCl) and ultrasonicated for 30 min.

Cell Viability Test: *E. coli* cells were separately incubated with Graphene and G-Fe_3O_4 nanocomposite dispersion in isotonic saline solutions at 37 °C for 2h at 200 rpm. The loss % of viability of *E. coli* cells was evaluated by colony counting method. Briefly, series of 10-fold cell dilutions (100 µl each) were spread onto nutrient agar plates, and left to grow overnight at 37 °C. Colonies were counted and compared with those on control plates to calculate changes in the cell growth inhibition. Isotonic saline solution containing *E.coli* only was used as control. All treatments were prepared in triplicates, and repeated at least on three separate occasions. Further, concentration and time dependent studies were also performed. 0-200 ppm of G-Fe_3O_4 was studied for concentration dependent studies and 0-90 minutes for time dependent studies.

Cell Morphology Observation: Treated *E. coli* cells were washed with ethanol and centrifuged at 2000 rpm to remove debris. The obtained pellet was washed twice and dispersed in sterile distil water. It was then mounted on a coverslip and left to dry at room temperature. The dried cells were sputter-coated for making it conductive for SEM imaging.

Biochemical assay of total cellular protein degradation due to effect of G-Fe_3O_4

To 1 ml aliquot of the treated and untreated *E.coli* suspension, 5 ml of the freshly prepared alkaline copper sulphate reagent was added; after 10 min 0.5 ml of Folin's reagent was added, mixed well and allowed to stand for 30 minutes for color to develop. Absorbance was recorded at 660 nm after setting the instrument with reagent blank, which contains 1ml 0.1 N NaOH instead of sample aliquot. Standard curve with graded concentration of Bovine Serum Albumin was plotted.[1]

Total protein degradation (by SDS-PAGE methods) of *E.coli* due to effect of G-Fe_3O_4

Extraction of Total Protein: 1 ml of treated and control cultures of *E. coli* was added to 5 ml of phosphate buffer and then centrifuged at 8000 rpm for 20 mins. Supernatant was collected and extraction step was repeated 4 times. The supernatants were pooled together and the volume was made to 50 ml with phosphate buffer. To 1 ml of the above extract, 1 ml 20% Tri chloro-acetic acid was added and after half an hour centrifuged at 8000 rpm for 20 min. Pellet was washed twice with acetone and again centrifuged. Supernatant was discarded and the pellet was mixed in 5 ml of 0.1 N NaOH.
Gel for SDS PAGE: 5% of Stacking gel and 10% of Resolving gel were used.

Protein Sample Preparation for Electrophoresis: The sample protein was denatured in 2 ml gel sample buffer containing 1.25 ml 0.5 M Tris-HCl (pH 6.8), 1 ml β-mercapto-ethanol, 2 ml glycerol; 0.4 ml 1% bromophenol blue to 0.4 g SDS and the final volume was made to 10 ml with distil water. 25 µl of the above mentioned protein samples were loaded in each well of the electrophoretic gel. HTP001 Himedia Protein Molecular Weight Marker™, having a range of 29- 205 kDa, mol. wt. was used as standard protein marker. Initially 10-15 mA current was applied for 10-15 min i.e. until the samples started traveling through the stacking gel. Then current supply was increased to 30 mA until the bromophenol blue dye reached near the bottom of the gel slab. The gel slab was kept in a trough containing staining solution (200 ml methanol + 35 ml glacial acetic acid + 1.25 g Coomassie Brilliant Blue R-250 made to 500 ml by adding distilled water) until clear bands were observed. Excess stain was removed by keeping the gel in de-staining solution (75 ml of glacial acetic acid + 50 ml of methanol final volume made to 1 liter with distilled water).[2]

Detection of Reactive Oxygen Species ($O_2^{\bullet}$)

The possibility of superoxide radical anion ($O_2^{\bullet}$) production was evaluated by monitoring the absorption of XTT (2,3-bis (2-methoxy-4-nitro-5- sulfophenyl)-2 H -tetrazolium-5-carboxanilide, Fluka).[3]

Glutathione oxidation assay

The concentration of thiols in GSH was quantified by the Ellman's assay[4]. Graphene and G-Fe_3O_4 dispersions was added into 225 µL of GSH (0.8 mM in the bicarbonate buffer) to initiate oxidation. All samples were prepared in triplicate. The GSH-graphene and G-Fe_3O_4 mixtures were transferred into a 24-well plate. The 24-well plate was covered with foil to prevent illumination, and then placed in a shaker with a speed of 150 rpm at room temperature for incubation of 2 h. After incubation, 785 µL of 0.05 M Tris-HCl and 15µLof DNTB (Ellman's reagent, 5, 5 0-dithio-bis-(2-nitrobenzoic acid), Sigma-Aldrich) were added into the mixtures to yield a yellow product.

Graphene and G-Fe_3O_4 was removed from the mixtures by filtration through a 0.45 μm polyethersulfone filter (Acrodisc Syringe Filters with Supor Membrane). A 250 μL aliquot of filtered solutions from each sample was then placed in a 96-well plate. Their absorbance at 412 nm was measured on a Varian microplate spectrophotometer. GSH solution without graphene-based materials was used as a negative control. GSH (0.4 mM) oxidization by H_2O_2 (1 mM) was used as a positive control. The loss of GSH was calculated by the following formula:

$$loss\ of\ GSH(\%) = \frac{Absorbance\ \ negative\ control}{\frac{Absorbance\ of\ sample}{Absorbance\ \ negative\ control}} X100$$

After 2 h incubation at room temperature, 98% of GSH in the positive control sample was lost, which was found consistent in all trials.

References

[1] S.K. Sawhney, R. Singh, Introductory Practical Biochemistry, Narosa Publisher, India, 2002.
[2] I.M. Walker, The Protein Protocol Handbook 1996, Humana press, Totowa, NJ.
[3] H. Ukeda, S.Maeda, T.Ishii, M. Sawamura, Anal. Biochem. 1997, **251**, 206–209.
[4] G. L. Ellman, Arch. Biochem. Biophys. 1959, **82**, 70–77.

XRD Pattern

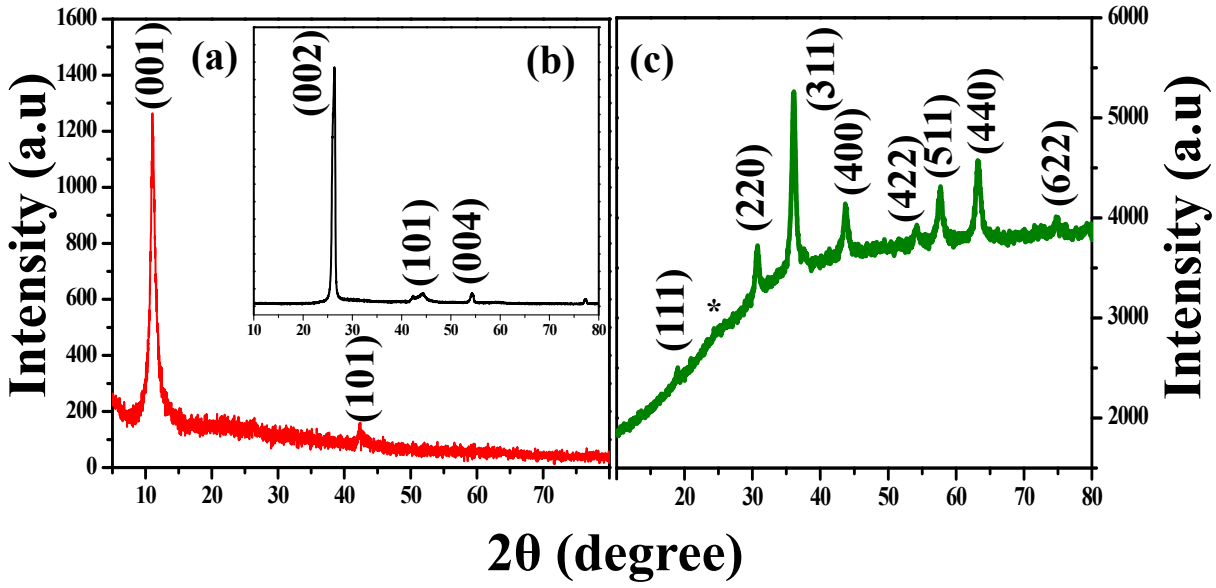

Fig. S1 XRD Patterns of (a) GO (b) Graphite powder (c) G-Fe_3O_4 nanocomposite.

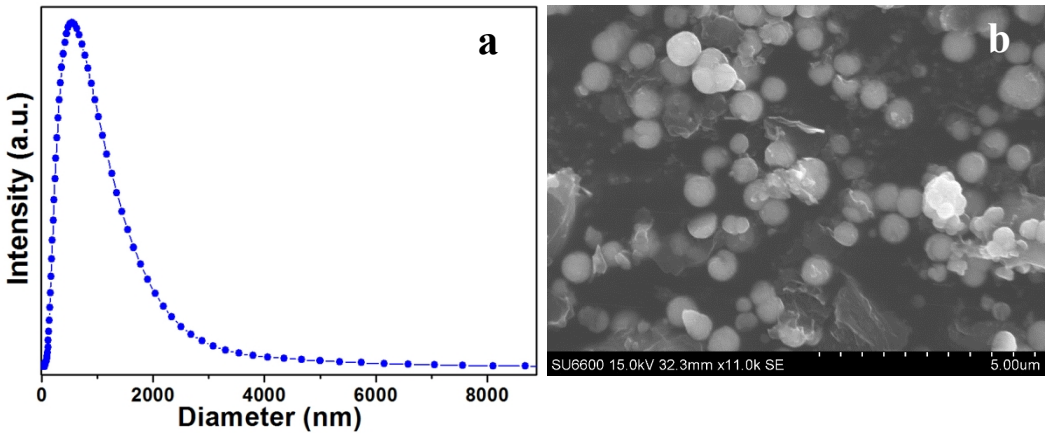

Fig. S2 (a) DLS measurements and (b) FE-SEM image of G-Fe_3O_4 nanocomposite.

TGA-DTA analysis

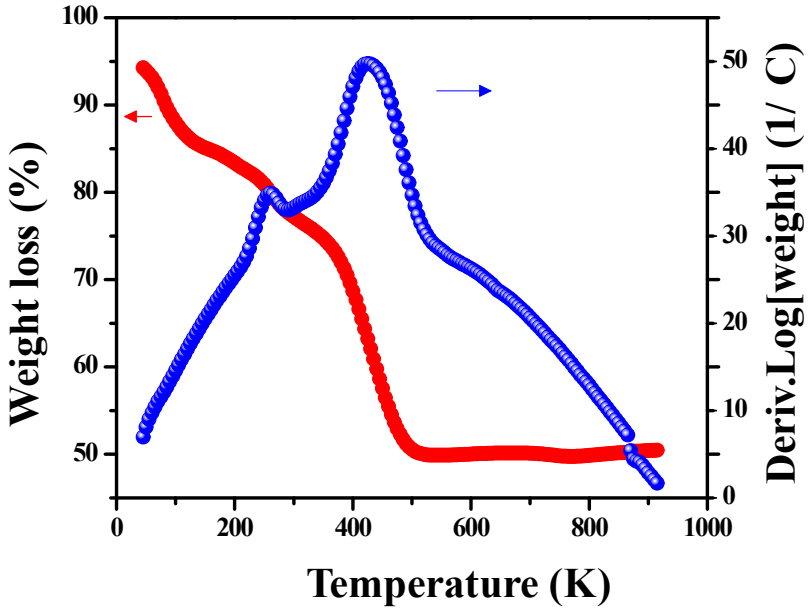

Fig. S3 TGA-DTA curves of as-prepared material (G-Fe_3O_4) composite at a heating rate of 5°C/min.

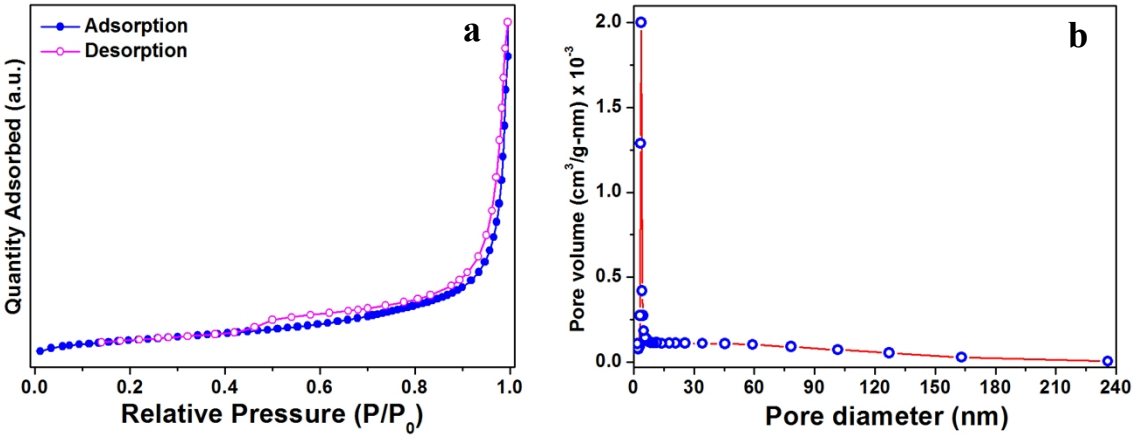

Fig. S4 (a) N_2 adsorption-desorption isotherm and (b) dV/dD pore volume vs. pore diameter curve of G-Fe_3O_4.

Adsorption parameters

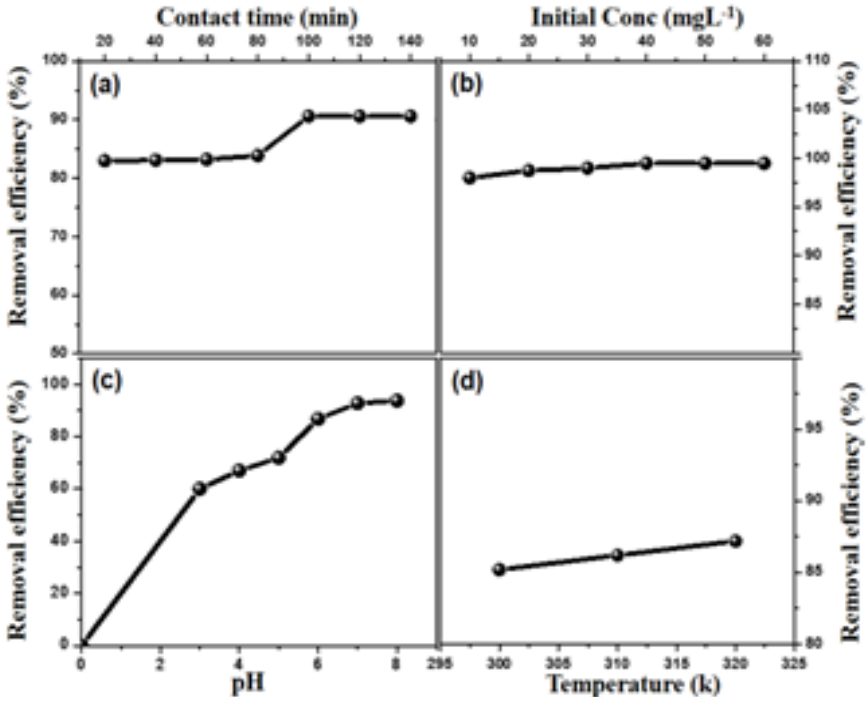

Fig. S5 Effect of adsorption parameters of lead ions adsorption onto G-Fe_3O_4 (a) contact time (b) Initial concentration (c) effect of pH and (d) effect of temperature.

Photodegradation

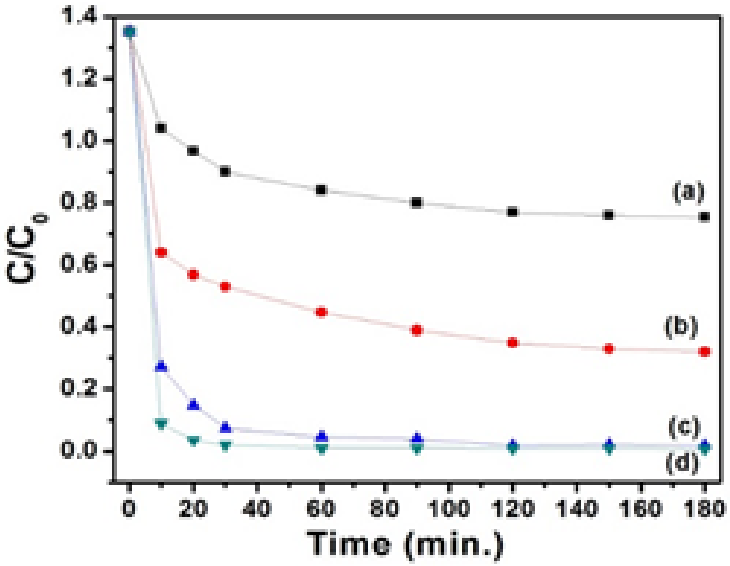

Fig. S6. Dye adsorption spectra of G-Fe_3O_4 composite using different concentrations (a) 0.1, (b) 0.2, (c) 0.3g/L and (d) graphene nanosheets at 0.2g/L

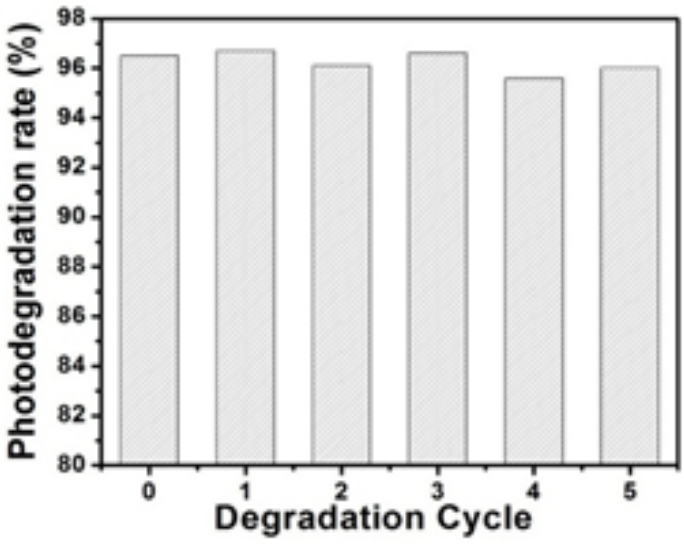

Fig. S7. Bar plot showing the Photo-Fenton degradation rate of dye for 5 cycles using 0.2g/L G–Fe_3O_4 composite in presence of visible light and H_2O_2.

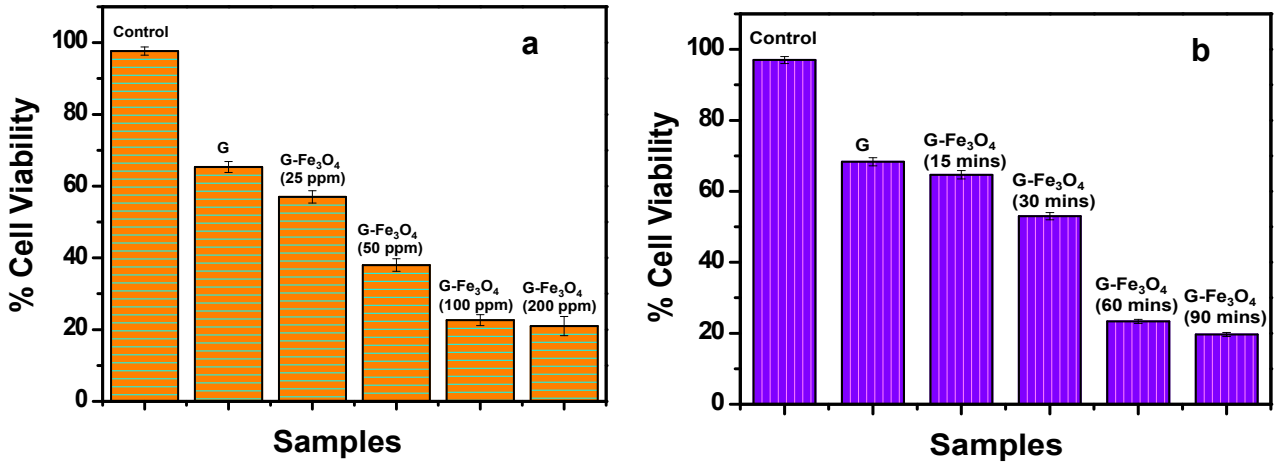

Fig. S8 (a) Concentration dependent study 0-200 ppm G-Fe_3O_4 treated with *E.coli* cells. (b) time dependent study 0-90 mins. G-Fe_3O_4 treated with *E.coli* cells.

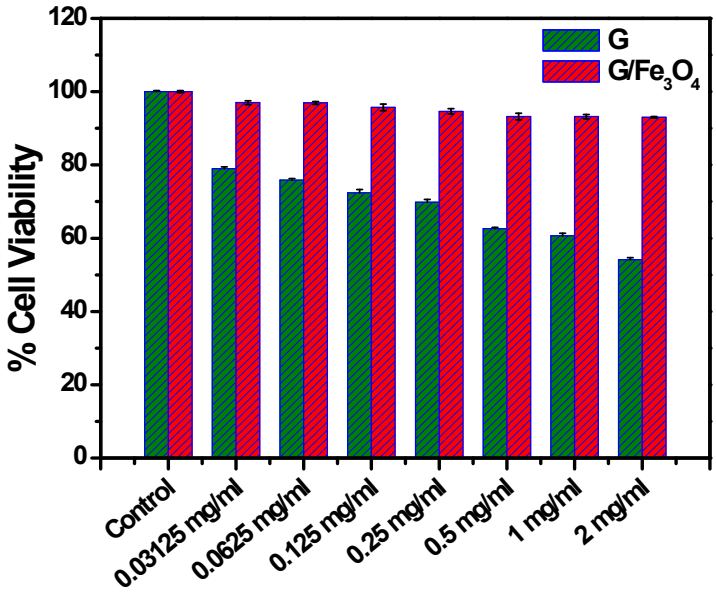

Fig. S9 Biocompatibility of Graphene and G-Fe_3O_4 on L9292 cells

Table 4 Total protein content in *E.Coli* after the treatment with graphene and G-Fe_3O_4

	Total protein degradation µg/ml
Control	236±1
Graphene	196±1
G-Fe_3O_4	86.66±0.57