Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Biosynthesis approach of nitrogen doped graphene by denitrifying bacteria CFMI-1

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Experimental Section

Chemicals

Graphite powder was purchased from Qingdao Zhongtian Company (Qingdao, China). Analytical-grade NaOH, $KMnO_4$, 98% H_2SO_4 and 30% H_2O_2 aqueous solution. All aqueous solutions were prepared with deionized water produced by laboratory water purification system. All other chemicals were analytical reagent unless stated otherwise.

Preparation of biomass

The preparation procedures of biomass is similar to that of other strains with slightly modification. The preculture (each 50 ml of Luria-Bertani medium in 250 ml flask) of the denitrifying bacteria CFMI-1 was inoculated with a single colony repeatedly isolated from nearby rivers. LB medium generally comprises of peptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L and then the mixture was incubated on a rotary shaker at 30 ^oC for 24 h. The expansile culture (each 250 ml of batch medium in 1000 ml flask) was inoculated with 10% *v*/*v* of the preculture and then, incubated on a rotary shaker at 30 °C for 76 h. Batch medium generally contains KNO_3 0.36 g/L, KH_2PO_4 1.5 g/L, sodium succinate 2.81 g/L, $Na_2HPO_4 \cdot 12H_2O$ 10.55 g/L, $MgSO_4 \cdot 7H_2O$ 0.1 g/L, trace element solution 2 ml/L. The trace element solution contains ethylene diamine tetraacetic acid (EDTA) 50 g/L, $ZnSO_4$ 2.2 g/L, $CaCl_2$ 5.5 g/L, $MnCl_2 \cdot 4H_2O$ 5.06 g/L, $FeSO_4 \cdot 7H_2O$ 5.0 g/L, $CuSO_4 \cdot 5H_2O$ 1.57 g/L, $CoCl_2 \cdot 6H_2O$ 1.61 g/L, pH7.0. The spent medium and biomass were prepared synchronously for further experiments by firstly centrifugation in 10000 r/min for 20 min and then filtration with 0.22 μm filter membrane.

Preparation of GO (graphene oxide)

GO was prepared from graphite powder using a modified method of Hummers and Offeman. Graphite powder (0.5 g) was mixed with 20 mL H_2SO_4 and 5 mL HNO_3 in an ice bath. $KMnO_4$ (3 g) was slowly added to the mixture. The solution was heated at 35°C for 3 h, and then diluted with 40 mL of deionized water. In two hours, the solution was further diluted by adding an additional 100 mL of deionized water, followed by slow adding 3.0 mL of H_2O_2 (30% *v*/*v*). After these steps, the black

graphite suspension was converted into a bright yellow graphite oxide solution. The precipitate of graphite oxide was isolated by centrifugation at 1500 r/min for 30 min and washed with, and then re-suspended in, deionized water. The aqueous graphite oxide solution was then sonicated for 2–4 h to facilitate the exfoliation of stacked graphite oxide sheets into monolayer or multi-layered GO sheets. The as-prepared GO yellow brown solution (0.1% m/m) was used for further experiments.

Preparation of N-doped graphene

Bacterially-reduced N-doped graphene was prepared according to the method described previously with slight modification. Briefly, the batch medium (contains KNO_3 0.36 g/L, KH_2PO_4 1.5 g/L, sodium succinate 2.81 g/L, $Na_2HPO_4 \cdot 12H_2O$ 10.55 g/L, $MgSO_4 \cdot 7H_2O$ 0.1 g/L, trace element solution 2 ml/L.) was mixed with GO suspension (0.1% m/m) in volume ratio of 1:1 in 1 L flask. The mixed solution was filled with nitrogen for 30 min to remove the dissolved oxygen. Then, the mixed medium was inoculated with seed liquid of 10% *v*/*v* from the preculture and finally incubated under anaerobic conditions in incubator at 30°C for 72 h (DNMG-1) and 120 h (DNMG-2). After the incubation period, the stable black dispersion was sonicated for 5 min to disperse the cells from graphene materials. The suspension was centrifuged at 10,000 r/min for 20 min to collect cells and precipitate. The precipitate was washed with 10% hydrochloric acid, 80% ethanol repeatedly and finally washed with deionized water until neutral pH. The pure products were dried overnight in a vacuum freezing drying oven.

Analytical methods and characterization

The mechanism experiment of GO-to-graphene was completed as below. As described previously, blank medium, spent medium, biomass and mixture were acquired from the procedure of preparation of biomass. The three components with blank control were mixed respectively with GO suspension (0.1% m/m) in the volume ratio of 1:1 in 20 mL serum bottles and the four flasks were incubated under anaerobic conditions at 30°C for 5d. Three sets of parallel experiments were carried out. Raman spectra were collected with a Renishaw Invia Raman Microprobe (laser wavelength 514 nm). X-ray photoelectron spectra (XPS) were carried out on a RBD

upgraded PHI-5000C ESCA system (Perkin Elmer) with Mg K_α radiation (hν=1253.6 eV). The XPS peaks were deconvoluted using Lorentzian-Gaussian components after a shirley background subtraction. Energy Dispersive X-ray Detector (NORAN SYSTEM7, Thermo scientific) were performed at 250kV. TEM image was obtained by Field emission transmission electron microscope (JEM-2100F JOEL). Samples for AFM imaging were prepared by drop-casting the dispersive DNMG onto freshly cleaved mica substrates, which were then allowed to dry in air. Field-emission scanning electron microscopy (FESEM) was performed with a LEO1550 microscope. Fourier transform infrared spectra (FTIR) were run on a Bruker Model IFS 66v/s spectrophotometer. The O.D. value of samples were collected by UV visible spectrophotometer (Thermo Fisher Evolution 220, the United States).

The cyclic voltammetry experiments were done with platinum electrodes. Firstly, the platinum electrodes were perpendicularly polished to form a mirror surface with alumina slurry. Secondly, platinum electrodes were rinsed with deionized water and dried by blowing nitrogen. Finally, a 10 μl suspension (containing sample of 10 mg, isopropyl alcohol of 5ml and a drop of Nafion) was carefully dropped onto the electrodes and dried at room temperature using 5 mM solution of potassium ferricyanide [$K_3(FeCN)_6$] containing 0.1 M KCl as electrolyte. Scanning speed is 5mV/s and scanning scope is from -0.3V-1.0V. (Electrochemical workstation, Tianjin Lan Li Ke Chemical Electronic High Technology Co Ltd, LK 2005A).

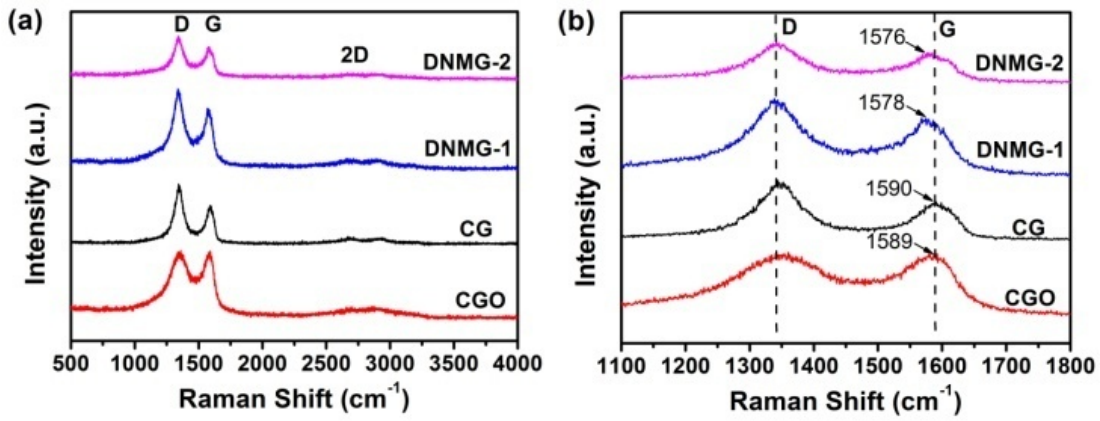

Fig. S-1. Raman spectra of (a) the wide spectrum and (b) high resolution spectrum.

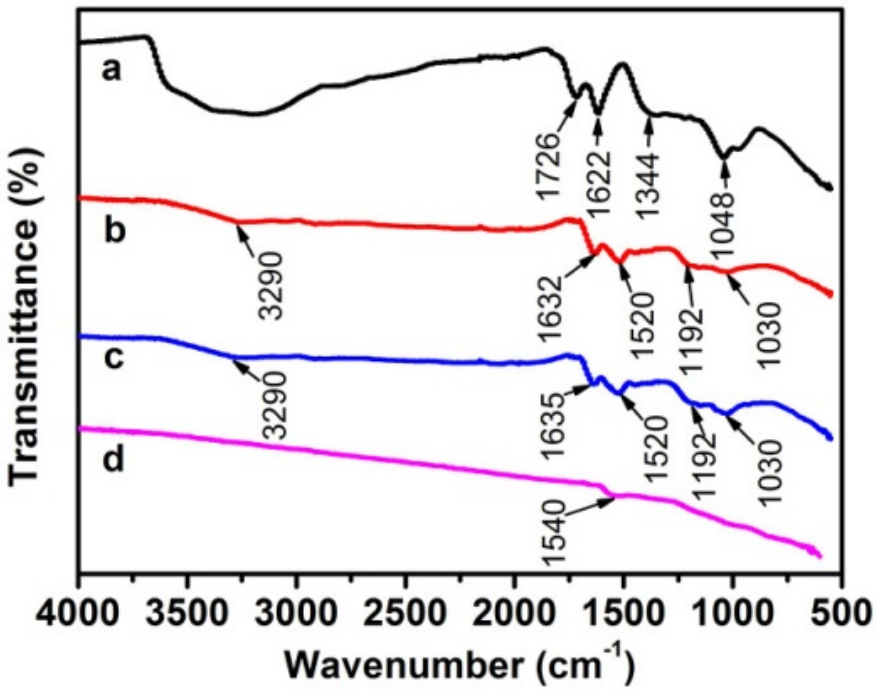

Fig. S-2. Fourier transform infrared spectra of (a) CGO, (b) DNMG-1, (c) DNMG-2, and (d) CG.

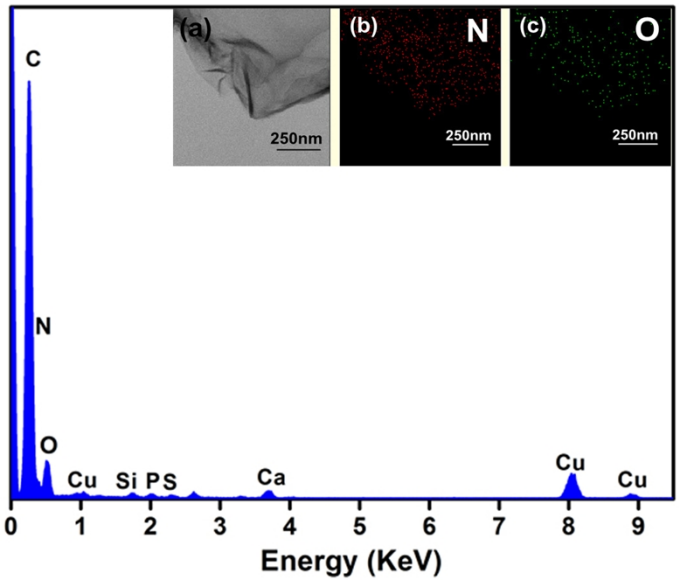

Fig. S-3. EDX spectrum of DNMG-1. (inset: TEM image of DNMG-1 (a), mapping of N (b) and mapping of O (c).)

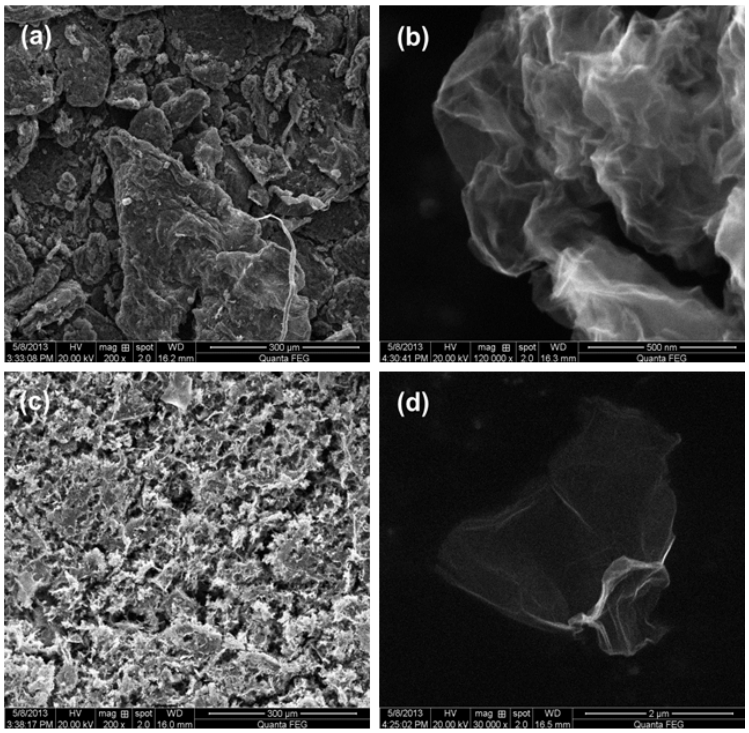

Fig. S-4. FESEM of images of (a ×200, b ×120 000) CG and (c ×200, d ×30 000) DNMG-1.

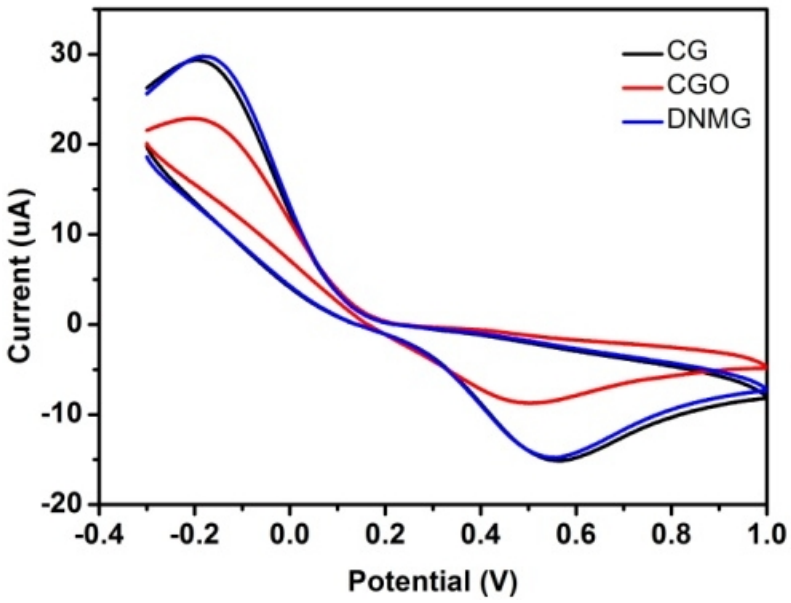

Fig. S-5. Cyclic voltammetry of CG, CGO, and DNMG.

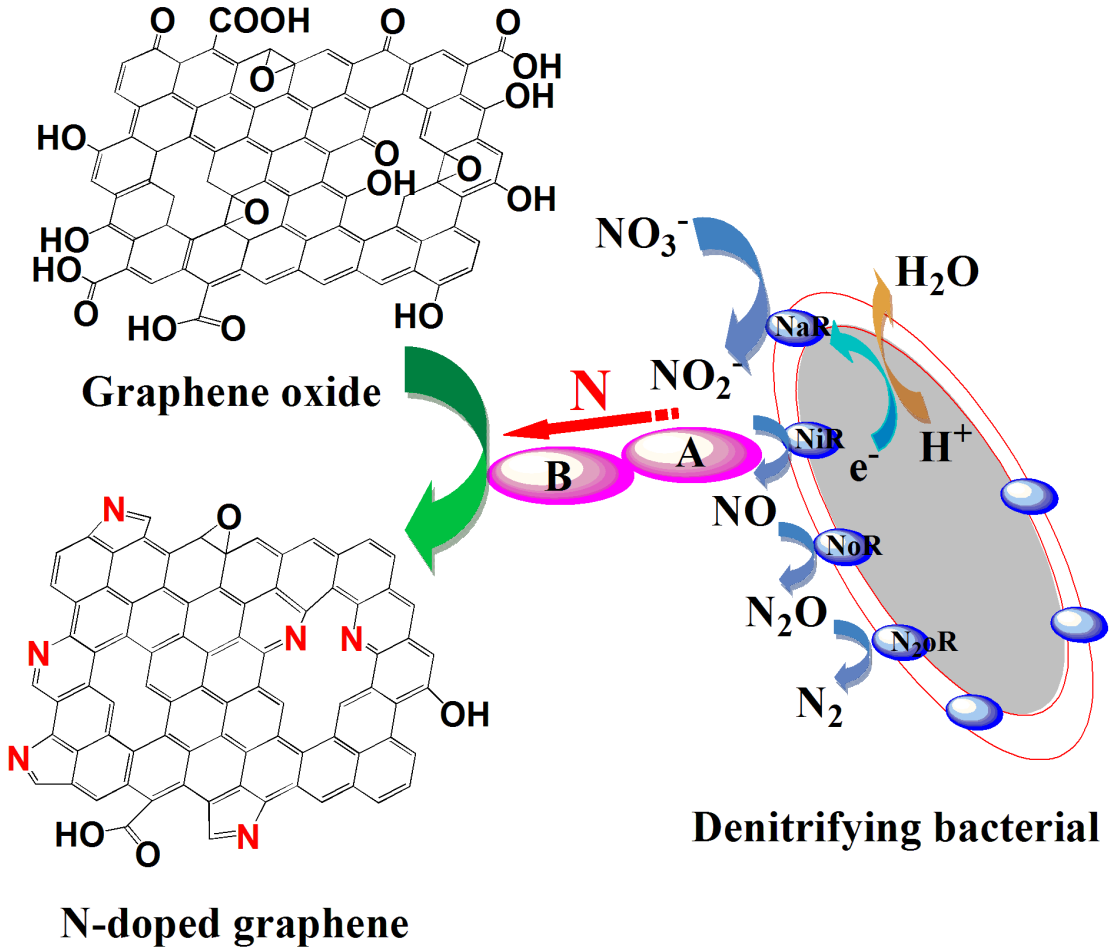

Fig. S-6. Proposed mechanism of biosynthesis of N-doped graphene by denitrifying bacteria CFMI-1. Blue dots represent a series of reductase, such as nitrate reductase (NaR), nitrite reductase (NiR), nitric oxide reductase (NoR) and nitrous oxide reductase (N_2oR). Pink dots (A and B) represent unknown outermembrane proteins, which may be involed in graphene oxide reduction and graphene N-doped.

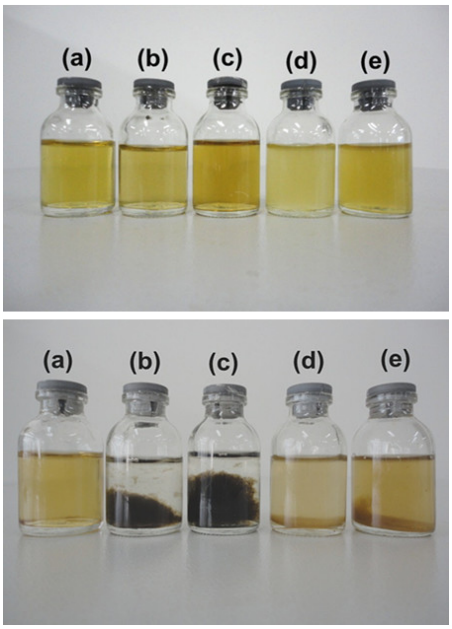

Fig. S-7. GO suspensions reduced by different components at 30℃ for 5d in constant temperature incubator: (a) blank control with denitrifying medium only, (b) and (c) spent medium without cells, (d) and (e) cells only.

Table S-1 Nitrogen atomic percent of DNMG prepared by denitrifying bacteria CFMI-1.

Sample	GO	DNMG-1	DNMG-2	CG
C% (atom %)	52.8	64.3	68.1	97.9
N% (atom %)	0	8.0	8.2	0
O% (atom %)	47.2	27.7	23.7	2.1

Table S-2 The peak area ratios of the oxygen and nitrogen-containing bonds to the C-C bonds for the as-prepared GO and the reduced GO by denitrifying bacteria CFMI-1.

Sample	GO	DNMG-1	DNMG-2
$A_{C\text{-}OH}/A_{C\text{-}C}$	0.52	0.36	0.20
$A_{C\text{-}O\text{-}C}/A_{C\text{-}C}$	1.05	0.36	0.33
$A_{O=C\text{-}OH}/A_{C\text{-}C}$	0.54	0.33	0.31
$A_{C\text{-}N}/A_{C\text{-}C}$	-	0.19	0.51

Table S-3 Green reducing agents and environment-friendly methods for reduction of GO

no.	reduction method or agents	react conditions	N content at. %	reference
1	vitamin C	95°C, 4 h, Water or common organic solvents (DMF or NMP)	-	1
2	polyphenols of green tea	40-80°C, 10 min, water	-	2
3	sugar	95°C, 30min, $NH_3 \cdot H_2O$, glucose	-	3
4	glucose	95°C, 30min, water	-	4
5	DMF reduction	153°C, 1 h, DMF+CO	-	5
6	solvothermal reduction	200°C, 24 h, 1:1 H_2O:DMF	-	6
7	*Shewanella*	anaerobic or aerobic conditions, room temperature, 72h	-	7
8	*Eschericchia coli*	anaerobic conditions, room temperature, 72h	-	8
9	Denitrifying bacteria (our method)	anaerobic conditions 30°C for 72 h	8.2	our method

Reference

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