'Template-free' Hierarchical MoS_2 Foam as Sustainable 'Green' Scavenger of Heavy Metals and Bacteria in Point of Use Water Purification

Paresh Kumar Samantaray [a, b], Sushma Indrakumar [b], Kaushik Chatterjee [a, b, c], Vipul Agarwal [b, d*] and Suryasarathi Bose [b*]

[a] Centre for BioSystems Science and Engineering, Indian Institute of Science, Bangalore, India

[b] Department of Materials Engineering, Indian Institute of Science, Bangalore, India; E-mail: sbose@iisc.ac.in

[c] Manipal Institute of Regenerative Medicine, Manipal Academy of Higher Education, Bangalore, India

[d] Centre for Advanced Macromolecular Design (CAMD), School of Chemical Engineering, University of New South Wales, Sydney, NSW 2052, Australia;
E-mail: agarwalvipul84@gmail.com

Supporting Information

Materials and Methods

Bovine serum albumin (BSA), sodium chloride (≥ 99 %), molybdenum (IV) sulfide (MoS_2) (< 99%), lead nitrate (99.999 %) and sodium meta arsenite (≥ 99 %) were procured from Sigma Aldrich. Dulbecco's Modified Eagle's Medium (DMEM) was procured from Lonza. Penicillin-streptomycin (Gibco) was procured from Fisher Scientific.

Exfoliation of BSA mediated MoS_2 and fabrication of 3D foam

BSA mediated MoS_2 exfoliation was performed as per previously reported protocol[1] with some modifications. Briefly, BSA (1 mg/mL) was dissolved in double-distilled water at room temperature (~23°C). The bulk MoS_2 (5 mg/mL) was dispersed in the dissolved BSA solution and sonicated using the bath sonicator (Electrosonic Industries, Model# EL6.LHI, 30±3 KHz and 100 Watts) for a period of 192 h to obtain the characteristic greenish-grey color dispersion. The dispersion was centrifuged at 13000 rpm for 2 min to remove any non-exfoliated bulk material.

The supernatant was further centrifuged to remove unbound protein. The final dispersion was lyophilized to obtain greenish-black foam product.

Material Characterization

The fabricated MoS_2 foam was characterized through Fourier transform infrared (FTIR) spectrum on a Perkin Elmer Frontier (wavelength from 4000 cm^{-1} to 400 cm^{-1}), XRD using X-Pert PRO PANalytical equipment using Cu target. Diffraction intensities were recorded as a function of 2-theta ranging from 10° to 60° at a step width of 0.03° and a count time of 2 s.

The morphology of the foam was assessed using the scanning electron microscopy (SEM) (Ultra55 FE-SEM Karl Zeiss) at an accelerating voltage of 5 kV. To quantify the amount of the bound protein, lyophilized exfoliated samples were re-dispersed in the water at the concentration of 1 mg/mL and subjected to colorimetric Pierce BCA protein assay kit (Thermo Scientific) as per manufacturer's protocol.

Inline Adsorption Studies

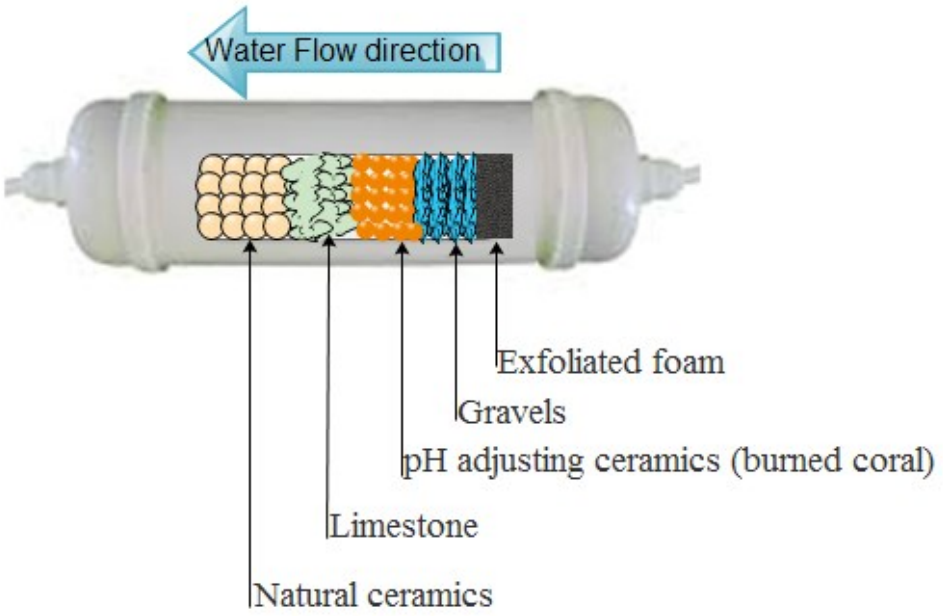
For the inline adsorption studies, four different concentrations of lead (Pb II) and arsenic (As III) were formulated in double distilled water i.e. 5 ppm, 10 ppm, 20 ppm and 50 ppm. The stock solution of 100 ppm for Pb (II) was made by dissolving exactly 0.1599 g of lead nitrate to 10 mL of 2 % (v/v) HNO_3 and then diluted in 990 mL of double-distilled water. This formulation was serially diluted to make different specific concentrations. For As (III), 1 L double distilled water was bubbled with nitrogen gas for 15 min to remove dissolved oxygen in it. To this, 175.14 mg of sodium meta arsenite was added. This was 100 ppm arsenic (III) solution. This stock was serially diluted to obtain other concentrations.

Experimental design

The inline adsorption based pre-filtration kit was designed as per the schematics shown in scheme-SI1. Each segment is separated by a retainer membrane of 0.1 μm poresize. In all the inline studies, the active exposed layer was the MoS_2 foam. For control experiments, bulk MoS_2 was taken in the place of MoS_2 foam. The influx flow rate was set at 0.05 LPM using a flow meter. Inductively coupled plasma optical emission spectrometry (ICP-OES) was performed on

the feed and permeate and the heavy metal rejection (in %) was determined using equation (1). Triplicates of each elution were taken for statistical comparisons.

% Rejection= 100- ((permeate conc. in ppm)/ (feed conc. in ppm)) (1)



Scheme-S 1: Inline adsorption kit as designed for the experiments.

Cell culture and cytotoxicity assays

The mouse lymphoid endothelial cell line (SVEC) was cultured in complete DMEM growth medium containing fetal bovine serum (FBS-10%; Invitrogen Gibco) and penicillin/streptomycin (1%, Invitrogen Gibco). Lyophilised BSA exfoliated MoS_2 was sterilized under UV and reconstituted in complete growth media. Different dilution of the reconstituted material was prepared (0, 5, 12.5, 50, 100, 200 µg/mL). The cells were incubated at 37 °C in a humidified atmosphere of 5 % CO_2. All experiments were carried out with cells between passages 5-6.

Cell viability was conducted using the LIVE/DEAD Assay Kit (Invitrogen, UK) as per manufacturer's protocol.[2-4] In brief, 1000 cells/well concentration were seeded in a 96 well plate and incubated to stabilize for 24h. The next day, the exfoliated material was added at the

specified concentration (0, 5, 12.5, 50, 100, 200 µg/mL) and incubated for 24 h in the humidified incubator at 37 °C with 5 % CO_2. Following which the cells were washed twice with PBS, stained with calcein AM (100 µL, 1 µM)/ ethidium bromide (100 µL, 2 µM) and incubated in the incubator for 30 min before imaging. Images were captured using an Olympus IX71 inverted microscope with a 10x objective with fixed exposure time. Both live and dead cells were counted using Image J software (NIH) with cell counter plugin. Data are presented as mean ± standard error mean (n = 4).

WST-1 assay (Roche Applied Sciences, USA) was employed to assess cell proliferation using the manufacturer's protocol. Briefly, 1000 cells/well concentration were seeded in a 96 well plate and incubated for 24 h. Next, exfoliated material was added at the specified concentration (0, 5, 12.5, 50, 100 and 200 µg/mL) made in complete media and the plates (individual for each time point) were incubated for 72 h in the humidified incubator at 37 °C with 5 % CO_2. At specific time point wells were washed with 1x PBS and supplemented with WST-1 solution (10 µL/100 µL media) in each well and incubated for 3 h in dark in the humidified incubator at 37 °C with 5 % CO_2 following which the plate was read under a plate reader at 450 nm excitation wavelength. Same protocol was followed at every time point for next 2 days. Data presented as mean ± standard error mean (n = 3). The results for cell viability and cell proliferation were analyzed for analysis of variance (ANOVA) and the significance was evaluated using Bonferroni and Turkey's post-hoc analysis at 95% confidence ($p < 0.05$). All the analysis was carried out using Origin statistical software.

Antibacterial Studies

The antibacterial study was performed using *E.coli* ATCC 25922 (gram-negative bacteria). Single colony was isolated from the streak plates of the bacteria and grown in the Luria Bertani (LB) broth at 37 °C and harvested at the mid-exponential growth phase. The cells were centrifuged, washed with 1x PBS three times and re-suspended in 1x PBS. The cells were normalized to 1.5 x 10^7 CFU/mL for plate count and ROS studies.

Standard plate count

The effect of concentration and the incubation time was studied for both bulk MoS_2 and fabricated MoS_2 foam. Different concentrations (5, 12.5, 50, 100 and 200 μg/mL) of bulk MoS_2 and the MoS_2 foam were prepared by dispersing the stipulated amount of the material in the bacterial suspension. These suspensions were incubated in a shaker incubator with 300 rpm and the incubation time was varied from 0.5 h to 4 h 37 ^{o}C. The viable cells after each incubation period were assessed by plating the suspensions via serial dilutions on freshly prepared nutrient agar plates and measured using the standard plate count method.

Intracellular reactive oxygen species

For 200 μg/mL of the material-bacterial suspension, the intracellular reactive oxygen species (ROS) generated was evaluated using dichlorodihydrofluorescein diacetate (DCFH-DA) assay as a function of time. After incubation for different time points, 50 μL of DCFH-DA dye was added to the material-bacterial suspension and kept in dark for 30 min to facilitate cellular uptake of the dye. The fluorescence intensity was measured at an excitation wavelength of 485 nm and an emission peak at 528 nm. The obtained ROS value (in counts per second) is then divided by the number of live cells in each case to obtain ROS/CFU. The control for this experiment was bulk MoS_2 of the same concentration.

Mode of action: DCFH-DA dye is taken up by the cells where it gets de-acetylated by cellular esterase to non-fluorescent 2′,7′-dichlorodihydrofluorescein (DCFH) which is rapidly oxidized by the reactive oxygen species (ROS) to highly fluorescent 2′,7′-dichlorodihydrofluorescein (DCF)).[2, 5] DCF emits fluorescence and the amount of fluorescence is directly co-related to the amount of the reactive species.

List of tables:

Table S1: Recent reports on 2D material and foams for lead and arsenic remediation in water.

Adsorbent System	Pb(II) removal(%)	As removal (%)
Low temperature exfoliated graphene[6]	95 %[a]	-
Magnetite reduced graphene oxide composite[7]	-	99.9 %[b]
Graphene oxide foam[8]	> 85 %	-
Few layer graphene sheets[9]	98 %[c]	-
3D graphene foam[10]	~90 %	~90 % for As (V)
Graphene oxide and polyethylenimine foam[11]	~98 %[d]	-
Phosphorylethanolamine- 3D graphene foam[12]	85%	-
Lignosulphonate modified graphene[13]	90 %	-
Graphene oxide/$CuFe_2O_4$ foam[14]	-	~95 % for As (III)
This work	99.9 %[e]	98.7 %[e]

[a]: for 5 ppm lead concentration; [b]: up to 7 ppm of arsenic was taken in this study; [c]: 120 mg/L of Pb (II) taken for the study; [d]: study performed at pH 5; [e]: concentration of lead and arsenic was 5 ppm. For all the other feed concentrations up to 50 ppm >98 % for lead and >97 % of arsenic removal was observed in our study.

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