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Chromium remediation and toxicity assessment of nano zerovalent iron against contaminated lake water sample (Puliyanthangal Lake, Tamilnadu, India)

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ABSTRACT

Since its high toxicity and mobility, hexavalent chromium is considered to be a high priority pollutant. This study was performed to carry out a remediation test along with toxicity assessment in a water sample collected from the saturated zone of a historically Cr (VI)-contaminated site known as a Puliyanthangal lake (Tamilnadu, India) using nanoscale zero-valent iron (nZVI). The water samples were examined before and after the nZVI application by means of microbial cultivation tests, phospholipid fatty acid analysis (PLFA) and toxicological tests. The present experimental results revealed that Cr (VI) is considerably adsorbed on nZVI nanoparticles and it could be a cost-effective method for the in situ remediations of Cr (VI). In addition, standard plate count assay showed the dose-dependent decrease in the bacterial cell viability in lake water sample after the addition of nZVI.

1. Introduction

Chromium is one of the most abundant heavy metals, causing pollution of groundwater and soil due to its frequent industrial application. Chromium occurs naturally mainly in the trivalent Cr (III) and hexavalent Cr (VI) forms. The majority of its adverse effects is caused by Cr (VI) because of its solubility, mobility and high oxidizing potential leading to generally higher toxicity causing health problems such as liver damage, pulmonary congestion, vomiting and severe diarrhea (Nriagu and Nieboer, 1988). On the other hand, Cr (III) is less reactive and toxic and can be readily precipitated out of solution. Therefore, the majority of in situ treatment methods employed at the present time utilizes geo-fixation of Cr (VI) by its reduction to Cr (III) and formation of insoluble Cr (III) compounds (Jardine et al., 1999). A number of articles have been published to date describing various applications of individual biological or chemical approaches to precipitate chromium into its insoluble Cr(III) form. One of the most promising methods is reduction using iron-based materials such as zero-valent iron and dissolved Fe (II) and solids containing Fe(II) (Barrera-Díaza et al., 2012). Interest has increased over the past few years in using zero-valent iron (Fe0) and its respective nano-scale form to reduce chromium (VI) contamination.

Zero-valent iron (ZVI) is a readily available and low-cost reducing agent that is also used extensively to remove a number of other kinds of contaminants, such as chlorinated compounds, pesticides, and heavy metals e.g. As(V). Although the efficiency of ZVI and especially nano-scale ZVI (nZVI) in reducing the concentrations of Cr(VI) and other pollutants is well documented, only a few works have focused on its ecotoxicity for native organisms in the soil (Ahamed et al., 2016).

This study was performed to carry out a remediation test along with toxicity assessment in the saturated zone of a historically Cr (VI)-contaminated site known as Puliyanthangal Lake (Tamilnadu, India) using nZVI. The water samples were examined before and after the nZVI application by means of microbial cultivation tests, phospholipid fatty acid analysis (PLFA) and toxicological tests.

Based on the available literature, not sufficient work was carried out on this study area Puliyanthangal Lake (Tamilnadu, India) and these combinations of study like remediation test along with toxicity assessment. These phenomena provide novelty of the research investigation.

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2. Materials and methods

2.1. Chemicals

$FeCl_3 \cdot 6H_2O$ (Analytical grade) was procured from SD Fine Chemicals Ltd (India). All other reagents used were of analytical grade.

2.2. Synthesis of nano zero valent iron particles

For the synthesis of nZVI; 0.5406 g $FeCl_3 \cdot 6H_2O$ was dissolved in a 4/1 (v/v) ethanol/water mixture (24 mL ethanol + 6 mL deionized water) and stirred well followed by the addition of 10 mL of *Parthenium hysterophorus* plant extract. Then the sample was incubated in dark for 24 h. After 24 h, the sample was measured for its maximum absorbance using UV–Visible spectrophotometry. The sample was then heat dried to obtain the synthesized nano zero valent iron particles for characterization (Ahamed et al., 2016).

2.3. Characterization of the synthesized nano zerovalent iron particles

Prepared nanoparticles were characterized by means of various physicochemical studies includes; UV–Visible spectroscopy analysis, Fourier transform infrared spectroscopy (FTIR) analysis, scanning electron microscopy (SEM) analysis, transmission electron microscopy (TEM) analysis and Dynamic Light Scattering (DLS) analysis (details in our previous report, Ahamed et al., 2016).

2.4. Chromium remediation test

Water and soil samples were collected from Puliyanthangal Lake (leather industrial site and study area). The initial and final water characterization including determination of pH, temperature, DO, TDS etc., was carried out using water quality analyzer (ELICO-PE 138, India). Metals concentration was determined after acid digestion according to the EPA (US-Environmental Protection Agency) method followed by atomic absorption spectrophotometry (AAS) analysis. The concentration of Cr (VI) was determined by the colorimetric method using diphenyl carbazide after alkaline digestion.

2.5. Phospholipid fatty acid analysis (PLFA)

Chromium contaminated soil samples for PLFA were extracted with a mixed chloroform-methanol–phosphate buffer (1:2:0.8). Phospholipids were separated using solid-phase extraction cartridges (LiChrolut Si 60, Merck, Germany) and the samples were subjected to mild alkaline methanolysis. The free methyl esters of phospholipid fatty acids were analyzed by gas chromatography-mass spectrometry (456-GC, SCION SQmass detector, Bruker, USA). Similarly, groundwater samples for PLFA analyses were prepared by filtration through microbial filters (0.2 μm). These filters were then extracted, fractionated and analyzed using the above-said method.

2.6. Cytotoxicity assessment

All the experiments were carried out on the dominant bacterial species *E.Coli,* isolated from the lake water and an initial cell count of 5×100 CFU mL^{-1} was maintained throughout the study. Bacteria interacted with three different low concentrations (0.05 ppm, 0.5 ppm, and 1 ppm) of nZVI dispersion.

All the experiments were carried out in parallel in two sets, under light and dark conditions. For light induced studies, test beakers were exposed to UV irradiation through UV lamps (Philips, 15 W, Poland) and for supporting non-illuminated studies, complete darkness was maintained. Control beakers without nZVI addition were kept for both light and dark conditions. Samples were analyzed after an interaction period of 3 h, 6 h, and 24 h respectively.

2.7. Cell viability assessment

Percentage reduction in cellular viability after the interaction period (3 h, 6 h, and 24 h) was determined using the standard plate count (SPC) assay as described by Mossman. (1983). In the recent past, the SPC assay has been employed as a method for analyzing the cellular viability of prokaryotes (Jardine et al., 1999). The decrease in bacterial viability in test samples was calculated with respect to control. Cell cultivability was determined using standard plate count assay on depicts nutrient agar medium.

2.8. Live–dead cells discrimination through fluorescence microscopy

Membrane permeability of treated cells was observed by fluorescence microscopy (Leica, DM-2500) after 3 h, 6 h and 24 h of NP interaction. Cells were stained with Acridine orange (AO) and Ethidium Bromide following the protocol described by Jakopec et al. (2006). 40 To 500 μL of bacterial suspension, 4 μL of AO (15 μg mL^{-1} in PBS) and 4 μL of EtBr (50 μg mL^{-1} in PBS) were added. After 5 min incubation, the cell suspension was centrifuged and the supernatant was discarded to eliminate unbound dyes. The cell pellet was resuspended in 500 μL PBS. The dark condition was maintained to avoid photobleaching of dyes. Fluorescence was detected by the BP 450–490, LP 590 filter; images were captured with a Leica-DFC-295 camera and processed using Leica-Application Suite 3.8.

2.9. Oxidative stress assessment

The fluorescence probe DCFH-DA (2'-7'-Dichlorodihydrofluorescein diacetate) was used to quantify generation of reactive oxygen species (ROS). DCFH-DA is membrane permeable and oxidizes to form the green fluorescent DCF in the presence of cellular esterases and ROS. Intracellular ROS generation was monitored in control and NP interacted bacterial cells at 3 h, 6 h and 24 h following the protocol described by Wang and Joseph with minor modifications (1999). 5 mL of cell suspension was incubated with DCFH-DA with a final concentration of100 μM at 37 °C for 30 min.

Fluorescence was measured using a Spectrofluorometer (SL174, ELICO, India) with excitation and emission wavelengths of 485 nm and 530 nm, respectively. A negative control of nZVI without cells was also analyzed to find out the auto fluorescence of nZVI that may interfere with the DCFH-DA dye.

Superoxide dismutase activity in interacted cells was measured by the method described by Winterbourne et al. using riboflavin as the O_2 generator (Wintherbourn et al., 1975). This method depends on the ability of the enzyme to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide, generated by the reaction of photo-reduced riboflavin and oxygen.

Glutathione reductase catalyzes the reduction of oxidized glutathione (GSSH) to reduced glutathione (GSH). This enzyme enables the cell to sustain adequate levels of cellular GSH, which acts as an antioxidant, reacting with free radicals and organic peroxides. The activity of glutathione reductase in interacted cells was monitored spectrophotometrically using a commercially available Glutathione reductase assay kit. Data were reported as percentage release of GSH compared to control.