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A novel bio-sorbent comprising encapsulated *Agrobacterium fabrum* (SLAJ731) and iron oxide nanoparticles for removal of crude oil co-contaminant, lead Pb(II)

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ABSTRACT

A novel adsorbent, iron oxide nanoparticles and *A. fabrum* strains encapsulated in calcium alginate, was prepared for the removal of lead. The crude oil degrading strain was isolated from core samples from Assam Oilfield, India and was able to resist lead up to 2900 mg L^{-1} thus proving to be a highly lead tolerant. Chemically synthesized Fe_3O_4 magnetic nanoparticles (10–20 nm) by co-precipitation method were characterized using various techniques. Vibrating Sample Magnetometer (VSM) showed saturation magnetization of 46.6 emu g^{-1}, indicating superparamagnetic nature of synthesized MNPs, which will aid in separation of biosorbent after adsorption. Lead removal was studied using the bio-sorbent synthesized by immobilizing biomass and MNPs in calcium alginate beads at 200 rpm and 37 °C, and the adsorption capacity was found to be maximum at pH 5.5. Adsorption kinetics was examined using pseudo first order, pseudo second order and intra-particle diffusion (IPD) models. The kinetic data agreed to pseudo second order model and the adsorption rate constant (k_2) decreased with an increase in initial concentrations. Initial adsorption rate (h, mg g^{-1} min^{-1}) was found to be kinetically controlled and the function of initial Pb(II) concentration. The IPD was found rate limiting step after 60 min of adsorption. Adsorption data were fitted well to Langmuir isotherm. The maximum adsorption capacity of the synthesized bio-sorbent was found to be 197.02 mg g^{-1}. Thermodynamic studies suggested that the adsorption process was spontaneous and endothermic in nature. Biosorbent showed good adsorption capacity even after repeatedly using them for five consecutive cycles without compromising much with adsorption efficiency.

1. Introduction

Petroleum and its products have proven to be beneficial since their discovery, but now, because of their complex hydrocarbon make-up, these are becoming a major cause of water and soil pollution [1]. Deliberate and accidental spills of crude oil have serious repercussions on the overall quality of the soil of the region [2]. Previous studies have already confirmed that many oil drilling sites are co- contaminated with heavy metals that act as an inhibitor for the natural biota [3]. The co-contamination of oil with heavy metals is found mostly in the sites affected by oil spills [4]. US Environment Protection Agency (EPA) has ranked combination of binary pollutants among top 12 contaminants of a major concern [5]. Heavy metals released into the surface and groundwater have been a major preoccupation for many years because of their increased discharge, acute toxicity, non-biodegradable nature and the tendency for bioaccumulation [6].

A study has recently reported that in the early stages of hydrocarbon degradation a group of Proteobacteria dominates the microbial assemblage, including the alpine and polar soils [7]. In a study [8] it has been found that symbiotic nitrogen fixing bacteria associated with legumes in wetlands are very sensitive to crude oil pollution. Hydrocarbon-degrading capability of the nitrifiers (Nitrosomonas and nitrrobacter) was affected the most due to it, but the hydrocarbon degrading capability of free nitrogen fixing bacteria like *Azotobacter* sp, *Bacillus polymyxa* and *Pseudomonas aeruginosa* was not affected. They effectively grew and utilized crude oil as the sole source of carbon and energy and hence bioremediate crude oil polluted soil [8].

As per earlier reports heavy metal remediation using bacteria isolated from contaminated sites gives an upper hand as these are resistant to high metal concentrations and also possess high

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binding affinity [9]. Bio-surfactants, produced extracellularly or as part of the cell membrane by bacteria, yeast and fungi, are widely applied in the area of environmental remediation, and few reports also suggested their suitability to remove heavy metals from solutions [9]. However, not many investigations about the metal adsorption on bio-surfactant producing microorganisms have been reported yet [9]. Thus our study with a bio-surfactant producing/oil-degrading bacteria may open up new fields of investigation.

A successful bioremediation program requires specifically tailored strategies that are a prerequisite for a method to work. Bio-sorption is on one of the emerging methods for bioremediation, in which accumulation of contaminant takes place on the surface of the adsorbent [6]. This process is a fast method of passive adsorption of metals from contaminated soil by non-growing biomass/adsorbents. Due to the affinity of metals ions for the adsorbent, they get bound on the surface by different mechanisms until an equilibrium is reached [10,11]. Choosing efficient biomass/bio-sorbent is the most important challenge for this process to make it inexpensive and proficient. Various metal binding sorbents such as magnetic nanoparticles encapsulated fungi beads, magnetic calcium alginate/maghemite hydrogel beads etc. on the basis of their selectivity had been used for bio-sorption process [6,12]. Microorganisms like bacteria, fungi, yeast and algae have been tested and found to be excellent bio-sorbents [10].

Field investigations have shown that iron oxide magnetite nanoparticles (MNPs) have gained considerable research attentions in environmental remediation, due to their high surface area and unique super-paramagnetism [13–15]. MNPs have also shown considerable potential as immobilization carrier [6,16]. Amalgamation of biotechnology with nanotechnology helps in increasing the practical use of nanomaterials as immobilization agents [6]. The immobilization can also increase the mechanical strength, porous structure and adsorption capacity compared to discrete constituents. Therefore the application of immobilized iron oxide MNPs with biomass is expected to be a potential bio-sorbent for bioremediation.

In this present study, a novel adsorbent using biomass isolated from petroleum core which was found to be highly lead resistant was immobilized using iron oxide nanoparticles and calcium alginate. The beads formed were investigated for batch adsorption studies of lead (Pb) at different concentrations, pH, contact time and temperature. Adsorption kinetics was studied using pseudo first order, second order kinetics and intra-particle diffusion model. Adsorption data were also fitted to Langmuir and Freundlich isotherms to determine the maximum adsorption capacity of the bio-sorbent. Regeneration and reusability studies were tested to find the efficiency of the biosorbent for five consecutive cycles.

2. Experimental

2.1. Materials

Iron(II) chloride tetrahydrate (Cat no. A16327) and Iron(III) chloride hexahydrate (Cat no. A16231) were bought from Alfa Aesar, India. Other chemicals used were of analytical grade and were procured from HiMedia, India. Double distilled water (18 MΩ, Millipore system) was used throughout the experiments.

2.2. Isolation and screening methods

Bacterial strains were isolated from petroleum core samples obtained at Assam oil field, Dibrugarh, India (27.4728° N, 94.9120° E). Isolation was done on Bushnell Haas agar media plates with 1% crude oil obtained from oil reservoirs as the sole carbon source. Positive colonies were then picked up and sub-cultured to obtain pure cultures of bacterial strains for further studies.

Bio-surfactant producing strains were screened using oil plating and oil displacement method. The strains with bio-surfactant producing ability were later tested alteration in surface tension. The strains were grown in Luria Bertini (LB) broth media (pH 7.4) at 37 °C for about 20 h and surface tension of the supernatant was measured using a tensiometer (Dataphysics, DCAT 11EC).

2.3. Determination of minimum inhibitory concentration

Nutrient Agar plates (pH 7.4 ± 2, 25 °C) of composition peptic digest of animal tissue (5 g mL^{-1}), sodium chloride (5 g mL^{-1}), beef extract (1.5 g mL^{-1}), yeast extract (1.5 g mL^{-1}) and bacteriological agar (15 g mL^{-1}) with different concentrations of lead ranging from 100 to 4000 mg L^{-1} were prepared, and inoculated with the isolated bacterial strain. The plates were inoculated at 37 °C for 48 h (Çolak et al. [24]). Nutrient agar plate without lead was used as positive control for the experiment. The concentration at which the bacterium ceases to grow is termed as minimum inhibitory concentration (MIC).

2.4. Identification and characterization of isolates

The isolates were identified on the basis of 16s rRNA sequencing. The bacterial DNA was first lysed and then amplified using Polymeric Chain Reaction (PCR), which was then sequenced in both directions i.e. forward and backward. The primer sequences used were AGAGTTTGATCMTGGCTCAG and TACGGYTACCTTGTTACGACTT. The consensus sequence generated was used to carry out Basic Local alignment Search Tool (BLAST) BLASTn with National Centre for Biotechnology Information (NCBI) database and a phylogenetic tree was constructed using neighbour-joining method by Mega6 software [60].

2.5. Preparation of bio-sorbent

In this part, firstly iron oxide magnetite nanoparticles (MNPs) were synthesized and characterized. Next, biomass was grown and lyophilized. Further, MNPs and biomass were encapsulated in calcium alginate and MBCaAb (MNP-Biomass-calcium alginate) beads were formed.

2.5.1. Synthesis and characterization of MNPs

Magnetic nanoparticles were chemically synthesized using co-precipitation method as mentioned elsewhere [17,18]. Briefly, 1.04 gm of $FeCl_2 \cdot 4H_2O$ and 2.64 gm of $FeCl_3 \cdot 6H_2O$ were gently mixed in 100 mL of deoxygenated water at room temperature. The above solution was continuously stirred mechanically at 600 rpm using a mechanical stirrer (Remi, India) at 80 °C under N_2 atmosphere. Formation of black colored precipitate on dropwise addition of reducing agent (25% NH_4OH) confirmed hydrolysis of iron precursors to MNPs during synthesis [19]. The solution was left on stirring for another 2 h to ensure complete reduction of iron salts to nanoparticles. Later, they were washed several times with water until neutral pH, separated in the presence of an external magnetic field, and dried overnight at 65 °C in a hot air oven.

The magnetic properties of synthesized MNPs were evaluated using vibrating sample magnetometer (VSM) instrument (Lakeshore, 7410 series) at room temperature. Atomic force microscopy (AFM, Agilent-5500 series) was used for nanoparticle's shape and size determination. AFM sample was prepared by ultra-sonicating (Vibra Cell, VC-505) the MNPs in water, drop casting them on a silicon wafer and drying at 37 °C. The dried silicon wafer with nanoparticles was imaged using a silicon nitride tip of <10 nm