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The efficiency of a novel bioreactor employing bacteria and chitosan-coated magnetic nanoparticles

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ABSTRACT

Heavy metals like lead, copper, nickel, and arsenic, which are known to have toxic effects at very low concentrations, have a tendency to bioaccumulate and end up as permanent additions to the environment. The objective of the present work was to investigate the efficiency of a combination of bacteria and chitosan-coated magnetic nanoparticles (CMNPs) in removing heavy metals from industrial effluents. To perform the study, we constructed a novel two-stage reactor containing bacteria and CMNPs, using a design theoretically calculated using Aspen HYSYS 7.2 process-modeling software. The strain of bacteria used was isolated from various samples of wastewater and identified using colony-forming assay. The bioreactor was tested with both synthetic and industrial effluents containing nickel. Optimal conditions in the bioreactor for both synthetic and industrial effluents were determined for retention time (20–60 min), pH level (0.5–9), CMNP dosage (0.09–1 g/L), and initial metal ion concentration (50–500 mg/L). Maximum removal rates for synthetic and industrial effluents of 83% and 92.1% were obtained.

1. Introduction

Numerous approaches have been tested for adsorption and separation of heavy metals to reduce their toxic effects, including biological methods [1], bioreactor processing [2], and chemical precipitation [3]. Polymer-coated magnetic nanoparticles (MNPs) have proven to be a good option because of the ease with which their surfaces can be modified to refine their functionality in relation to specific bioactive molecules. Among the MNPs, iron oxide is useful and suitable for separation technology because of its easy fabrication and functionalization. In some cases it has demonstrated superparamagnetic properties when used to remove pollutants, exhibiting magnetic properties only within magnetic fields [4–7].

Research on the use of dried herbs and other plants to remove heavy metals has been conducted on synthetic wastewater. In a previous study of the ability of plants to remove heavy metals, especially nickel, from wastewater we compared activated carbon and nonactivated carbon [8]. In another study we used a bioreactor that utilized algae nanoparticles (NPs) for removal of heavy metals [5,9]. For the current study we designed a reactor employing bacteria and chitosan-coated iron oxide NPs to treat actual wastewater as well as synthetic wastewater [9]. The advantages of this reactor configuration include lower cost, availability of materials, and increased adsorption capacity. CMNP reactors for the treatment of drinking water and wastewater have potential as an effective and cost-efficient means of separating and recycling solids and extracting organic pollutants from hostile industrial wastewaters [10]. Recently, the use of MNPs has drawn significant attention due to their specific characteristics [11,12]. NPs coated by magnetic particles with other functionalized materials have potential use in bioreactors for removal of both organic and inorganic pollutants in wastewater [12,13]. CMNPs made up of different types of magnetic and polymer materials have been used recently for heavy metal removal from wastewater [14,15].

For this study, we designed a reactor containing bacteria and CMNPs fabricated in the presence of ammonia, using a design theoretically calculated using Aspen HYSYS process-modeling software. The bacteria were isolated from various samples of wastewater and the strain identified using colony-forming assay. The removal behavior of these sorbents was investigated using nickel as the target metal contaminant because of its extensive environmental impact. Both synthetic and industrial effluents containing nickel were processed in the bioreactor. The effects of several factors such as retention time, pH, NP dosage, and initial nickel concentration were analyzed with Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and ultraviolet-visible spectrophotometry (UV–vis).

2. Materials and methods

2.1. Materials

This study was conducted in Shahr-e-Rey, Tehran, Iran. The samples were sterilized by autoclave and transferred to the laboratory.

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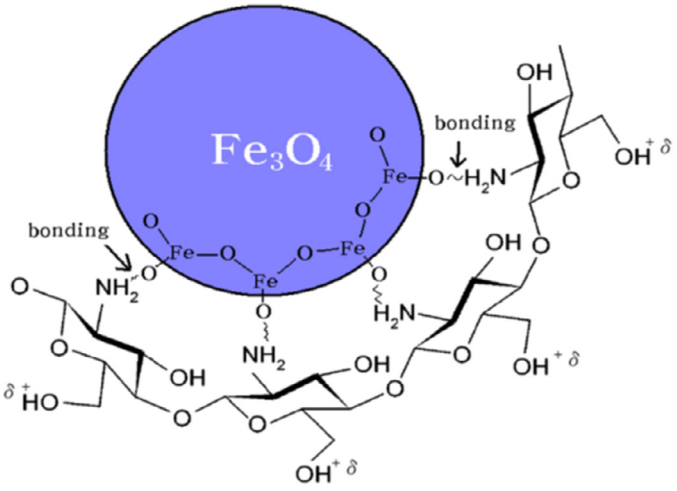

Fig. 1. Coating polymer chitosan around the nanoparticles iron oxide.

Industrial effluents were diluted (7%) and their pH adjusted to normal (pH 7) using 1 M HCl/1 M NaOH. Fe(II) chloride tetrahydrate (99%), Fe (III) chloride hexahydrate (97%), and chitosan polymer (150 kDa) were purchased from Merck of Germany. Müller Hinton broth; nutrient agar; and agar–agar medium containing casein peptone, meat peptone, and glucose were purchased from Sigma–Aldrich, USA.

2.2. Preparation of MNPs

Magnetic Fe_3O_4 NPs were coprecipitated by combining NH_4OH with a ferrous complex. A solution of $FeCl_2.4H_2O$ and $FeCl_3.6H_2O$ [Fe^{2+}: Fe^{3+} = 1:2] was dissolved in 200 mL deionized water and heated to 70 °C under continuous ultrasonic agitation and then added drop by drop into NH_4OH under strong ultrasonic agitation and bubbling N_2 gas for 30 min. The chemical reaction can be described as follows:

$$2FeCl_3 + FeCl_2 + 8NH_3 + 4H_2O \rightarrow Fe_3o_3 + 8NH_4Cl$$

A permanent magnet was used to decant the precipitated black Fe_3O_4 particles, which were then washed repeatedly in deionized water [16].

2.3. Preparation of Fe_3O_4 CMNPs

The CMNPs were prepared in the Fe_3O_4 aqueous suspension using ultrasonic irradiation. In this method 10 mg chitosan was added to 50 mL of 0.02 M HCl and then the pH was adjusted to 5.5 by increasing the amount of 0.1 M NaOH in the solution. The maximum surface adsorption of the chitosan molecules was achieved by addition of 10 mg of Fe_3O_4 NPs, stirred for 30 min by mechanical stirrer (Fig. 1). The precipitate was separated and washed several times in 250 mL of 0.1 M phosphate buffer [17].

2.4. Isolation and cultivation of bacteria

The culture medium was obtained using brain-heart infusion (BHI) agar, combining 37 g of BHI powder with 1000 mL of distilled deionized water at 25 °C and stirring at 400 rpm for 10 min. The solution was transferred to an autoclave at a temperature of 121 °C and sterilized for 1 h. For preparation of the pour-plate culture, we diluted the industrial effluents containing our bacterial sample by transferring loopfuls of the specimen from media tube to media tube, with fewer bacteria ending up in each successive tube. The BHI deeps (15 mL) were boiled and liquefied (agar medium will liquefy at 100 °C) and then cooled in a water bath (Without cooling the hot agar medium down, the bacterial specimens would be cooked). After adding the bacterial sample, the agar was poured into sterile petri dishes and incubated for 72 h at 38 °C.

Several tests were performed on the bacterial samples: egg yolk agar, iodine test for starch, urease agar, skim milk, Simmons citrate agar, nitrate reductase, sulfide indole motility medium, methyl red–Voges-Proskauer, gelatin hydrolysis, glucose, glucose screening, arabinose fermentation, d-xylose absorption, and mannitol fermentation. Eight sterile flasks containing the broth culture medium BHI were prepared and sterilized in an autoclave. Four tubes containing 1 mL industrial effluent solution and the other four tubes were inoculated with 100 μL of bacterial suspension and incubated at 37 °C for 7 days. We measured the optical density (OD) three times daily on a spectrophotometer (ELICO SL 207) at a wavelength of 600 nm. Growth was calculated using the following formula:

$$\text{Percentage growth inhibition} = \frac{\text{OD of control}}{\text{OD of control} - \text{OD of test}} \times 100$$

As can be seen in Fig. 2a and b, the highest percentage of growth for both the control and industrial effluent samples occurred between 19 h and 20 h.

2.5. Theoretical bioreactor design

Based on a review of the literature, we selected a nonrandom, two-liquid model to calculate thermodynamic properties. The Aspen HYSYS 7.2 simulator used is beneficial for research, development, design, and production. We were able to use it to design a pilot plant and develop various alternatives (Fig. 3a). In research the software helps to reduce the required number of laboratory experiments. It can be employed for risk-free analysis of various what-if scenarios. We theoretically calculated a new bioreactor using the Aspen HYSYS software and used the data generated to construct a bioreactor for removing nickel from wastewater (Fig. 3b).

2.6. Removal of synthetic and industrial effluents

The synthetic solutions were all prepared using deionized water and analytical-grade salt (Merck). Adsorption was studied using different dosages of NPs in 1000 mg/L solution at initial concentrations of 20, 40, 60, 80, 100, 120, 160, and 200 mg/L and initial pH 8.5. Samples were tested with an atomic adsorption spectrometer. All the adsorption experiments were carried out at room temperature (23.2 °C). The pH of the sample solution was adjusted to the determined optimal values for heavy metal removal by adding 1 M HCl or 1 M NaOH during the equilibrium period using an agitation speed of 150 rpm for 2 h. An optimal concentration of 50 mg/L was selected.

For controlling pH, 20 mL of nickel solution (50 mg/L) was vigorously stirred with 0.1 g NPs. Values of pH between 0.5 and 9 were evaluated for their effect on adsorption of nickel, and a pH of 8.5 was determined to be optimal. All adsorption experiments were carried out using an agitation speed of 150 rpm [18,19].

Industrial wastewater was pumped from the feed tank to the first tank, where it was retained for treatment with bacteria. Following this, the wastewater entered the second tank and was exposed to NPs under the previously determined optimal conditions. In this section the wastewater was stirred vigorously for 2 h. The removal efficiency was measured at 92.1%.

2.7. Determination of the nickel content

The concentration of nickel in the solution was determined by atomic absorption spectrometry, using a GBC-932 Plus Perkin Elmer AAnalyst 300 atomic absorption spectrometer with an air–acetylene burner and equipped with deuterium as background corrector, and