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Biosorption potential of Gum ghatti-g-poly(acrylic acid) and susceptibility to biodegradation by *B. subtilis*

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ABSTRACT

This article reports the biosorption potential of Gum ghatti (Gg)-grafted-acrylic acid (AA) polymer and its susceptibility to biodegradation by *Bacillus subtilis* (BS) in two different liquid media, i.e. phosphate buffered saline (PBS) and mineral salt medium (MSM). The progress of biodegradation was monitored after every 15 days using FT-IR and SEM techniques. The degradation of the polymer was further evidenced by a loss of weight of 23.2% and 27% in BS-MSM and BS-PBS, respectively, after 60 days. The AA-grafted polymer was then utilized for the removal of Pb(II) and Cu(II) from aqueous solution. The adsorption isotherm data were studied using Langmuir, Freundlich, Temkin, Flory–Huggins and Dubinin–Kaganer–Radushkevich isothermal models. High values of correlation coefficients confirmed the applicability of Langmuir isotherm model used to determine the adsorption capacity of the AA-grafted polymer. The maximum adsorption capacity was found to be 84.74 mg/g for Cu(II) and 310.55 mg/g for Pb(II). Kinetic data were evaluated using pseudo first order, pseudo second order, Elovich, intraparticle diffusion and liquid film diffusion models. The experimental kinetic data fitted well with the pseudo second order rate model.

1. Introduction

Contamination of water by different pollutants like heavy metal ions is one of the major environmental concerns faced by the developing countries. Natural erosion of soils and anthropogenic activities are mainly responsible of the dispersion of toxic heavy metals in surface waters. Most of the heavy metal ions are toxic in nature and even very low concentrations of heavy metal ions like Pb(II) and Cu(II) ions have adverse effects to the human health [1,2]. Different methods like adsorption, flocculation-coagulation, photocatalysis, ion exchange, biological degradation and chemical oxidations have been used previously for the treatment of metal ions contaminated water but the adsorption of metal ions on the surface of some solid material is the method of choice. Among affordable adsorbents often used for removal of heavy metal ions in diluted solutions, biopolymers based biodegradable adsorbents are of particular interest. Biopolymers based adsorbents have been extensively used by researchers for the removal of pollutants from solutions and have exhibited different adsorption capacities and affinity towards metal ions [3–6]. Investigating metal ion adsorption potential of crosslinked poly (acrylamido glycolic acid), Rivas et al. [7] observed that this particular resin had higher affinity for lead; in separate study, Navarro et al. [8] modified cellulose by graft polymerization in order to improve its metal adsorption capacity; the resulting polymer showed better adsorption capacity than cellulose.

The choice or synthesis of a particular polymer for adsorption purposes must often be informed by the nature of pollutant and the susceptibility to biodegradation. Polymers that may persist in the environment after use must be discouraged in bioremediation processes, because they are likely to become a burden in the future. Biodegradation of polymers by microorganisms such as bacteria, fungi and algae basically occurs through two major steps, namely biodeterioration and biofragmentation [9]. In the environment, the first step occurs in combination with abiotic factors; the growth of microorganism on the polymer is mainly responsible of the degradation at this stage and begins with deposition, adhesion and colonization resulting in biofilm formation [10]. The second step mainly results from enzymatic activities; enzymes produced by microorganisms cleave several bonds of the biodegradable polymer chain yielding monomers [9–11].

Gum ghatti is an anionic complex gum polysaccharide also known as Indian gum. It is obtained as the exudate of *Anogeissus latifolia* tree belonging to the *Combretaceae* family. It is mainly obtained as grey to reddish-grey powder, granular or light to dark brown lump and is almost odourless. The structure of Gg is very

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complex and the exact molecular weight is not yet known; Gum ghatti is composed of sugars such as L-arabinose, D-galactose, D-mannose, D-xylose and D-glucuronic acid in a 48:29:10:5:10 molar ratio. The main structure of Gg has alternating 4-O-substituted and 2-O-substituted α-D-mannopyranose units and chains of 1→6 linked β-D-galactopyranose units with side chains which are most frequently single L-arabinofuranose residue [12,13]. Gg is mainly used as a thickening agent in the foods stuffs and as an emulsifier due to its proteinous molecular components [14].

The aim of this study was to synthesize a biodegradable polymer of Gum ghatti (Gg) with acrylic acid (AA) using potassium persulphate (KPS) as an initiator and N,N′-methylene-bis-acrylamide (MBA) through graft co-polymerization technique with high metal adsorption capacity and investigate the degree of susceptibility to degradation by *Bacillus subtilis* in controlled environment.

2. Experimental

2.1. Materials

Gg, AA, KPS and MBA procured from Sigma Aldrich were used as received without further purification. All the solutions were prepared in triple distilled water. The other chemicals used were of analytical grade used as received.

2.2. Synthesis

The synthesis of the AA-grafted polymer was carried out by adding 1.0 g Gg to 15 ml of distilled water in a 100 ml beaker, 24 h before the reaction for the better dissolution of polysaccharide in water. Then 0.05 g KPS and 0.1 g MBA was added in the beaker and stirred vigorously. In the last step, 2.5 ml AA was added with continuous stirring. The beaker was kept undisturbed for 3 h at a temperature of 60 °C. After 3 h, the beaker was allowed to cool at room temperature and the homo-polymer was removed by successive extractions with acetone. Finally, the crosslinked polymer was dried at 50 °C in a hot air oven.

2.3. Biosorption experiment

2.3.1. Preparation of metal solutions

Stock solutions of Pb(II) and Cu(II) were prepared by dissolving appropriate amounts of lead nitrate and copper sulphate in 1000 ml of distilled water. Adequate volumes of the stock solution were further diluted in distilled water to make working solutions of various concentrations.

2.3.2. Metal ion removal

The batch experiments for the adsorption of Pb(II) and Cu(II) ions were done in thermostat water bath at 28 °C and 120 rpm for 24 h in 100 ml plastic bottles. Optimization of solution pH and polymer dose was done to get optimized adsorption conditions. Effect of pH on the adsorption of dye was studied over the pH range of 3–11 and the pH was adjusted by using 0.1 M NaOH and 0.1 M HCl solutions. In the typical batch experiments, 30 mg of the polymer was added in 50 ml of each metal ion solution (50 mg/l) taken in plastic bottles and shacked for 24 h over the thermostatic water bath. After 24 h, the bottles were taken out and the suspensions were filtered. Concentration of the unadsorbed metal ions was analyzed using atomic adsorption spectrophotometer. Polymer free solutions were run concurrently as controls to account for precipitation. Adsorption efficiency was calculated using the equation [15]:

$$q_e = \frac{(C_o - C_e)V}{m} \quad (1)$$

where q_e is the equilibrium adsorption of the metal ions per unit mass of the adsorbent (mg/g), m is the weight of the adsorbent (g) and V is the volume of the metal ion solution taken (l).

After the optimization of pH of the metal ions solution, effect of polymer dose on the adsorption of the metal ions was studied at optimum pH under the same experimental conditions described above with varying concentrations of the polymer dose i.e. 10, 20, 30, 40 and 50 mg. Respective biomass of polymer was mixed with 50 ml of metal ion solutions (50 mg/l) and the similar adsorption process was followed as used for the optimization of pH. Adsorption efficiency was calculated using Eq. (1).

Adsorption isotherm studies for the adsorption of Pb(II) and Cu(II) using Gg-cl-PAA was carried-out at 28 °C by adding optimized amount of the polymer in 50 ml of the metal ion solutions of various concentrations (20–80 mg/l). The metal ion solutions were placed in the water bath shaker and shaked for 24 h. The equilibrium adsorption was calculated using Eq. (1).

For the kinetics studies, adsorption of Cu(II) and Pb(II) onto Gg-cl-PAA was conducted over predefined time intervals; a mass of 0.2 g of the modified polymer was added to the 1000 ml (50 mg/l) solutions of Pb(II) and Cu(II) ion solutions. The solutions were continuously stirred at a speed of 120 rpm at 25 °C. Sample aliquot of 5 ml was collected from the start of the experiment and at predefined time intervals, then filtered and the filtrate was analyzed. Metal ion adsorbed at a particular time interval was calculated using the following equation [16]:

$$q_L = \frac{C_o - C_t}{m} \times V \quad (2)$$

where q_t is the amount of metal adsorbed per unit mass of the adsorbent (mg/g) and C_t is the concentration of metal (mg/l) at time t.

2.4. Biodegradation

2.4.1. Bacterial culture

Single colonies of *B. subtilis* were inoculated in nutrient broth ('Lab-Lemco' powder: 1.0 g/l; yeast extract 2.0 g/l; peptone 5.0 g/l; sodium chloride 5.0 g/l; pH 7.4 ± 0.2 at 25 °C; Merck Chemicals, SA) and incubated at 37 °C for 16 h (log phase) in an incubator with shaker (160 rpm). The cells were then harvested by centrifuging the culture at 8867 g for 10 min.

2.4.2. Degradation experiment

A biomass of 200 mg of polymer added to 100 mg of *B. subtilis* in 20 ml of liquid medium [phosphate buffered saline (137 mM NaCl, 10 mM PO_4, 2.7 mM KCl, pH 7.4) or mineral salt medium (50 mg/l KH_2PO_4; 5 mg/l $MgSO_4$; 100 mg/l $Na_2HPO_4{\cdot}12H_2O$; 20 NH_4NO_3)]. They were then mixed properly by gentle stirring and kept in the dark (to avoid photolytic degradation) at room temperature (28 °C). The mixture was stirred once a day and the medium replaced after four days. The experiment was carried out for 15, 30, 45 and 60 days, after which the content of petri dishes was filtered, dried in an oven at 90 °C for 24 h, then weight to determine the percentage weight loss. Controls consisting of bacteria free solutions were run concurrently to account for degradation resulting from hydrolysis. Experiments were carried out in triplicate and the average value was recorded.

2.4.3. Characterization of polymer by analytical methods

The structural determination, confirmation of grafting of poly(acrylic acid) onto Gg and different stages of biodegradation was done through ATR-FTIR (Perkin-Elmer Spectrum 100 spectrometer) in the spectral range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}. The changes in the morphology of Gg-cl-PAA before and after biodegradation with BS-MSM and BS-PBS were studied using