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MICROBIAL COMPOSITE IN TRIVALENT CHROMIUM REMEDIATION FROM EFFLUENTS

Abhilash, M. Prakash, K.D. Mehta, B.D. Pandey

CSIR-National Metallurgical Laboratory(CSIR-NML), Jamshedpur, India

Abstract

Chromium (III) in the industrial effluents is considered hazardous due to oxidation and therefore its remediation by alternate methodology such as biosorption has recently gained importance. Though sorption of Cr(III) on algae and fungi have been studied, their applications as fungal-clay composite is not much investigated. Growth of *A.niger* with bentonite showed release of spores and development of full cells in <72 h facilitating good metal uptake. In this study, *Aspergillus niger* and bentonite have been used to develop a composite sorbent and removal of Cr (III) from a model effluent solution of 100 ppm Cr(III) was investigated. A maximum sorption of 85 % Cr(III) was achieved with 1 % (w/v) composite (1:6 ratio of biomass to bentonite) of <50µm size within a contact time of 5 min at 2.5 pH and 35 °C temperature. Increasing the pH up to 4.0 caused elevation in the degree of sorption. The composite was thus found to be effective sorbent to treat chromium (III) containing waste solutions.

Keywords: – effluent, chromium (III), bentonite, *Aspergillus niger*, microbial composites, biosorption

Introduction

Chromium (III) available in effluents of tanning units and chemical plants, exists in different hydrolyzed forms as a function of pH, time, temperature, hydrolysis conditions, etc., and is considered a hazardous substance because of its conversion to hexavalent state under aquatic environment [1]. Generally, hexavalent chromium is treated by chemical/electrochemical method to reduce it to trivalent form followed by precipitation as a hydroxide. Besides precipitation, other methods such as adsorption, ion-exchange/solvent extraction, and reverse osmosis are other options for chromium removal.

Many yeast, algae, bacteria and various aquatic flora are known to be capable of concentrating metal species from dilute aqueous solutions. This uptake is often considerable and at times selective, and occurs via a variety of mechanisms including active transport, ion-exchange or complexation, and adsorption or inorganic precipitation [2]. Biosorbent may be used as an ion-exchange material. Adsorption occurs through interaction of the metal ions with functional groups that are found in the cell wall as biopolymers of either living or dead organisms. Researchers typically utilize dead organisms so that the metal uptake is only due to adsorption onto the fungus through interactions with the chemical functional groups found on the cell wall [2, 3]. The biosorption of uranium and other radionuclides by microbes has received attention. It seems that physicochemical interactions are responsible for majority of total uptake. In particular, the sorption of metal ions by fungal biomass arises from the coordination of the ions to the available functional groups acting as binding sites. Structural components include proteins, lipids, carbohydrates, polysaccharides, minerals as major components and essential oligoelements, free aminoacids, vitamins as minor components [3].

Clay minerals possess a layered structure and are typically suspended in aqueous solutions as fine particles having average diameter of about 2µm. Clays especially montmorillonite and bentonite are widely studied for the adsorption of metals. These clays are chosen to avoid pollutant release into the environment owing to their high surface areas, low cost and ubiquitous presence in most soils [4]. Several metal uptake mechanisms of trivalent metal ions have been proposed on clay minerals which includes sorption on edge sites, sorption on interlayer sites and the formation of lamellar nucleation phases. Bentonite as a representative clay mineral is a clay mainly composed of montmorillonite, a 2:1 type of aluminosilicate. Bentonites are valued for their sorptive properties. Affinity of some metals such as Cr (III) to bentonite is also of special interest for adsorption applications. Under certain conditions, the clay particles may aggregate leading to the variations of important properties which causes

difficulties in its practical usage and the applications to adsorption. It becomes necessary, therefore, to add certain additives, or polymers, to stabilize clay particles and prevent this behavior. Composites may display effective adsorbent behaviour than the inorganic and organic components [5–7]. To prepare composite ion exchangers or composite adsorbents, both inorganic and organic materials may be used as binding materials [8]. Composite adsorbents with inorganic binding matrices may have binders such as clay materials, cement, etc.

Adsorption of Cr (III) on zeolites and clays has been reported and so is the biosorption with algae and fungi [4, 9]. Most studies on metal ion removal have concentrated on the uptake of metals by either clay minerals or microbial biomass as individual and separate entities. There are reports on the sorption of metals to mixtures of microalgal/clay and cyanobacterial-clay. However, little has been paid to the investigation of metal accumulation by composites of fungi with clays [4].

In this study, the biomass of a fungus, *Aspergillus niger* and bentonite have been taken to develop a composite adsorbent and used for the removal of Cr (III) from a model effluent solution.

Materials and Methods

The reagents used in the experiment were of analytical reagent (AR) grade (Merck). Ultra pure water (resistivity $18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$) was used in the experiments. A solution of 1000 ppm of Cr(III) was prepared from $\text{CrCl}_3\cdot 6\text{H}_2\text{O}$ (Merck) by dissolving the salt in distilled water. The stock solution was diluted to prepare model effluent. The pH of each test solution was adjusted to the required value with dilute H_2SO_4 and NaOH solutions. Reagent blank were run for every sample solution. Buffering was not used due to unknown effects of buffer compounds on adsorption.

Aspergillus niger was obtained from IMTECH, Chandigarh. For the sorption studies, harvested fresh fungal samples were washed extensively with distilled water to remove the salts (sodium, potassium, magnesium and calcium) and then dried in an oven at 90°C for 24 h. The dry biomass was ground and sieved using a British Standard 300 mesh sieve ($<53 \mu\text{m}$). This biomass sample was stored in polypropylene container in a desiccator and used to prepare the composite adsorbent.

A commercial sample of bentonite in powdered form was obtained from M/s Ashapura Group, Mumbai. Na bentonite was dry-sieved under open laboratory conditions using a British Standard 300 mesh sieve ($<53 \mu\text{m}$) and stored in a polypropylene container in a desiccator. The sample was analysed as (%): SiO_2 :45.5; Al_2O_3 :16.1%; Fe_2O_3 :12.8; CaO :0.1; MgO :1.39; Na_2O :2.06; K_2O :0.5, with nearly 18.5% as loss due to ignition. The sample was characterized for its various physical properties influencing sorption, which are detailed in table 1.

Table 1

Characteristics of Bentonite

PROPERTY	DETAILS
Bulk Density	0.96 g/ml
Alkalinity	8.16%
Organic Matter	0.34%
Exchangeable Cations (mM)	$\text{Na}^+ = 42.6$, $\text{K}^+ = 1.4$, $\text{Mg}^{2+} = 3.7$, $\text{Ca}^{2+} = 22.9$
Water Soluble Cations (m eq/100g)	$\text{Na}^+ = 1.20$, $\text{K}^+ = 0.033$, $\text{Mg}^{2+} = 0.27$, $\text{Ca}^{2+} = 0.78$
CEC (mM/100g)	70.6518

Preparation of fungal-clay composite as adsorbent

The saprophytic fungus, *Aspergillus niger*, and clay, the bentonite were used to prepare as a composite adsorbent. Dry biomass powder (*A.niger*-150 mg) was mixed with 900 mg bentonite. The mixture was wetted with pure distilled water and thoroughly mixed. After mixing, the paste was heated in an oven at $105\pm 5^\circ\text{C}$ for 6 hours to dry the mixture. The wetting and drying step was repeated to maximize contact between *A.niger* and bentonite, thereby improving the composite efficiency. Then, the composite of *A.niger*- bentonite briquette was

then broken and passed through a 150 µm sieve. The composite was then dried in an electric oven at 105°C for several hours before using for the sorption experiments.

The swelling percentage of composite was obtained by adding 5 g of dry sample to pure water in a 100 ml graduated cylinder and recording the volume before and after 24 h. The swelling percentage of composite adsorbent was found to be 1.0 ml/g.

Batch sorption experiments

All the sorption experiments were performed using the composite sorbent. The prepared composite adsorbent (1g) was suspended in 100 mL of Cr(III) solution in an Erlenmeyer flask at selected pH. The solution was separated from the solids by filtering by Whatman No.42 Filter paper and if necessary by centrifugation for 15 minutes at 6000 rpm. The residual Cr(III) concentration in aqueous solution was determined by AAS. The amount of adsorbed chromium (III) was calculated from the difference of the chromium concentration in aqueous solution before and after sorption. The percent sorption (%) of Cr(III) was calculated by the equation (1)

$$Adsorption (\%) = \frac{C_i - C_f}{C_i} \times 100 \tag{1}$$

where C_i and C_f are the concentration of the Cr(III) in the initial and final solutions (mg/l), respectively. All experiments were performed in duplicates, and ±2% was found to be the limit of experimental error.

Results and Discussions

A. Effect of bentonite on growth of *A.niger*

Fungi are ubiquitous soil micro-organisms and are often the major component of the soil biota, especially in acidic soils of pH<5. In the soil environment, they exist in close and intimate contact with the clay minerals present [9].

To investigate the effect of clay on fungal growth, *A.niger* was grown in Czapek Dox Broth in presence of 1% (w/v) bentonite for a period of 72 h and the microscopic studies for effect of bentonite on fungal growth is detailed below. The pictorial presentation of the variations is cited in fig. 1.

Time	Microscopic Observations
<24 h	Well defined mycelium with formation of sporangium; clear deposition of clay on fungal surface
<48 h	Budding and visible branching of mycelium, sporangium bulbs seen
<72 h	Release of spores and presence of fully grown fungal cells in large groups in 1000X

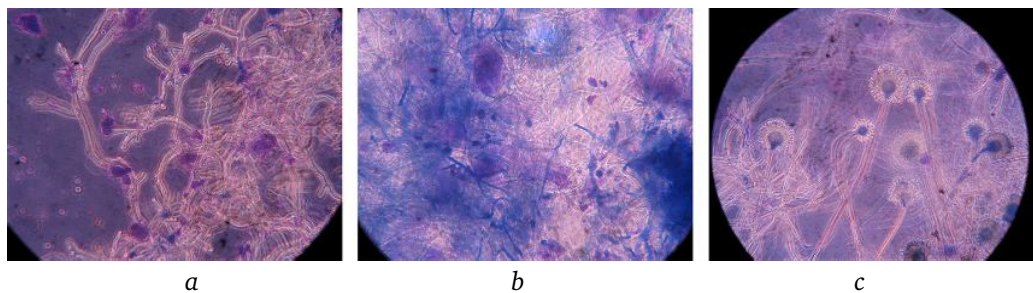


Fig. 1. Effect of bentonite on fungal growth (a:<24 h; b:<48 h; c:<72 h)

The fully grown fungus in less than 72h indicated that no microscopic damage was brought on to the cells.

B. Sorption of Cr(III) on the fungus-bentonite composite

B.1.Effect of mixing ratio of *A.niger* and bentonite in composite on sorption of Cr(III): Ratio of the components of composite was varied and its effect on the sorption is recorded in fig.2 (a, b). As depicted in fig.2a, the ration of bentonite substantially improved

sorption properties of the composite due to intercalation of clay in the biomass and strengthening of binding properties of the composite as a whole [4]. It was observed that in samples with high bentonite content, the amount of sorbed chromium increased. *A.niger*: Bentonite composite ratio of 1:6 would be used for biosorption experiments. On contrary in fig.2b, the increase in ration of fungus caused agglomeration and loosening in composite structure, rendering lesser degree of sorption.

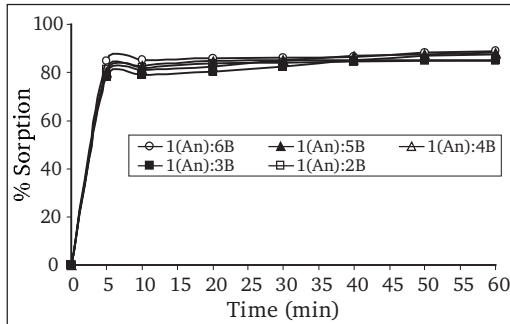


Fig. 2a. Effect of increasing bentonite ratio in composite on the sorption of chromium

[Cr(III) conc. = 100ppm, pH 2.5, T = 35°C, contact time = 1 h, m = 1 g, V = 100 ml {An-*A.niger*, B-Bentonite}]

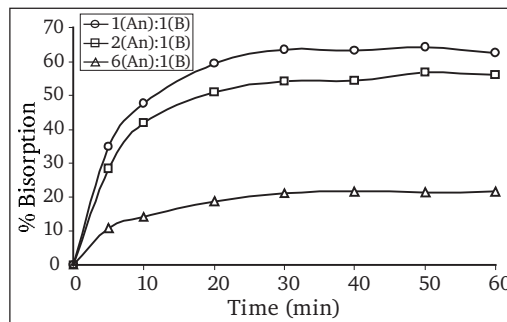


Fig. 2b. Effect of increasing ratio of fungus in composite on the adsorption of chromium

B.2. Effect of pH of solution on sorption of Cr(III) using 1:6 (An: Ben) composite

The effect of pH on sorption of 100ppm Cr(III) on composite was studied by varying the pH of model effluent solution from pH 2.0 to 5.0. The results are shown in fig.3 below.

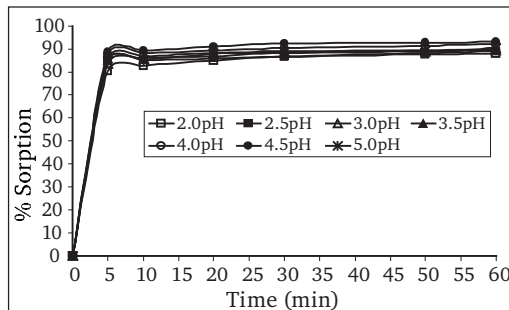


Fig. 3. Effect of pH on the adsorption of chromium by 1 (An)-6 (B) composite
[Cr(III) conc. = 100ppm, T = 35°C, contact time = 1 h, m = 1 g, V=100 ml {ratio: 1An-6B}]

The percent sorption increases with increase in pH up to 4.0–4.5 beyond which a decrease was observed mainly due to precipitation of Cr(III) as Cr(OH)₃. Relatively low degree of sorption at low pH was mainly attributed to the strong H⁺ ions competition for available exchange sites. At low pH, the oxides of aluminium, calcium, iron, silicon with their hydroxylated surfaces results in a positive surface charge [8]. This also adds to the lower sorption of Cr³⁺ on the sorbent surface. Hence, pH 4.0 (92% sorption in 1h) was selected to be the optimum pH in further studies.

Conclusions

1. *Aspergillus niger* was found adaptable to bentonite.
2. The ratio of 1:6 for *A.niger* to bentonite was found effective for biosorption.
3. 85% removal of Cr(III) in 5min was achieved from 100ppm feed at pH 2.5 and 35°C temperature with A: R ratio of 1:100.
4. Sorption rate increases with increase in pH from 2.0 to 4.5, after which Cr(III) precipitates. pH 4.0 was considered to be optimum for Cr(III) biosorption on the microbial composite.

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