Bull. Environ. Contam. Toxicol. (2004) 72:617–624

DOI: 10.1007/s00128-004-0288-1

Environmental Contamination and Toxicology

Bioremediation of Arsenic Contaminated Groundwater by Modified Mycelial Pellets of *Aspergillus fumigatus*

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Received: 12 June 2003/Accepted: 30 November 2003

A high degree of industrialization, urbanization and other anthropogenic activities has led to the contamination of various pollutants to ground water. In particular, metals pose a serious threat to human health due to its non bio-degradability and high toxicity even at low concentrations. Arsenic and iron contaminations in groundwater has been reported by researchers in many places especially in eastern states of India, particularly West Bengal and Bangladesh (Navarro et al. 1993; Mandal et al. 1996; Dhar et al. 1997; Biswas et al. 1998; Thompson et al. 1999; Acharyya 2002). Major portion of population in this part of world is totally dependent on ground water for drinking purpose and so are highly prone to diseases caused by arsenic (Pal et al. 2002). Chakraborty and Saha (1987) and Biswas et al. (1998), reported high rate of arsenic related diseases among these people. Skin carcinoma, black foot disease, diffused melanosis, spotted melanosis, diffused keratosis, spotted keratosis, hyper keratosis, nonpitting oedema, Bowen's disease, gangrene etc. are the health hazards caused by arsenic contamination (Biswas et al. 1998).

Microbial biomass as potential sorbent for removal of metals from industrial and municipal wastewater has been proposed as a promising alternative to conventional metal management strategies in past decades (Gabriel et al. 2001). At this juncture using dead fungal biomass as biosorbents for metal removal has received considerable attention since this represents a significant by product from several fermentation processes and produces less sludge (Volesky et al. 1993). Use of these biosorbents in treatment of potable water is not widely studied. But using microbial biomass for such purpose will prove to be effective and highly economical as the presently existing technologies like reverse osmosis, electrodialysis, ion exchange etc. are very expensive to treat water containing trace amounts of metals. The present study deals with treatment of arsenic contaminated ground water by the filamentous fungus *Aspergillus fumigatus* (Subramanian, 1983) in batch mode process.

MATERIALS AND METHODS

Ground water sample was collected from Ramnagar Old Hotkola, Kolkata, West

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Bengal, India in pre cleaned 5L plastic containers. The metal contents were estimated spectrophotometrically using Phenenthroline for Fe(II) (APHA 1995), ammonium molybdate for As(III) (Bassett et al. 1994) and ammonium molybdate and rhodamine B for As(V) (Palanivelu and Ramakrishna 1990).

For the preparation of biosorbent, 1mL (10^6 spores) of *A. fumigatus* spore suspension was inoculated into 100 mL of Czapex-Dox broth in 250 mL Erlenmeyer flasks and incubated at room temperature ($27^0 \pm 3^0$ C) for 5 days in an orbital shaker at 125rpm. At the end of fifth day, the mycelial pellets were separated by filtration through Whatman No.1 filter paper. Mycelial pellets were then washed with generous amount of deionized water until free from the media components. The washed pellets were autoclaved at 15 lbs for 15 min. A portion of the autoclaved mycelial pellets was mixed with 15 mg/L of $FeCl_3$ solution for 90 min. The autoclaved and $FeCl_3$ treated mycelial pellets were then tested for their efficacy to remove As and Fe from ground water. To determine the adsorption capacity of the mycelial pellets, 1.0g of the biosorbent was added to 50ml of sample and agitated in a rotary shaker at 150rpm for predetermined time intervals at 30^0C. The adsorbate and biosorbent were separated by centrifugation at 10,000 rpm for 20min. The remaining metals in the adsorbate were analyzed spectrometrically. The study was carried out with different dosages of biosorbent for an equilibrium time. Rate constant and Freundlich isotherm were determined from the data obtained in the equilibrium studies. Control experiments were carried out in absence of the biosorbent in order to find out whether there is any adsorption on the container walls. No adsorption on to the container walls was observed.

RESULTS AND DISCUSSION

As(III) and As (V) content of the sample was estimated to be 1.3 and 0.9 mg/L. But the permissible level of total arsenic in drinking water prescribed by WHO is 0.05 mg/L, which is far below than the level detected in sample. Biswas et al. (1998) has reported the occurrence of various health problems to the people consuming tube well water containing 0.05-1.0 mg/L of arsenic. In case of Fe(II) 0.3 mg/L was seen in the sample and the permissible limit is also 0.3 mg/L. This shows that there is no possibility for any health hazard by Fe(II). But still the efficacy of the biosorbent to remove Fe(II) was also tested as the sample contained all the three metals.

Metal uptake increased with increase in contact time but remained constant after an equilibrium time. Equilibrium time varied with metals due to the difference in initial metal concentration and affinity of biosorbent for a particular metal ion. In the groundwater sample, Fe(II) was completely removed in 60 min by autoclaved mycelial pellets and 105 min by $FeCl_3$ treated mycelial pellets; whereas, for As(III) and As(V) the equilibrium time was 150 min for autoclaved mycelial pellets (Fig. 1). Equilibrium time for As(III) and As(V) in $FeCl_3$ treated mycelial pellets was 135 and 150 min respectively. Equilibrium time required by

Penicillium purpurogenum biomass was 4h (Say et al. 2003). The equilibrium time required by the adsorbent used in the present study to remove As(III) is very less when compared to other reported adsorbents (Senthilkumar, 1998). This result is interesting because equilibrium time is one of the important considerations for economical water and wastewater treatment applications. Ghimire et al. (2002) reported that the As(III) removal was high in the $FeCl_3$ treated orange juice residue than the untreated orange juice residue. Iron(III) is considered to be adsorbed on the surface of the adsorbent by cation exchange mechanism and the arsenic can be adsorbed on the iron(III) immobilized onto the adsorbent by the mechanism of ligand exchange (Ghimire et al. 2002). This was concurrent with our results. The experiment was carried out with different biosorbent dosage up to equilibrium time (Fig. 2). It was noted that after the adsorbent dosage level of 1.75g/50ml, adsorption of As(III) and As(V) was either constant or very low. Fe(II) was completely removed. Consequently, this biosorbent dosage level was selected for further studies. Based on data obtained, the optimum conditions for removal of metals from groundwater were standardized.

The Freundlich adsorption isotherm was also applied for the adsorption of metal ions by modified mycelial pellets of *A. fumigatus*.

$$X/m = k_f C_e^{1/n} \text{ ------------------------------------ } (1)$$

Rearranging the equation (1) gives,

$$\log X/m = \log k_f + {}^{1/n} \log C_e \text{ ------------------ } (2)$$

where, C_e, is the equilibrium concentration (mg / L)

X/m, is the amount adsorbed at equilibrium time (mg / g) and

k_f & n are Freundlich constants (n gives an indication of the favorability and k_f[mg / g $(l/mg)^n$], the capacity of the adsorbent) (Pollards et al. 1991).

Linear plots of log C_e Vs log X/m show that the adsorption of metal ions onto modified pellets of *A. fumigatus* follows Freundlich isotherm model (Fig. 3). It also indicates that the average energy of adsorption decreases with increasing adsorption density. Similar results were reported by Senthilkumar (1998). Values of n and k_f were calculated from the slope and intercept, and are presented in Table. 1. The values of n between 1 and 10 represent good adsorption of the adsorbate onto the adsorbent (McKay et. al. 1982).

The rate constant of adsorption was determined from the following first order rate expression.

$$\log(q_e - q) = \log q_e - k_{ad}/2.303 \times t \text{ --------- } (3)$$

where, q and q_e are amounts of metal adsorbed (mg / g) at time, t (min) and at

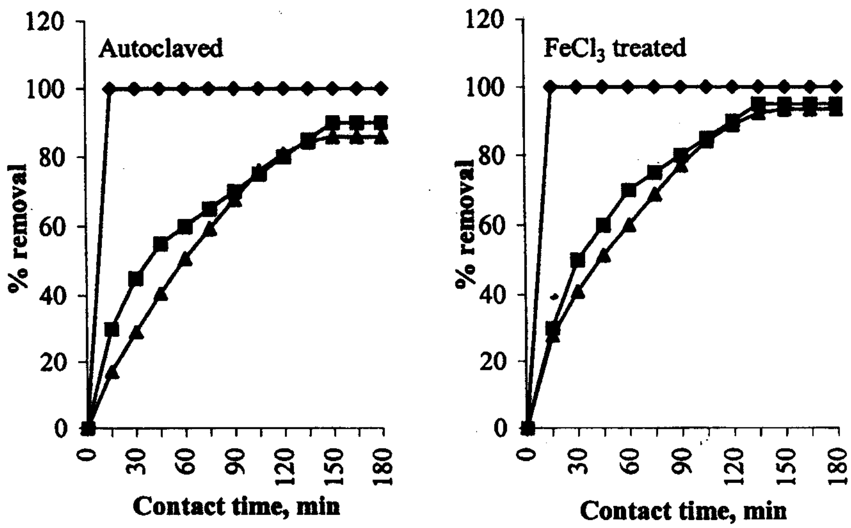

Figure 1. Effect of Contact time on removal of metals

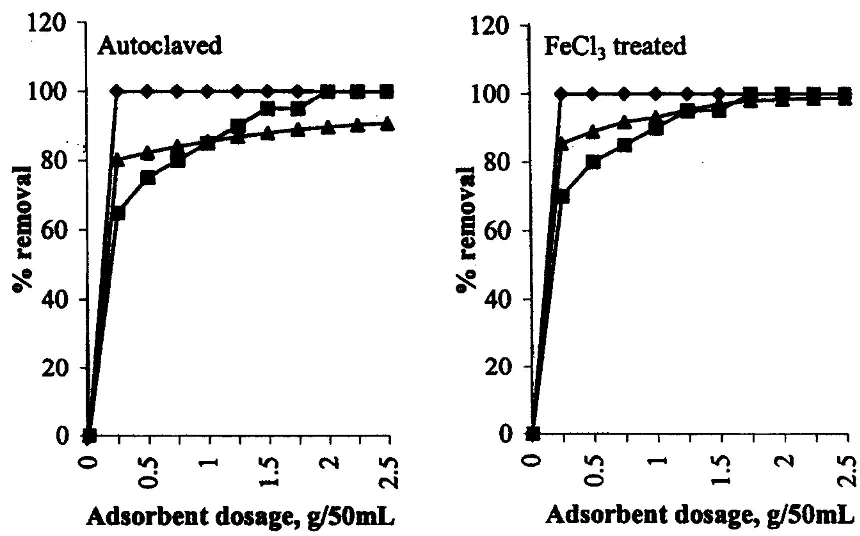

Figure 2. Effect of adsorbent dosage on removal of metals

Fe(II) As(III) As(V)

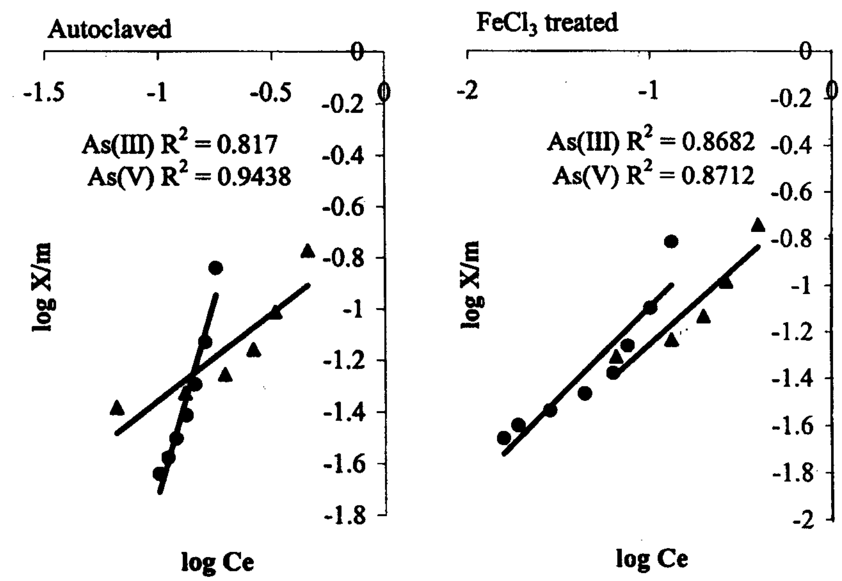

Figure 3. Freundlich plots for metal removal

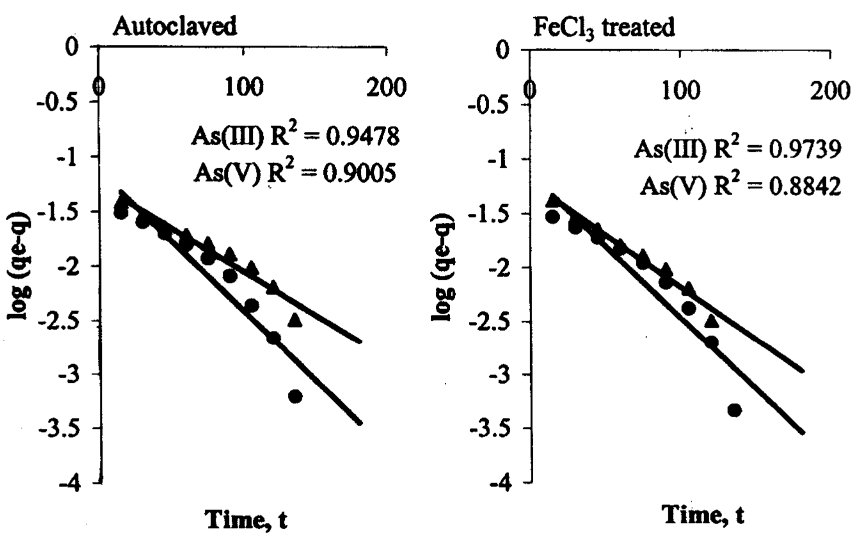

Figure 4. Lagergren plots for metal removal

▲ As(III) ● As(V)

Table 1. Freundlich and Lagergren constants for As(III) and As(V) removal

Metal	Treatment	Freundlich constants		Lagergren constant
		k_f [mg / g (l/mg)^n]	n	k_{ad}
As(III)	Autoclaved	0.2115	1.4646	0.0184
	$FeCl_3$ treated	0.2837	1.4205	0.0223
As(V)	Autoclaved	21.742	0.3281	0.0297
	$FeCl_3$ treated	0.4928	1.2765	0.0306

equilibrium, respectively

k_{ad}, is the rate constant for adsorption (1 / min).

The straight line plots of $\log(q_e - q)$ Vs t for different metal ion concentration indicate the applicability of the above equation (Fig. 4). Values of k_{ad} were calculated from the slope of the linear plots and are given in Table. 1. These are comparable with the k_{ad} values reported by Oscarson et al (1983) and Senthilkumar (1998). After treatment, the treated and untreated groundwater samples were plated on Czapex- Dox agar plates to check any contamination of *A. fumigatus* in treated sample. Similar colonies were observed on both samples. *A. fumigatus* was absent due to the use of autoclaved and $FeCl_3$ treated wet mycelial pellets as biosorbent. This validates the use of autoclaved-$FeCl_3$ treated mycelial pellets as a safe method for treatment of groundwater.

The study leads to the conclusion that $FeCl_3$ treated mycelial pellets of *A. fumigatus* is efficient in removing Fe(II), As(III) and As(V) from ground water. In batch mode studies, adsorption was dependent on contact time, initial metal ion concentration and biosorbent dosage. Adsorption of metal ions followed Freundlich isotherm model and the rate constant data would be useful for the fabrication and designing of water treatment plants. As the fungus *A. fumigatus* is easily cultivable and is also available as waste from certain fermentation industries, its utility as a biosorbent will be economical and can be viewed as a waste management strategy. Moreover, as the mycelial pellets are easily biodegradable, the metals can be desorbed after adsorption and the pellets can be taken for composting which is not possible in other chemical adsorbents.

REFERENCES

Acharyya SK (2002) Arsenic contamination in groundwater affecting major parts of southern West Bengal and parts of western Chhattisgarh: Source and mobilization process. Current Sci 82:740-744

APHA (1995) Standard methods for the examination of water and waste water. 19th edition. American Public Health Association, Water Pollution Control Federation, Washington, DC.

Bassett J, Denny RC, Jeffery GH, Mendham J (1994) Vogel's text book of quantitative inorganic analysis 5th edition. Longman Press, London

Biswas BK, Dhar RK, Samanta G, Mandal BK, Chakraborti D, Faruk I, Islam KS, Md. Moniruzzaman chowdhury, Islam A, Roy S (1998) Detailed study report of Samata, one of the arsenic-affected villages of Jessore District, Bangladesh. Current Sci 74:134-145

Chakraborty AK, Saha KC (1987) Arsenical dermatosis from tube well water in West Bengal. Indian J Med Res 85:326-328

Dhar RK, Biswas BK, Samanta G, Mandal BK, Chakraborti D, Roy S Jafar A, Islam A, Gulshan A, Kabie S, Khan AW, Ahmed SA, Hadi A (1997) Groundwater arsenic calamity in Bangladesh. Current Sci 73: 48-59

Gabriel J, Baldrian K, Hladikova, Hakova M (2001) Copper sorption by native and modified pellets of wood-rotting basidiomycetes. Letts Appl Microbiol 32:194-198

Gimire KN, Inoue K, Makino K, Miyajima T (2002) Adsorptive removal of arsenic using orange juice residue. Sep Sci Technol 37(12): 2785-2799.

McKay G, Blair HS, Gardner TK (1982) Adsorption of dyes on chitin. J Appl Polymer Sci 27:3043-3057

Mandal BK, Chowdhury TR, Samanta G, Basu GK, Chowdhury PP, Chanda CR, Lodh D, Karan NK, Dhar RK, Tamili DK, Das D, Saha KC, Chakraborti D (1996) Arsenic in groundwater in seven districts of West Bengal, India – The biggest arsenic calamity in the world. Current Sci 70:976-986

Navarro M, Sanchez M, Lopez H, Lopez MC (1993) Arsenic contamination in Waters, Soils and Sludges in Southeast Spain. Bull Environ Contamin Toxicol 50:356-362

Oscarson DW, Huang PM, Hammer UT (1983) Oxidation and sorption of arsenite by manganese as influenced by surface coatings of iron and aluminium oxides and calcium carbonate. Water Air Soil Pollut 20:233-244

Pal T, Mukherjee PK, Sengupta S (2002) Nature of arsenic pollutants in ground water of Bengal basin – A case study from Baruipur area, West Bengal, India. Current Sci 82:554-561

Palanivelu K, Ramakrishna TV (1990) Separation and spectrophotometric determination of trace amounts of arsenic as the ternary complex with molybdate and rhodamine B. Indian J Technol 28:67-70

Pollards SJT, Sollars CJ, Perry R (1991) A low cost adsorbent from spent bleaching earth in the selection of an activation procedure. J Chem Technol Biotechnol 50:265-275

Say R, Yilmaz N, Denizli A (2003) Biosorption of cadmium, lead, mercury and arsenic ions by the fungus *Penicillium purpurogenum*. Sep Sci Technol 38(9), 2039-2053

Senthilkumar S (1998) Recycling of industrial solid "waste" Fe(III)/Cr(III) hydroxide for the adsorption of toxic metals from aqueous and its applications to industrial wastewaters. Ph.D. thesis, Bharathiar University, Coimbatore, India

Subramanian CV, (1983) Hyphomycetes – Taxonomy and biology. Academic press, London, p 502

Thompson TS, Lee MD, Kasick AR, Macaulay TJ (1999) Arsenic in Well Water supplies in Saskatchewan. Bull Environ Contamin Toxicol 63:478-483

Volesky B, May PH, Holan ZR (1993) Cadmium biosorption by *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 18:1215-1218