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Arsenic biosorption by mucilaginous seeds of *Hyptis suaveolens* (L.) Poit

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Hyptis suaveolens seeds could serve as natural immobilized source of agriculturally based polysaccharide. Maximum adsorption capacity (6 gl^{-1}) of the seeds for arsenic adsorption has been found at pH 3.5 - 4.5. Among the tested interfering common metal ions, Ca^{++}, Mg^{++} and Cl^{-} decreased adsorption rate about 48%, 54% and 30% respectively, whereas Zn^{++} and Co^{++} ions have no significant effect. Adsorption isotherm studies revealed a better correlation with Langmuir isotherm plot.

Keywords: Arsenic biosorption, *Hyptis suaveolens*, Mucilaginous seed

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Introduction

Millions of people in the world are suffering with skin lesions, cancers and other related diseases due to consumption of arsenic contaminated groundwater[1]. Many people have died and hundreds of millions are access at serious risk in Bangladesh, India, China, Vietnam, Taiwan, Japan, Poland, Hungary, Romania, Slovakia, Belgium, Chile, Argentina and North Mexico[2-4]. Long term geochemical changes led to the release of arsenic from arsenopyrites caused by its oxidation by air reaching underground aquifers through the tubewell conducts[5]. Arsenic is also released by current or historical mining activities and oxidation of sulfide minerals[6]. Arsenic is accumulated in human beings from drinking water as well as from agricultural crops and vegetables[7]. Sequestration of arsenic by biological material is receiving attention in a biotechnological context since microbe based technologies[8] and phytoremediation[9-10] is cost effective and environment friendly. Microbial exopolysaccharides, which may be excreted into the medium or extracellular gelatinous mucilage layer or capsule around the cell used in the treatment of aqueous heavy metal and radionuclic waste[11], and agricultural based polysaccharides are attractive biosorbent for heavy metal treatement[12].

Present study identifies a cost effective possible bioremediation process for arsenic contaminated water cleanup technology using mucilaginated seeds of *Hyptis suaveolens* (L.) Poit. Seed coat mucilage containing acidic polysaccharide is a highly branched L-fuco-4-O-methyl-D-glucurono-D-xylan[13].

Materials and Methods

Pretreatment of Seeds

H. suaveolens (L.) Poit. (wilayati tulsi) is an aggressive weedy species, widely distributed in the tropics and subtropics[14]. Seeds, collected from natural population of IIT Kharagpur campus, were allowed to swell in deionized (Milli-Q) water for 30 min. Swollen seeds were pretreated for 30 min in water baths containing (a) boiling water (70-80ÚC), (b) 1M NaOH, (c) 1N H_2SO_4, (d) alcohol (95%). All treated preparations were extensively washed with deionized water to remove NaOH, H_2SO_4 and alcohol. Seeds are flat and irregular in shape. Average size of the seeds (3.5±0.16 mm × 2.5±0.12 mm) increases when steeped into water; seeds become swollen (5.8±0.22 × 4.5 ± 0.16mm). Swelling percentage $P = V_s - V_d / V_d \times 100$ is 125%, where V_s is volume of soaked seed and V_d is volume of dry seed (mean volume measured by measuring cylinder of 100 seeds in triplicates). Outer surface of swollen seeds are

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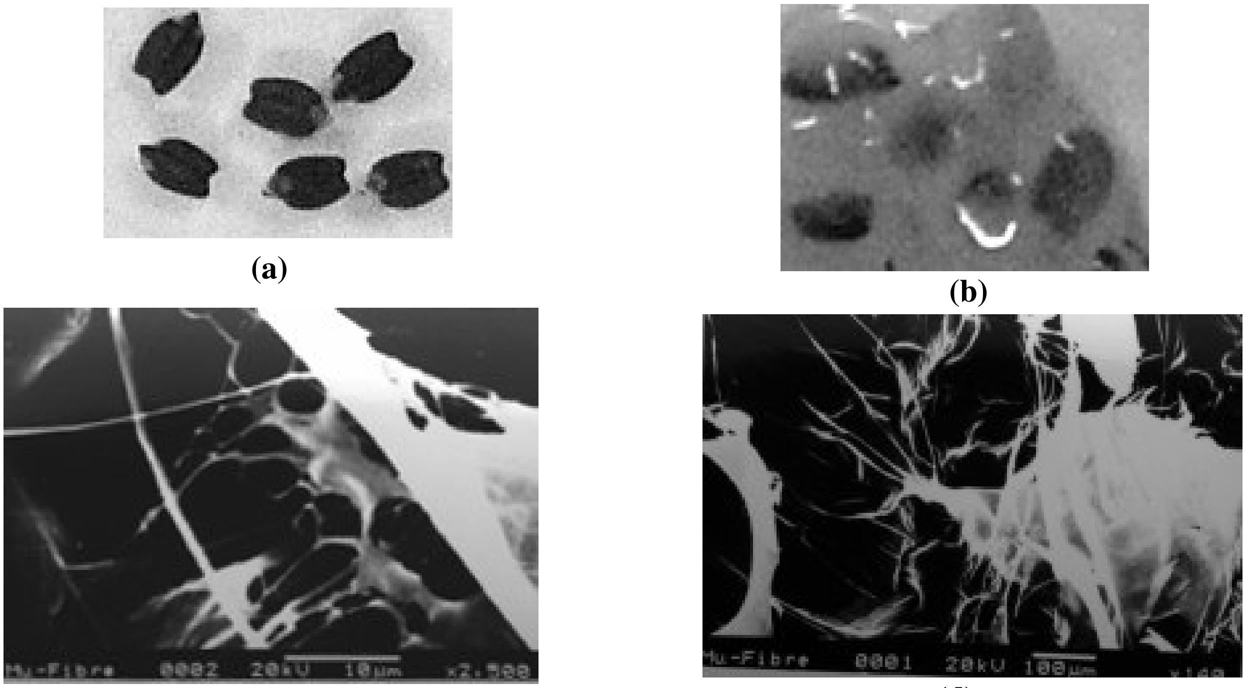

Fig. 1 — Seed characteristics of *H. suaveolens*: (a) Normal seed; (b) Soaked seeds (30 min in water); (c) Meshwork formation in the outer layer and (d) Fibril nature of mucilage

mucilaginous consisting of thread like microfibrillar structure forming a meshwork surrounding seed coat (Fig. 1).

Instruments and Reagents

A digital pH meter (EA-940, Orion research Inc., USA) was used for pH measurement, and rotary incubator shaker (Innova-4200, USA) was used for shaking. Absorbances were measured with (Beckman Coulter, DU 640B) spectrophometer equipped with 1 cm quartz cuvette. SEM pictures were taken on a Scanning Electron Microscope (JSM-5800, JEOL). Energy Dispersion Analysis by X-Ray (EDAX) studies were conducted using the Energy Dispersive Microanalysis system attached with SEM.

All reagents were of analytical grade. Methylene blue (MB) (Qualigens, India) was purified by the crystallization from alcohol. Sodium dodecyl sulfate (SDS) (SRL, India), $NaBH_4$ (Spectrochem, India) was freshly prepared in ice-cold water. Arsenic (V) solutions were prepared using Na_3AsO_4 (Merck Ltd., UK). The concentration of unadsorbed arsenic in the medium was determined spectrophotometrically[15].

Uptake Studies in Batch Process

Arsenic uptake studies were performed in a batch process. Treated and control swollen seeds (0.2g dry wt) were taken in 100 ml Erlenmeyer flask containing 50 ml of arsenate solution (conc. 25 µg/ ml). Flasks were placed on a rotary shaker and shaken at 150 rpm at 25±2°C. After desired incubation time (40 min), supernatant was analyzed for arsenic concentration.

Effect of pH and Adsorbent Dose

Aqueous arsenate solution (0.2 g dry seed in 50 ml arsenate solution) adjusted to the required pH (1-8) using 0.1 N HCl or 0.1 N NaOH and for adsorbent dose, weights of dry seeds were varied (2-10 gl^{-1}) at pH 3.5-4.5. In both cases, seeds were pretreated with boiling water. Flasks were shaken with 150 rpm at 25±2°C for 40 min.

Equilibrium Adsorption Dose

Equilibrium adsorption experiments were performed with pretreated seeds (boiling in water) equivalent to 6 gl^{-1} in flasks containing arsenate solution (5-50 mg/ l). Flasks were agitated with 150 rpm at 25±2ÚC. The uptake (q) for the construction an isotherm was calculated using the equation $(C_i - C_f)$ x V/M, where C_i is initial arsenic concentration, C_f is equilibrium arsenic concentration, V is volume of solution (l) and M is mass of biomass (g).

Effect of Other Ions on Arsenic Uptake

Uptake of arsenate by the treated (boiling water) seeds was studied in the presence of other ions with equal concentration of arsenate (conditions are same).

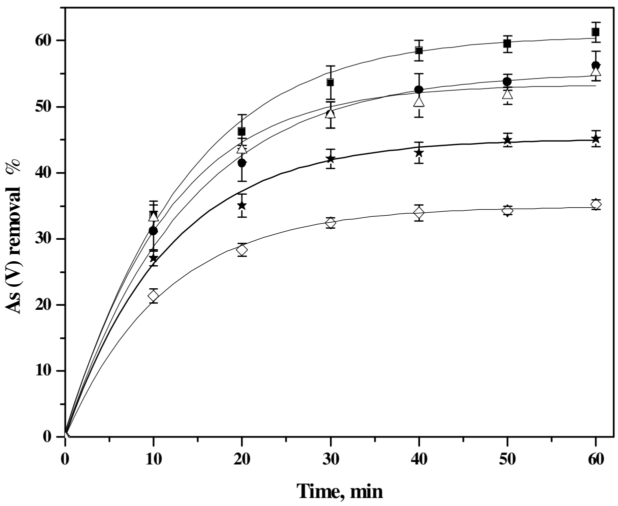

Fig. 2 — Kinetics of As (V) uptake by different pretreated seeds: (△) water; (■) boiling water; (◆) NaOH; (◇) H_2SO_4; (*) alcohol

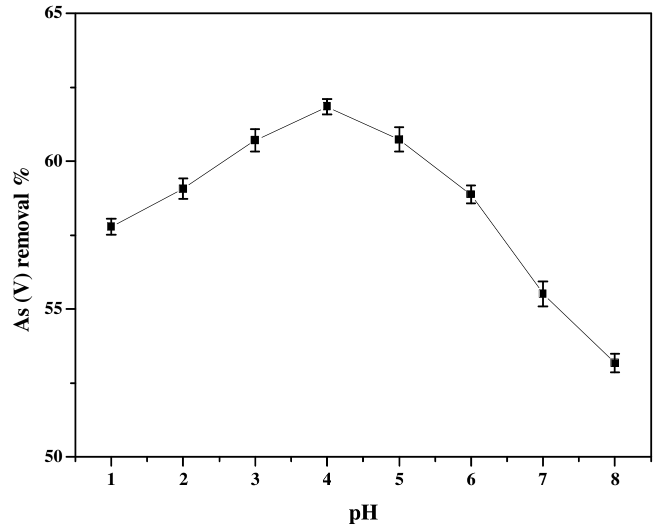

Fig. 3 — Effect of pH on biosorption of As (V) by *H. suaveolens* seeds pretreated with boiling water

Scanning Electron Microscopy and EDAX Study

Wetted seeds from agitated flask were dehydrated by multiple washing with progressively concentrated acetone (20, 30, 60, 80 and 100%) for 20 min each. These samples were then fixed onto a graphite stub and then kept in a E5200 Auto sputter coater (UK) under vacuum in 15 min. Photographs were taken at 20 kV accelerating voltage. For EDAX study, seeds were taken after absorption in optimum condition and dried at 40°C in oven and then one seed placed onto graphite stub for x-ray dispersion analysis.

Statistical Analysis

All data represents the mean of triplicates. Values are subjected to regression analysis using origin software package, version 6.1.

Results and Discussion

Pretreatment Effect on As (V) Uptake

Seeds pretreated with boiling water (70-80°C) exhibited maximum rate of adsorption of As (V) (Fig. 2). The mucilaginous polysaccharide might loose its viscosity with raising the temperature, resultant the thick compact polysaccharide to be fragile and easily dispensable in boiling water. Seed coat polysaccharide is prepared to have L-fuco-4-O-methyl-D-glucurono-D-xylan moiety with extensively acidic side chain[13] and it was probable opened in hot water. Muralikrishna *et al*[16] showed that few plants derived mucilaginous polysaccharide decreased their viscosity with increasing temperature up to a certain level. Seeds pretreated with 1M NaOH in water bath showed slightly better adsorption capacity compare to control. Pretreatment of microbial biomass or other agricultural biomass with alkali has shown to enhance metal adsorption capacities[17]. Seeds pretreatment with NaOH, showed outer mucilaginous surface slightly fragile[12] but few acidic side chains become neutralized. H_2SO_4 treatment resulted in a decrease in biosorption rate because of outer mucilaginous polysaccharide hydrolysis; as a result the side chain broke down. Alcohol treatment also decreases uptake rate due to its dehydrating nature; outer mucilaginous fiber like structures become slightly closed up and decrease the volume percentage.

Effect of pH on As (V) Biosorption

Arsenic uptake by adsorbent varies with pH[18]. Removal efficiency was maximum at pH 3.5-4.5 (Fig. 3). Adsorptivity decrease with increase in pH might be because at higher pH range there is a competition between arsenate and OH^- ions. Similar trends have been reported for microbial biomass[19].

Effect of Adsorbent Dose on As (V) Removal

Adsorbent dose has significant effects on the removal of arsenic[20]. Adsorption of As (V) reached maximum level at seed dose (6 g/l, dry wt.); after that, removal efficiency slightly decreases (Fig. 4). The higher biomass concentration formed a dense outer layer by the mucilaginous fiber of seed coat, blocking the binding site from arsenate ions.

Adsorption Isotherm

Arsenate uptake by seeds might be the result of chemical co-ordination between arsenate and acidic

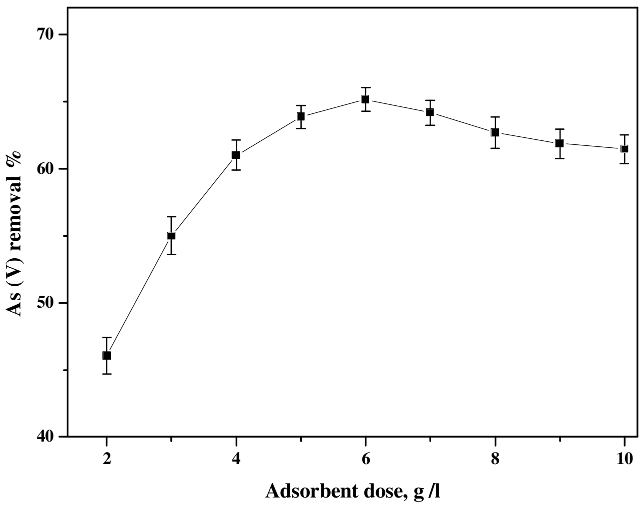

Fig. 4 — Effect of adsorbent dose for As (V) removal

Fig. 5— Langmuir isotherm plot of As (V) adsorption on *H. suaveolens* seeds boiling in water

polysaccharide of the seed. The relation between the amount adsorbed by an adsorbent and the equilibrium concentration of the adsorbate at a constant temperature were best fitted with a linear model of Langmuir isotherm (Fig. 5). This showed a correlation coefficient (R^2) = 0.9866.

Effect of Other Ions on As (V) Removal

EDAX study exhibited that control system (no interfering metal ions) has potential (64.38 ± 2.40) for arsenate uptake (Fig. 6). As (V) removal by various metals was found as follows: Zn^{++}, 62.42 ± 3.26; Ca^{++}, 31.16 ± 0.52; Mg^{++}, 35.72 ± 0.85; Co^{++}, 60.44 ± 1.65;

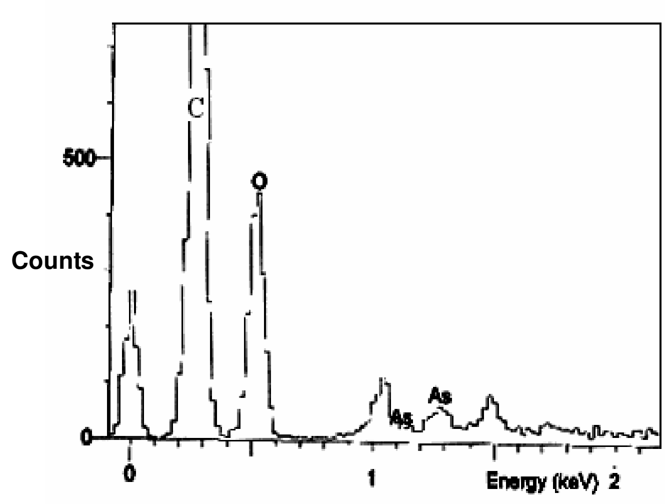

Fig. 6— Energy dispersion analysis by X-Ray for As (V) uptake by the seeds boiling in water (without other interfering ions)

and Cl^-, 46.08 ± 1.66%. Decrease (50%) in removal efficiency by Ca^{++} and Mg^{++} ions might be due to insoluble metal hydroxide formation onto mucilage layer surface that should have affected surface pH. Using common ion (Cl^-), arsenate removal capacity decreases (30%) due to stronger affinity of Cl^- ion competing with arsenate ions.

Packed bed reactors are not amenable due to irregular shape and size of seeds. Main disadvantage is that without rotation these highly mucilaginated pretreated seeds were dense and blocked the binding site. Relatively little is known about the potential for biological removal of arsenic. Compared to reports on arsenic removal such as phytochelation[21], rhizofiltration[22], genetic evaluation of hyperaccumulator[23], high biomass phytoremediators[24] and combustion of waste product[25], seeds of *H. suaveolens* in this paper may have potential advantages. This is because seeds have high amount of acidic polysaccharide and adsorb arsenate in aqueous medium. Seeds are easily available throughout the tropics and subtropics. It might be used for large-scale water treatment due to its mechanical stability to vigorous stirring.

Conclusions

H. suaveolens seeds have been found an effective adsorbent to remove arsenic as arsenate from water. Adsorption isotherm follows Langmuir isotherm. Seeds pretreated with boiling water were able to remove arsenic up to 64%. Metal ions like Zn^{++} and CO^{++} do not affect arsenic removal but it is affected by the presence of Ca^{++},

Mg^{++}, and Cl^{-} ions in water. Arsenic removal is efficiently performed at lower pH (3.5-4.5). Overall, technology is cost effective and might be used for large-scale water treatment system.

References

1 Chaterjee A, Das D & Mandal B K, Arsenic in groundwater in six districts of west Bengal, India. The biggest arsenic calamity in the world. Prt 1. Arsenic species in drinking water and urine of affected people, *Analyst*, **120** (1995) 643-650.

2 Niu S, Cao S & Shen E, The status of arsenic poisoing in China, in *Arsenic: Exposure and Health Effects*, edited by C O Abernathy *et al* (Chapman and Hall, London) 1997, 78-83.

3 Chowdhury T R, Basu G K, Mandal B K, Biswas B K, Samanta G, Chowdhury U K, *et al*, Arsenic poisoing in Ganges Delta, *Nature*, **401** (1999) 545-546.

4 Lindberg Anna-L, Goessler W E, Gurzau K K, Rudnai P, Kumar R, Fletcher T, Leonardi G, Slotova K, Gheorghiuc E & Vahter M, Arsenic exposure in Hungary, Romania and Solvakia, *J Environ Monit*, **8** (2006) 203-208.

5 Mandal B K, Chowdhury T R, Samanta G, Basu G K, Chowdhury P P, Chanda C R, *et al*, Arsenic in groundwater in seven districts in West Bengal, India- the biggest calamity in the World, *Curr Sci,* **70** (1996) 976-987.

6 Bowel R J, Morely N H & Din V K, Arsenic speciation in soil pore waters from Ashanti mine, *Ghana Appl Geochem,* **9** (1994) 15-22.

7 Das H K, Mitra A K, Sengupta P K, Hossain A, Islam F & Rabbani G H, Arsenic concentration in rice, vegetables, and fish in Bangladesh: A preliminary study, *Environ Int,* **30** (2004) 383-387.

8 Satishkumar M, Murugesan G S, Ayyasamy P M, Swaminathan K & Lakshmanaperumalsamy P, Bioremediation of arsenic contaminated groundwater by modified mycelial pellets of *Aspergillus fumigatus, Bull Environ Contam Toxicol*, **72** (2004) 617-624.

9 Ma L Q, Kumar K M, Tu C, Zhang W, Cai Y & Kennelly E D, A fern that hyperacumulates arsenic, *Nature*, **409** (2001) 579.

10 Francesconi K, Visoottiviseth P, Sridokchan W & Goessler W, Arsenic species in an arsenic hyperacumulating fern, *Pityrogramma calomelanos*: A potential phytoremidiator of arsenic contaminated soils, *Sci Total Environ,* **284** (2002) 27-35.

11 Gutnick D L & Bach H, Engineering bacterial biopolymers for the biosorption of heavy metals: New products and novel formulations, *Appl Microbiol Biotechnol,* **54** (2000) 451-460.

12 Melo J S & D' Souza S F, Removal of chromium by mucilaginous seeds of *Ocimum basilicum*, *Bioresour Technol,* **92** (2004) 151-155.

13 Aspinall G O, Capek P, Carpenter R C, Gowda D C & Szafranck J, A novel L-fuco-4-O-methyl-D-glucurono-D-xylan from *Hyptris suaveolens*, *Carbohydr Res,* **214** (1991) 107-113.

14 Willis J H, *Dictionary of Flowering Plants and Ferns* (Cambridge University Press, London) 1973, 587.

15 Kundu S, Kavalakatt S S, Pal A, Ghosh S K, Mandal M & Pal T, Removal of arsenic using hardened paste of Portland cement: batch adsorption and column study, *Water Res,* **38** (2004) 3780-3790.

16 Muralikrishna G, Bhat R & Tharanathan R N, Functional characteristics of the mucilaginous polysaccharides derived from cowpea (*Vigna sinensis*), black gram (*Phaseolus mungo*) and linseed (*Linum usitatissimum*). *Starch-strake*, **39** (2006) 107-109.

17 Azab M S & Peterson P J, The removal of cadmium from water by the use of biological sorbents, *Water Sci Technol,* **21** (1989) 1705-1706.

18 Zeng L, A method for preparing silica containing iron (III) oxide adsorbents for arsenic removal, *Water Res*, **37** (2003) 4351-4358.

19 Say R, Yilmaz N & Denizli A, Removal of heavy metal ions using the fungus *Penecillium canescens*. *Adsorpt Sci Technol*, **21** (2003) 643-650.

20 Goel R, Kapoor S K, Misra K & Sharma R K, Removal of arsenic from water by different adsorbents, *Indian J Chem Technol*, **11** (2004) 518-525.

21 Pickering I J, Prince R C, George M J, Smith R D, George G N & Salt D E, Reduction and coordination of arsenic in Indian mustard, *Plant Physiol*, **122** (2000) 1171-1177.

22 Grabisu C, Hernandez A J, Barrutia O, Alkorta I & Becerril J M, Phytoremediation: A technology using green plants to remove contaminants from polluted areas, *Rev Environ Health*, **17** (2002) 173-188.

23 Dhanker O P, Li Y, Rosen B D, Shi J, Salt D, Senecoff J F, *et al*, Enginnering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and gamma-glutamyl cysteine synthetase expression, *Nat Biotechnol*, **20** (2002) 1140-1145.

24 Doucleff M & Terry N, Pumping out the arsenic, *Nat Biotechnol*, **20** (2002) 1094-1095.

25 Cullen W R & Reimer K J, Arsenic speciation in the environment, *Chem Rev*, **89** (1989) 713-764.